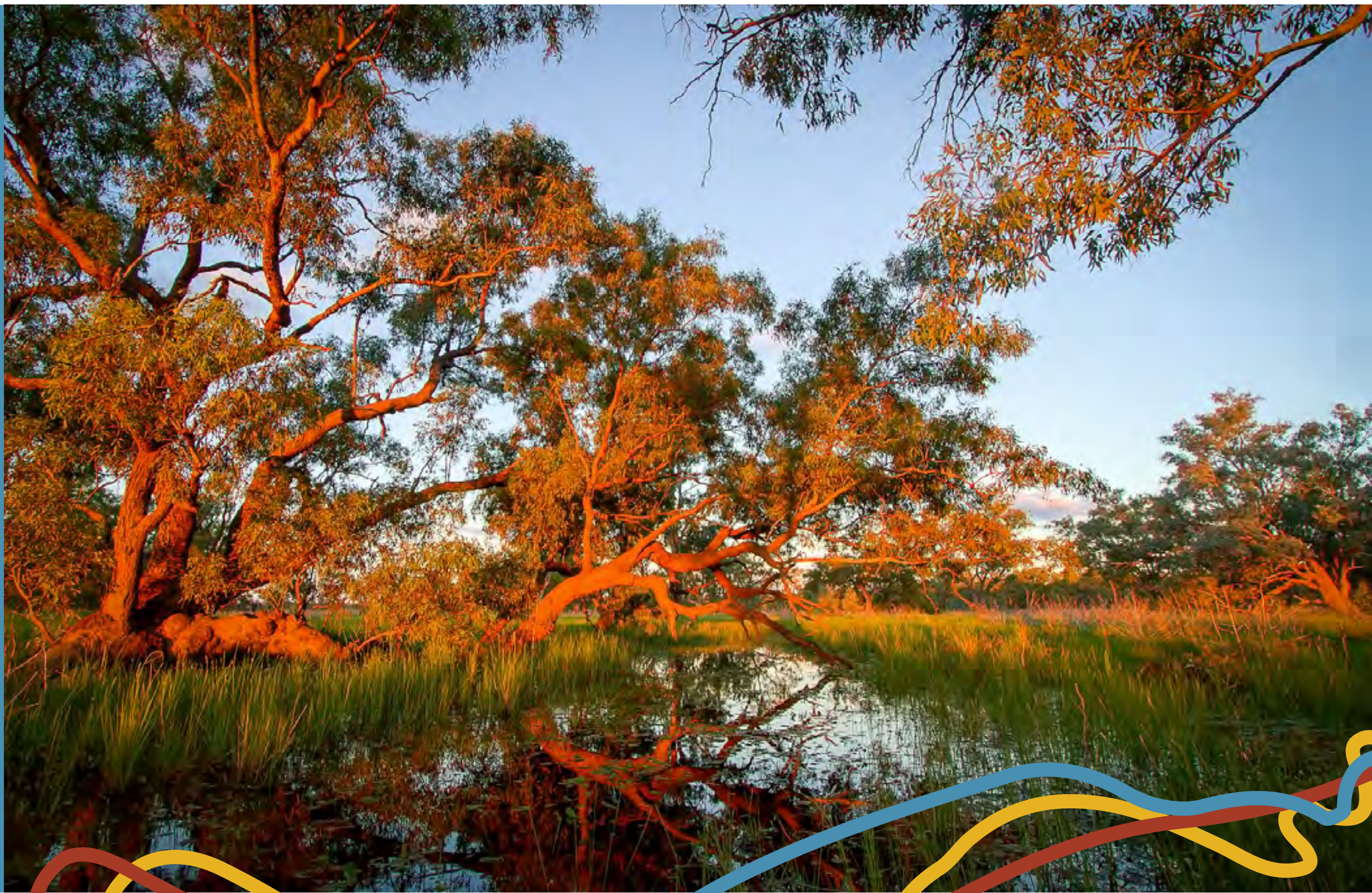
RESEARCH **15** **2017-054**

WHAT ARE THE CARP VIRUS BIOCONTROL RISKS AND HOW CAN THEY BE MANAGED?

NATIONAL CARP CONTROL PLAN

Social, economic and ecological risk assessment for use of Cyprinid herpesvirus 3 (CyHV-3) for carp biocontrol in Australia

VOLUME 1: Review of the literature, outbreak scenarios, exposure pathways and case studies



This suite of documents contains those listed below.

NCCP TECHNICAL PAPERS

1. Carp biocontrol background
2. Epidemiology and release strategies
3. Carp biocontrol and water quality
4. Carp virus species specificity
5. Potential socio-economic impacts of carp biocontrol
6. NCCP implementation
7. NCCP engagement report
8. NCCP Murray and Murrumbidgee case study
9. NCCP Lachlan case study

NCCP RESEARCH (peer reviewed)

Will carp virus biocontrol be effective?

1. 2016-153: Preparing for Cyprinid herpesvirus 3: A carp biomass estimate for eastern Australia
2. 2018-120: Population dynamics and carp biomass estimates for Australia
3. 2017-148: Exploring genetic biocontrol options that could work synergistically with the carp virus
4. 2016-170: Development of hydrological, ecological and epidemiological modelling
5. 2017-135: Essential studies on Cyprinid herpesvirus 3 (CyHV-3) prior to release of the virus in Australian waters
6. 2020-104: Evaluating the role of direct fish-to-fish contact on horizontal transmission of koi herpesvirus
7. 2019-163 Understanding the genetics and genomics of carp strains and susceptibility to CyHV-3
8. 2017-094: Review of carp control via commercial exploitation

What are the carp virus biocontrol risks and how can they be managed?

9. 2017-055 and 2017-056: Water-quality risk assessment of carp biocontrol for Australian waterways
10. 2016-183: Cyprinid herpesvirus 3 and its relevance to humans
11. 2017-127: Defining best practice for viral susceptibility testing of non-target species to Cyprinid herpesvirus 3
12. 2019-176: Determination of the susceptibility of Silver Perch, Murray Cod and Rainbow Trout to infection with CyHV-3
13. 2016-152 and 2018-189: The socio-economic impact assessment and stakeholder engagement
    Appendix 1: Getting the National Carp Control Plan right: Ensuring the plan addresses
    community and stakeholder needs, interests and concerns
    Appendix 2: Findings of community attitude surveys
    Appendix 3: Socio-economic impact assessment—commercial carp fishers
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    Appendix 7: Socio-economic impact assessment—recreational fishing sector
    Appendix 8: Socio-economic impact assessment—koi hobbyists and businesses
    Appendix 9: Engaging with the NCCP: Summary of a stakeholder workshop
14. 2017-237: Risks, costs and water industry response
15. 2017-054: Social, economic and ecological risk assessment for use of Cyprinid herpesvirus 3
    (CyHV-3) for carp biocontrol in Australia
    Volume 1: Review of the literature, outbreak scenarios, exposure pathways and case studies
    Volume 2: Assessment of risks to Matters of National Environmental Significance
    Volume 3: Assessment of social risks
16. 2016-158: Development of strategies to optimise release and clean-up strategies
17. 2016-180: Assessment of options for utilisation of virus-infected carp
18. 2017-104: The likely medium- to long-term ecological outcomes of major carp population reductions
19. 2016-132: Expected benefits and costs associated with carp control in the Murray-Darling Basin

NCCP PLANNING INVESTIGATIONS

1. 2018-112: Carp questionnaire survey and community mapping tool
2. 2018-190: Biosecurity strategy for the koi (*Cyprinus carpio*) industry
3. 2017-222: Engineering options for the NCCP
4. NCCP Lachlan case study (in house) (refer to Technical Paper 9)
5. 2018-209: Various NCCP operations case studies for the Murray and Murrumbidgee river systems
   (refer to Technical Paper 8)

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Biocontrol of European Carp

Ecological risk assessment for the release of Cyprinid herpesvirus 3 (CyHV-3) for carp biocontrol in Australia

Volume 1: review of the literature, outbreak scenarios, exposure pathways and case studies

Sam Beckett, Peter Caley, Matt Hill, Sam Nelson and Brent Henderson
February 2020

Data61, Synergy Building, North Science Road (Black Mountain Site), Acton, Canberra, Australia.

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Volume 1: review of the literature, outbreak scenarios, exposure pathways and case studies

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We would also like to thank the NCCP project teams whose work has provided critical insights into the biomass of carp on a continental scale, the likely impacts of carp mortality on water quality, and aspects of the likely behaviour of CyHV-3 as a biocontrol for carp across a range of freshwater ecosystems.

Over-and-above these teams, we would like to recognise the outstanding contributions of the many key researchers and water managers whose expertise in a range of areas has enabled us to make judgements about ecological risk. We are particularly grateful to Dr Jon Marshall for his comments on, and contributions to, the case study for the Moonie River Catchment; and to Dr Danial Stratford for his invaluable editorial and scientific review.

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INTRODUCTION

The Australian Government has invested in the development of a National Carp Control Plan (NCCP) to explore the potential use of *Cyprinid herpesvirus 3* (CyHV-3) for the biological control of carp in Australia. Carp occur in every state and territory except the Northern Territory and are now the dominant fish species within the Murray-Darling Basin. The ecological impacts of carp include increased turbidity, intensified algal blooms and reduced abundance of macrophytes, invertebrates and some native fish.

One of the core objectives of the NCCP is to undertake research and development to address knowledge gaps, and to better understand and manage risks to support the potential release of CyHV-3, subsequent clean-up, and the recovery of native fish and ecosystems. The ecological and social risk assessment detailed in the three volumes of this report was one of the projects funded through the NCCP.

The **ecological** component of the assessment was undertaken in two parts:

(a) A compilation of the science and epidemiology of CyHV-3 and an assessment of outbreak scenarios, exposure pathways and case studies (Volume I of this report)

(b) An assessment of the risks that the proposed release of CyHV-3 may pose to the assets that have been described under the EPBC ACT[1] as Matters of National Environmental Significance (MNES) (Volume 2 of this report).

Part (a) above provided the underpinning for part (b), but also gave a more comprehensive assessment of the environmental risks that may be associated with the release of CyHV-3. Part (b) was directed specifically at the needs of the Strategic Assessment that will be required by the Department of Environment and Energy, under the EPBC Act, if the release of CyHV-3 is judged to be feasible.

The standalone **social** component of the assessment is documented in Volume 3 of this report. This assessment included two forms of stakeholder interaction and was undertaken to evaluate perceptions about the risks attached to the proposed release of CyHV-3.

KEY FINDINGS

The **ecological risk assessment** made use of outbreak scenarios, exposure pathways and case studies to evaluate risks to species and ecological communities at a national scale. The development of outbreak scenarios enabled key aspects of the epidemiology of CyHV-3 to be considered in an Australian context. This included a range of outbreak settings, such as ephemeral

[1] Environment Protection and Biodiversity Conservation Act 1999

wetlands, perennial and disconnected river systems and lakes and other impoundments, as well as consideration of the implications of high-flow and lower-flow seasons. The outbreak scenarios were informed by the spawning behaviour of carp, and by the importance of aggregations and water temperature to the perpetuation of CyHV-3.

- Although aggressive outbreaks of CyHV-3 were considered possible in most settings, impacts on water quality are likely to require a relatively high carp biomass density and relatively poor connectivity of the waterway in which the outbreak occurs. In this context, impacts on water quality may include a reduction in dissolved oxygen as a result of increased biological oxygen demand (possibly to the point of anoxia), an increase in the likelihood of widespread cyanobacterial blooms as a result of an increase in phosphorous and dissolved organic carbon, and an increase in the risks associated with proliferating waterborne spoilage and other microorganisms. Native fish (small-bodied and large-bodied) and crustaceans are most at risk from low dissolved oxygen – in particular, species with a limited geographic range or a reliance on a small number of local populations. These and other aquatic and terrestrial water users, including waterbirds, will also be at risk from cyanobacterial blooms. Colonial-nesting waterbirds are more at risk than those that nest individually, as are the waterbird functional groups (such as the piscivorous seabirds and large waders) that are most closely associated with water.
- High-risk settings for the impacts of an outbreak of CyHV-3 on food webs include ephemeral wetlands during high-flow seasons, when the floodplains are inundated and a maximal number of breeding piscivorous waterbirds are present; and some permanent lakes and irrigation reservoirs that may act as a refugia for breeding waterbirds during lower-flow seasons. The emphasis on breeding (as opposed to nesting) waterbirds is relevant, as chicks are more likely to be stressed by the removal or suppression of juvenile carp than are adult birds. The removal of juvenile carp may result in piscivorous waterbirds switching to other prey species – including the juvenile large-bodied native fish, adult or juvenile small-bodied native fish, frogs and frog spawn, crustaceans and turtle eggs or hatchlings – and this may place stress on some important local populations.
- Botulism outbreaks in wildlife follow a highly-probabilistic process and are potentially a concern in any wetland, lake or waterhole where carcass accumulation occurs in the presence of large numbers of waterbirds. Although most terrestrial and aquatic species will be at risk in the event of an outbreak of type C (or C/D mosaic) botulism, fatalities are generally most striking amongst waterbirds – in particular, those that include insects in their diet.

These assessments speak to the risks faced by individuals, and by local populations. The risks faced by a species as a whole will reflect the exposure of individuals (as above) as well as a raft of population-level factors, such as the strength and geographic distribution of its population across Australia and the effectiveness of its recruitment or rejuvenation strategies.

A range of mitigations was considered for each of the identified exposure pathways. Following the dictates of the Department of Environment and Energy, these addressed, in decreasing order of preference, the avoidance, mitigation and offsetting of risk and ongoing adaptive management of residual threats. Risks to native species or communities can be avoided chiefly through the strategy for release of CyHV-3, which should consider the implications of high-flow and lower-flow seasons for impacts in different settings and geographical locations. The partial removal of carp from waterways, ahead of the release of the virus, may provide another means by which water quality risks can be avoided. This strategy is likely to be particularly attractive in waterways that are prone to low flows or to the formation of disconnected waterholes. Risks that cannot be avoided may be mitigated, and this will be chiefly through the removal of carp carcasses or the use of water regulation to flush carcasses or the products of carcass decomposition (including cyanobacterial blooms) from sensitive areas. Offsetting the harm from any remaining risks will focus largely on the release of farmed species at strategic locations. The effectiveness of this will in most cases be bolstered through wild-caught broodstock. Ongoing adaptive management will include programmed monitoring of water quality data from the Murray-Darling Basin and beyond, as well as the programmed monitoring of key or indicator species.

The **assessment of EPBC Act MNES** covered the breadth of natural and built assets that might be exposed through the proposed release of CyHV-3. This included threatened and migratory species, as well as threatened ecological communities, Ramsar wetlands, World Heritage Properties, National Heritage Places, Commonwealth Marine areas, the Great Barrier Reef Marine Park and Commonwealth Lands. The assessment for threatened and migratory species focussed on the likelihood of a Major impact at a national level, while the balance of assessments was undertaken using the Department of Environment and Energy's significant impact criteria. With risk mitigations in place, Medium risks remained for some large-bodied and small-bodied native fish, shorebirds, large waders and native frogs. Species that are micro-endemic within areas that also include a high biomass density of carp were maximally exposed from a geographic standpoint, although the risk estimates also reflected exposure to (as relevant) poor water quality, food web disturbances and an outbreak of botulism. The only non-negligible residual risks for MNES assets other than threatened and migratory species were attributed to a range of Ramsar wetlands and two of the National Heritage properties (the Cowra Japanese Gardens and Cultural Centre and Centennial Park).

The **social risk assessment** was undertaken to evaluate perceptions about ecological and other risks attached to the proposed release of CyHV-3. The assessment showed that while communities are accepting of the use of CyHV-3 to control invasive carp in Australian waterways, their acceptance is dependent on familiarity with the NCCP, personal interactions with waterways, knowledge of carp impacts and values, and their sense of community responsibility towards environmental stewardship. Uncertainty underpinned baseline concerns about the possible impact of CyHV-3 on humans, and on animals other than carp. The results underscored the need for proactive and effective communication across a range of social strata, with clear messaging about both strategy for the release of the virus and site-specific plans for the clean-up and disposal of carcasses.

ECOLOGICAL RISK ASSESSMENT

The ecological risk assessment was informed by an assimilation of the grey and published literature, and by the outputs of companion projects undertaken through the NCCP.

The review (Volume I of this report) encompassed the characteristics of Australia's freshwater waterways, the species and ecological communities that may be at risk in the event of an outbreak of CyHV-3, the ecology of carp in Australia and the underpinning for its success as an invasive species, the epidemiology of CyHV-3 in farmed and wild carp, and the impacts of fish kills on water quality, risk of botulism and food webs. The review provided an assimilation of key works from the published and grey literature. As they became available, the reports of other NCCP projects were also included. These included, in particular, the carp biomass modelling study (Stuart *et al*., 2019), and the water quality research (Walsh *et al.*, 2018) and modelling (Hipsey *et al*., 2019) studies. The report of the epidemiological modelling study (Durr *et al*., 2019a, 2019b and 2019c) was released in draft format immediately prior to the release of this risk assessment. An abstract from the epidemiological modelling study was included and key assumptions and conclusions were cross-checked for consistency with the outcomes of this risk assessment.

An expert elicitation study was embedded within the review and sought to clarify some of the critical questions concerning the role that juvenile carp may play as a food source for nesting piscivorous waterbirds. A quantitative joint-distribution modelling study was also carried out, with the aim of exploring the likelihood that other invasive species would rebound given the removal or suppression of carp. This study identified goldfish, tench, redfin perch, roach, Oriental weatherloach and eastern mosquitofish as invasive species whose habitat is currently correlated with that of carp. Dietary overlap, affinity for the highly-turbid waters that are likely to remain for the short-medium term, and shifts in pressures on zooplankton and phytoplankton, are all factors that may influence the likelihood that one or more of these species would benefit substantially from the removal or suppression of carp. Goldfish and eastern mosquitofish already coexist with carp in robust populations that include, in the case of goldfish, some hybridisation. Tench compete directly with carp and occupy a similar ecological niche and, although currently inhibited by carp, and likely to benefit from their removal or suppression, are considered far less destructive from an ecological standpoint. Redfin perch are a predatory species, and their success in the event of carp removal or suppression will relate to their ability to feed in turbid waters. As redfin perch also predate on other juvenile non-native fish, this may lead to secondary impacts that are more difficult to predict. Less is known about the ecology of roach or Oriental weatherloach in Australia, although both are substantially smaller fish than carp and neither is likely to recruit as effectively in high-flow seasons.

The assessment of ecological risk (Volume I of this report) built on the reviews and evaluations described above. One of the most striking characteristics of the assessment was the breadth of ecological assets (including species, communities and places) and settings that it was required to encompass in order to evaluate the possible impacts of CyHV-3 at a national scale. A national scale was chosen as the virus is likely to spread naturally from the place(s) of introduction, or through the translocation of affected live fish or carcasses, during one or several seasons. In order to address the breadth of concerns associated with release at a national scale, the assessment included outbreak scenarios for a range of freshwater environments, nine key exposure pathways describing the ways in which the release of CyHV-3 could result in harm to the environment, and a series of detailed case studies.

The outbreak scenarios focussed individually on ephemeral wetland settings, lakes and reservoirs, and riverine settings. Across these, the importance of aggregation events, carp biomass density and water temperature were underscored. The scenarios focussed on the events that are likely to unfold under a maximally aggressive outbreak. In most settings, this will correspond to the period immediately after release of the virus. In disconnected riverine environments, however, outbreaks are more likely to be aggressive during the dry season following from reconnection of the river system – that is, after affected fish have had an opportunity to be redistributed through the population.

Although aggressive outbreaks of CyHV-3 were considered possible in most settings, impacts on water quality are likely to require a relatively high carp biomass density and relatively poor connectivity of the waterway in which the outbreak occurs. In this context, impacts on water quality may include a reduction in dissolved oxygen as a result of increased biological oxygen demand (possibly to the point of anoxia), an increase in the likelihood of widespread cyanobacterial blooms as a result of an increase in phosphorous and dissolved organic carbon, and an increase in the risks associated with proliferating waterborne spoilage and other microorganisms. High-risk settings for impacts on water quality include spring or autumn outbreaks within the seasonally-disconnected waterholes that characterise many dryland river systems (for example, the Moonie River in Queensland), and spring and summer outbreaks within ephemeral wetlands during lower-flow seasons when aquatic biota are concentrated in available wetland or off-channel habitat (for example, the Barmah-Millewa Forest in New South Wales and Victoria). Conversely, it is relatively less likely that water quality will be diminished in the event of an outbreak of CyHV-3 within deep and flowing waterways such as the Murray River channel. Although there is a range in the susceptibility of individual species to low dissolved oxygen, most will be affected if levels lower than 3 mg/L persist. The Basin Plan target of ≥50 percent saturation (or a dissolved oxygen of approximately 4.5 mg/L at 20C) is widely regarded as the appropriate critical value for Australian freshwater river channels and anabranch creeks.

The accumulation of decomposing carcasses may also initiate a widespread cyanobacterial bloom. Some species of cyanobacteria are toxic, and this will have a direct impact on aquatic and terrestrial animals – including livestock and humans. As cyanotoxins may also bioaccumulate in animal tissue, a toxic threshold can be breached through repeated low-dose exposure. When the conditions change, or the substrate is depleted, the bloom will collapse and die. This results in a substantial increase in biological oxygen demand and a precipitous drop in dissolved oxygen. The impact of a collapsed bloom on dissolved oxygen is likely to exceed the impact of carcass decomposition (above) and will have a marked effect on water-breathing aquatic life.

Relatively less is known about the impact of decomposing fish carcasses on the proliferation of waterborne microorganisms. Carp gut flora and spoilage organisms may be present in high numbers, as might *E. coli*, some *Pseudomonas spp* and other opportunistic microorganisms. Shiga toxin-producing *E. coli* (STEC) has been isolated from ponds, streams, wells and water troughs, and have been found to survive for months in manure and water-trough sediments. *Aeromonas spp* have also been found in irrigation water, rivers, springs, groundwater, estuaries and oceans and are of public health concern. The decomposition of carp carcasses in mesocosms has resulted in a decrease in signature lake bacteria, and an increase in environmental copiotrophs and fish gut bacteria. Potentially, some changes to the bacterial flora may persist once waterways have returned to an otherwise healthy state. Aquatic and terrestrial animals that have faced other challenges arising from an outbreak of CyHV-3 (for example, a cyanobacterial bloom) are likely to be stressed and immunocompromised and may have a diminished resistance to waterborne microorganisms that are pathogenic for their species or functional group. These possibilities notwithstanding, very little evidence was found within the published or grey literature to substantiate a link between substantive fish kills in Australian freshwater environments and the proliferation of, or disease resulting from, waterborne microorganisms.

The mitigation of risks associated with diminished water quality will rest largely on the timely removal of carp and other carcasses, noting that carcasses will in general only float for 1 to 3 days following death. Although the timely removal of carp carcasses is likely to be a practical proposition in some settings (for example, urban lakes and some irrigation reservoirs), the magnitude of the task or the accessibility of waterways may in other settings be problematic. This is likely to be the case in some seasonally-disconnected dryland rivers, for example, where the population is sparse and the monitoring of, and access to, individual disconnected waterholes, may not be practical. The collection of carcasses may also be difficult in some wetland settings – in particular, during a high-flow season when the floodplains are inundated, and access is limited to shallow-draft water craft. As an alternative, or adjunct, to the removal of carcasses and carcass materials, it may be practical in some situations to make use of regulatory structures to flush carcass materials or cyanobacterial blooms from affected areas and to refresh the quality of the water. Within the Chowilla Floodplain in South Australia, for example, the sophisticated Chowilla regulator and ancillary structures enable water to be directed to particular parts of the wetland complex, even when flows through the Murray River channel are relatively low.

High-risk settings for the impacts of an outbreak of CyHV-3 on food webs include ephemeral wetlands during high-flow seasons when the floodplains are inundated, and a maximal number of breeding waterbirds are present (for example the Macquarie Marshes in New South Wales); and some permanent lakes and irrigation reservoirs that may act as a refugia for breeding waterbirds during lower-flow seasons (for example, Kow Swamp in Victoria). In this context, the effects on food webs may include stress to the chicks of (in particular) colonial-nesting piscivorous waterbirds following the removal or suppression of juvenile carp, as well as an impact on native species as a result of prey-switching.

Mitigation of the food web effects of an outbreak of CyHV-3 on breeding waterbirds will largely be limited to consideration of the timing of virus introduction into a naïve population of carp. In some catchments, for example, it may be beneficial to ensure that the virus is introduced during a relatively lower-flow season. The situation is complex, however, as two caveats to this approach are that: (a) breeding waterbirds taking dry-season refuge in permanent lakes and impoundments within the same catchment may then be exposed; and (b) the impacts of the virus on water quality may then be more significant. Mitigation of the effects of prey-switching will again rest on timing, with the aim being to avoid high-flow seasons when a wide range of native species will be taking advantage of inundated floodplains. It may also be beneficial to plan for the restocking of key ecological communities with (in particular) juvenile native fish. This strategy may in turn be aided by sourcing broodstock from key catchments and wetlands, to ensure that restocked juveniles have an optimum local fitness when released.

Botulism outbreaks in wildlife follow a highly-probabilistic process and are potentially a concern in many wetland, lake and waterhole settings. Most terrestrial animals (including livestock) are susceptible to type C (or C/D mosaic) botulinum toxin, the most likely form of botulism in Australian wildlife. Humans, however, are not susceptible, and fish are only partly susceptible. Waterbirds are commonly the most affected, and while all waterbird species are susceptible those that consume insects and those that are more closely affiliated with water are likely to be most at risk. Botulism outbreaks in wildlife may arise in two key ways: (a) through the death of animals carrying spores within their gastrointestinal tracts, and the initiation of what is termed the 'carcass-maggot cycle'; or (b) through the germination of spores within the environment. In both cases, the germination of spores is triggered by anaerobic conditions and the presence of a suitable organic substrate. Under the first pathway (a) large numbers of carp carcasses might result in the initiation of an outbreak of botulism. Under the second pathway (b) the accumulation of carp carcasses might result in a drop in dissolved oxygen within an aquatic environment; or might result in the initiation of a widespread cyanobacterial bloom, which then dies and results in a drop in dissolved oxygen. The mitigation of risks associated with botulism in wildlife will again focus on the timely removal of carcasses and the possible use of regulatory structures to divert water to affected areas. These considerations notwithstanding, Agriculture Victoria (for example) have investigated numerous major blackwater events and fish kills in Victorian waterways and wetlands and have not to-date identified any cases of botulism in associated waterbirds. The peer-reviewed literature is also absent of robust evidence for the role of large fish kills as initiators of botulism outbreaks in natural settings.

ASSESSMENT OF EPBC ACT MNES

The assessment of risks to assets defined under the EPBC Act as Matters of National Environmental Significance (MNES) was undertaken to provide the core material for a Strategic Assessment (Volume 2 of this report). The Strategic Assessment will be required under the EPBC Act if the Australian Government considers the proposed release of CyHV-3 to be feasible and chooses to take it forward.

The assessment of EPBC Act MNES included the following:

- Threatened species
  - Critically endangered species
  - Endangered species
  - Vulnerable species
- Migratory species
- Threatened ecological communities
  - Critically endangered communities
  - Endangered communities
  - Vulnerable communities

- Ramsar wetlands of international importance
- World Heritage Properties
- National Heritage Places
- Commonwealth Marine areas
- Great Barrier Reef Marine Park
- Commonwealth Lands.

The assessment for threatened and migratory species was undertaken using a five-point likelihood scale and a risk scenario that represented Major impact at a national level. The assessments for the balance of MNES assets were undertaken using a simpler dichotomous scale based on the existence of a real chance or possibility of observing a significant impact. Criteria for significant impacts on each category of MNES are provided by the Department of Environment and Energy.[2]

For assessments other than for Commonwealth Lands, evaluation was undertaken: (a) without risk management measures; and (b) with risk management measures (that is, residual risk). Risk management included measures to avoid, mitigate and offset risks and to provide for ongoing adaptive management. Throughout the evaluation of risk management measures, it was assumed that resources would be sufficient to encompass the activities in the location(s) described. Although the evaluation focused on outcomes following directly from the release of the virus, it was also assumed that resources would encompass surveillance and (if required) ongoing mitigation during years subsequent to the release of the virus.

A summary of the outcomes of the assessments for threatened and migratory species is given in Figure 1 (unmanaged risks) and Figure 2 (managed or residual risks). No unmanaged risks were considered Extreme. High unmanaged risks were recorded for large- and small-bodied native fish, shorebirds, large waders and native frogs. With management measures in place, no High risks remained, although a range of Medium risks remained for large-bodied and small-bodied native fish, shorebirds, large waders and native frogs. These included risks associated with poor water quality (whether from low dissolved oxygen [DO], widespread cyanobacterial blooms or proliferating microorganisms), food web disturbances (including the removal of juvenile carp as a dominant and stable food source, and the impacts of prey-switching as a result of this) or an outbreak of type C (or C/D mosaic) botulism. Under the assessment framework used for threatened and migratory species a Medium risk equated to the view that a Major impact at a national level is unlikely – that is, uncommon, although the outcome has been known to occur in a range of circumstances.

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[2] Matters of National Environmental Significance, Significant Impact Guidelines 1.1 (see: https://www.environment.gov.au/epbc/publications/significant-impact-guidelines-11-matters-national-environmental-significance)

Actions on, or impacting upon, Commonwealth land, and actions by Commonwealth agencies, Significant impact guidelines 1.2 (See: https://www.environment.gov.au/epbc/publications/significant-impact-guidelines-12-actions-or-impacting-upon-commonwealth-land-and-actions)

Figure 1 Summary of unmanaged risks for threatened and migratory species

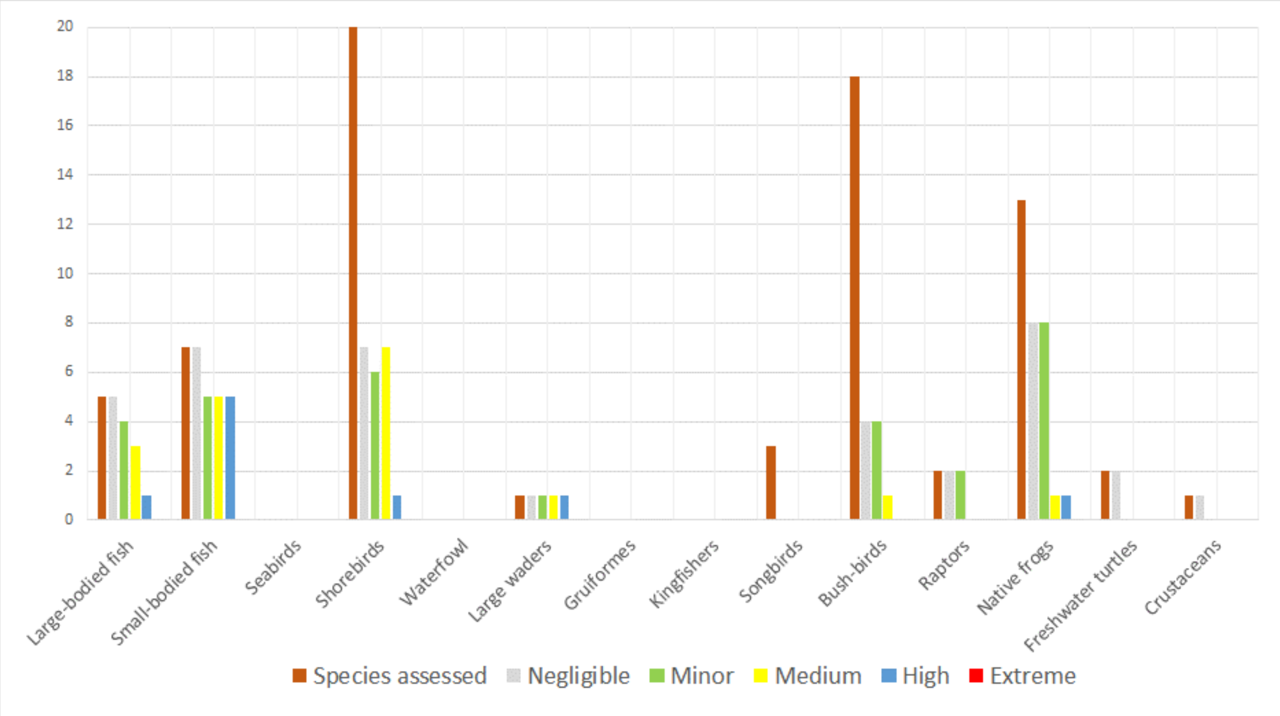
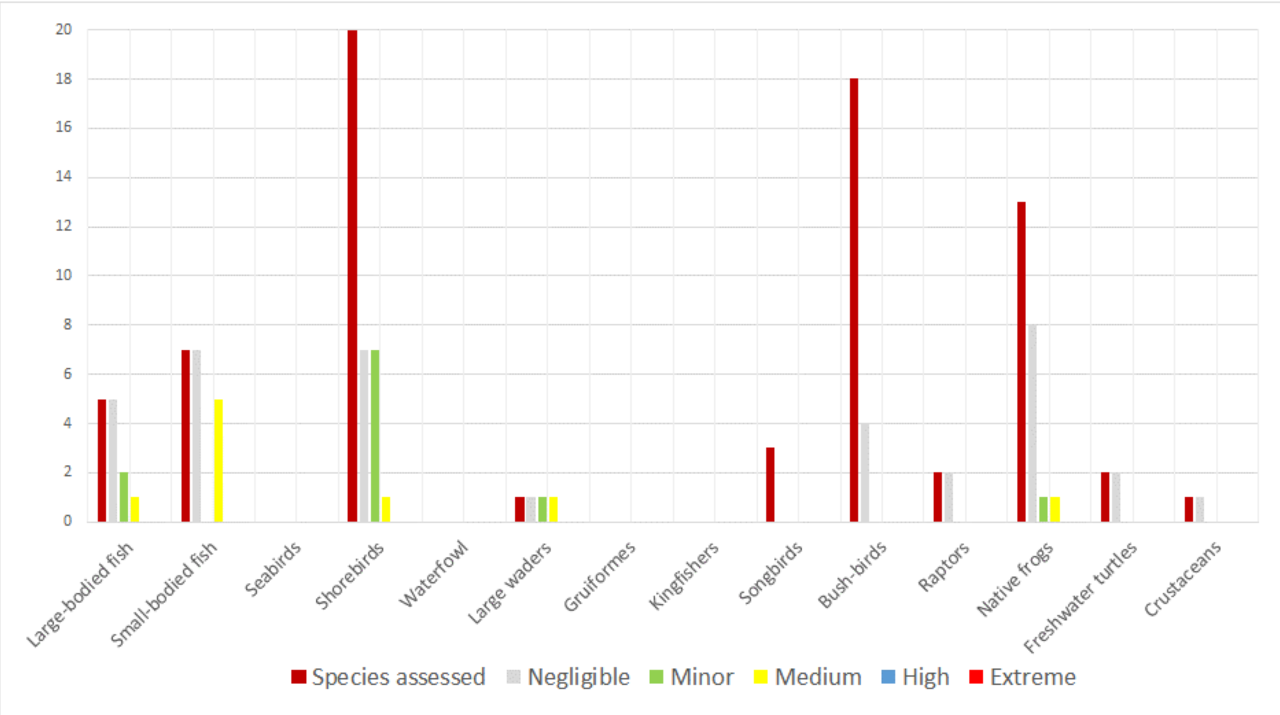


Figure 2 Summary of residual risks for threatened and migratory species



When management measures were considered, the only non-negligible risks for the balance of the MNES assets were attributed to Ramsar wetlands (including Ramsar wetlands of the northern Murray-Darling Basin, Ramsar wetlands of the southern Murray-Darling Basin and Wetlands within the Coorong and Lakes Alexandrina and Albert Wetland) and to two of the National Heritage properties (the Cowra Japanese Gardens and Cultural Centre and Centennial Park).

Additional planning could be undertaken to protect both the Cowra Japanese Gardens and Cultural Centre and Centennial Park, or to enable any harm that resulted from an outbreak of CyHV-3 to be rectified. In the case of the Cowra Japanese Gardens and Cultural Centre this might include vaccination of valuable ornamental Koi carp, a provision for restocking, or the use of effective biosecurity measures. Carp are a pest species within the Centennial Park ponds, and mitigation in this context would include additional resources for the immediate removal of carcasses and minimisation of harm to the amenity values of the park. The management of Ramsar wetlands will be more complex and is likely to require the development of a plan for each individual site. This plan would reiterate the values of each site, and the measures that can be taken to ensure that those values are protected or restored. These measures would address threats arising from the water quality effects of an outbreak of CyHV-3, as well as impacts on food webs or the risk of an outbreak of botulism. Additional analysis may be warranted to clarify the assets at stake within some categories of Commonwealth Land held by the Department of Defence and the Department of Finance.

RESIDUAL UNCERTAINTY

The breadth of this ecological risk assessment was considerable and, without any direct experience of the epidemiology of CyHV-3 in an Australian context, a degree of residual uncertainty is inevitable. In particular, this concerned the likely behaviour of the virus in a range of Australian freshwater settings and key components of the identified exposure pathways. Although largely beyond the scope of this assessment, there was also some residual uncertainty about the likely efficacy and practicality of some mitigations when applied in certain settings.

The likely behaviour of CyHV-3 was encapsulated in the detailed outbreak scenarios discussed above. Although the assumptions underpinning these scenarios concurred, in broad terms, with the NCCP's epidemiological modelling, it was recognised that the behaviour of an exotic disease in such diverse and complex settings cannot be predicted with certainty. It is possible, for example, that CyHV-3 will not penetrate local carp populations to the extent envisaged. It is equally possible, however, that the virus will be more successful than expected, or that particular characteristics of its epidemiology (such as the higher sensitivity of juvenile carp) will lead to an impact on carp populations that is more marked than modelling and qualitative assessment have suggested.

As noted, residual uncertainty also exists in respect of the identified exposure pathways.

The tolerance of each lifecycle stage of every native water-breathing aquatic species that may be exposed to low DO is not known, although this may be inferred with varying degrees of confidence from the literature about blackwater events. Similarly, whilst the NCCP water quality modelling studies showed that a dangerously low DO was only likely to occur within partially-connected or disconnected waterways, with a very high carp biomass density, there remained a degree of uncertainty about the importance of local conditions. A similar situation existed for widespread cyanobacterial blooms, with inference in that case based on the development and impacts of blooms that have occurred naturally throughout Australian freshwater waterways. Substantial uncertainty also surrounded the assessment of waterborne microorganisms that may be released into waterways with the decomposition of carp carcasses. In this case, uncertainty included the species of microorganisms that are likely to be involved, and their pathogenicity for particular functional groups and native species, as well as the persistence of epidemics within waterways after the dissolution of carcass materials.

In addition to the water quality pathways, substantial uncertainty remained in respect of the impact of CyHV-3 on food webs – in particular, in settings that include large numbers of nesting piscivorous waterbirds. The two aspects of this scenario included the putative effects of removing a stable and plentiful food source (juvenile carp), and the likelihood that piscivorous waterbirds would then switch to native fish, crustaceans, frogs and turtle eggs and young as an alternative source of food. Very little is currently known about the likelihood, and likely severity, of either pathway, and this was reflected in the conservative estimates.

Botulism in wildlife is considered to be an inherently probabilistic process, with relatively few outbreaks observed in Australia given the ubiquity of spores and the frequent alignment of suitable conditions. Compounding this is a paucity of reports specifically linking fish kills to outbreaks of type C (or C/D mosaic) botulism in waterbirds, despite the fact that substantial fish kills (as a result of blackwater events and other processes) are not uncommon within Australian waterways. This notwithstanding, it was recognised that concurrent outbreaks of CyHV-3 across a catchment or river system have the potential to create a uniquely high-risk scenario – in particular, given the co-occurrence of: (a) carp at a relatively high biomass density; and (b) large numbers of nesting waterbirds. In view of this, conservative estimates were assigned to this pathway. Type E botulism was ruled out of the case studies and assessment of MNES on the grounds that there is no evidence that it exists within Australia. This was considered a practical and realistic standpoint, although it was also noted that there has not been a systematic search for type E *C. botulinum* across Australian waterways, and that none of the experts consulted was willing to state categorically that type E is an exotic strain. The importance of type E is twofold: (a) it is primarily a disease of fish (although waterbirds are severely impacted), and therefore more likely to arise in the context of a widespread and multifocal fish kill; and (b) it is highly-toxic (frequently fatal) to humans.

SOCIAL RISK ASSESSMENT

The social risk assessment was undertaken to evaluate perceptions about ecological and other risks attached to the proposed release of CyHV-3. This standalone work was based on qualitative and quantitative analysis of stakeholder surveys.

The qualitative survey focussed on interviews with a range of stakeholders, including recreational fishers and water sports enthusiasts, farmers and irrigators, retirees, Indigenous Australians and the general public more broadly. Respondents were members of local communities who in many cases possessed both local knowledge and practical experience dealing with the effects of significant environmental issues such as blackwater events. The quantitative component of the social risk assessment focussed on the deployment and analysis of a national survey. The survey was informed by an analysis of social groups and demographic profiling, with a focus on those who lived on or close to major waterways and those from urban settings. In total, 2,026 people participated in an online survey that was developed and administered by Taverner Research (an online market-research provider).

The social risk assessment showed that while communities are accepting of the use of CyHV-3 to control invasive carp in Australian waterways, their acceptance is dependent on familiarity with the NCCP, personal interactions with waterways, knowledge of carp impacts and values, and their sense of community responsibility towards environmental stewardship. The assessment showed that those who agree in general that carp control is necessary – and can recognise potential ecological benefits of carp control – are also more likely to accept the release of CyHV-3 as a possible means to this end. This trend meant that people who live within the Murray-Darlin Basin and are closely involved with the river system, better appreciate the need for carp control. This group, however, was also attuned to the ecological and other risks associated with the proposed release of CyHV3 – in particular, the risks associated with the accumulation of decomposing carp carcasses. Uncertainty underpinned baseline concerns about the possible impact of CyHV-3 on humans, and on animals other than carp. These concerns extended to agricultural products irrigated with water from waterways in which the virus was active. Whether linked to these concerns, or to the effects of carcass accumulation and decomposition, anxiety about the control of carp using CyHV-3 was negatively correlated with acceptance of the virus. This result underscored the need for proactive and effective communication across a range of social strata, with clear messaging about both strategy for the release of the virus and site-specific plans for the clean-up and disposal of carcasses.

Part I Introduction

Overview of the ecological risk assessment for the release of cyprinid herpesvirus 3 (CyHV-3) for carp biocontrol in Australia

1 Background

The Australian Government has invested in the development of a National Carp Control Plan (NCCP) to explore the potential use of the virus known as Cyprinid herpesvirus 3 (CyHV-3; frequently called the 'carp virus' or Koi herpesvirus) for the biological control of common carp (*Cyprinus carpio*; hereafter, 'carp') in Australia.[3] CyHV-3 is a double-stranded DNA virus of the family *Alloherpesviridae*. Carp occur in every state and territory except the Northern Territory and are now the dominant fish species within the Murray-Darling Basin. The ecological impacts of carp include increased turbidity, intensified algal blooms and reduced abundance of macrophytes, invertebrates and some native fish.

The objectives of the NCCP are to:

- Undertake research and development to address knowledge gaps and to better understand and manage risks to support the potential release of CyHV-3, subsequent clean-up and recovery of native fish and ecosystems
- Plan for an integrated approach to control carp in Australia's waterways
- Build community awareness and support for the proposal to release CyHV-3, and identify and address stakeholders' and communities' concerns about the proposal
- Develop detailed strategies for release of CyHV-3 and subsequent clean-up
- Support national coordination on all elements of the NCCP's development.

The potential release of CyHV-3 will not occur before completion of a range of legislated national and state and territory approvals processes. Should a decision be made to implement the NCCP and proceed with a release of CyHV-3, this will be managed by the relevant state and territory governments through existing interjurisdictional governance structures.

The NCCP administers funds for research across three themes:

1. Environment
2. Communities
3. Informing possible implementation.

The ecological risk assessment documented within this report is one of the projects undertaken through the NCCP within the environment theme.

The ecological risk assessment seeks to evaluate risks to species, ecological communities, properties and places that may follow from the release of CyHV-3. The scope of the assessment extends to the breadth of the Strategic Assessment that will be required by the Australian Government if it determines that the release of CyHV-3 is a feasible proposition, and subsequently seeks environmental approval for release of the virus from the Department of Environment and

[3] See: https://www.carp.gov.au/-/media/Fish-NCCP/Research-Report/NCCP-RTP-030517.ashx?la=en

Energy.[4] The Strategic Assessment is a requirement of the Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) and examines risks to listed Matters of National Environmental Significance (MNES). These include:

- Threatened species and ecological communities
- Migratory species protected under international agreements
- Wetlands listed under the Ramsar Convention
- World Heritage Properties
- National Heritage Places
- Commonwealth Marine Areas
- The Great Barrier Reef Marine Park
- Commonwealth Lands.

The risk assessment was approached in three key steps.

The first step (Part II of this report) focussed on the assimilation of published and grey literature, as well as the outcomes of other projects undertaken under the NCCP. An expert elicitation study and a quantitative evaluation of the rebound of other invasive species were also undertaken.

The second step (Part III of this report) focussed on the assessment of ecological risk. This included the development of outbreak scenarios for a range of freshwater environments, the evaluation of nine key exposure pathways describing the ways in which the release of CyHV-3 could result in harm to the environment, and the examination of a series of detailed case studies.

The third step (Part IV of this report) used the outcomes of the ecological risk assessment to inform an evaluation of possible impacts on the EPBC Act MNES. The purpose of the third step was to provide the information that will be required by the Government if the proposed release of CyHV-3 is determined to be feasible and environmental approval is sought from the Department of Environment and Energy.

[4] Noting that a range of other approvals processes will also be required

2 Approach to the ecological risk assessment

2.1 Overview

This ecological risk assessment followed the principles put forward by Hayes (2002) in a guide to best practice in ecological risk assessment for genetically modified organisms (GMOs). Applying the principles of this approach, the assessment rested on the identification of ecological endpoints, the identification and elaboration of exposure pathways and the identification of key sources of variability and uncertainty. Although the treatment of risks, and the nature and extent of any residual risk, fell largely beyond the scope of the assessment, consideration was also given to mitigation strategies.

Following from this arrangement, the risk assessment was undertaken in three steps (Figure 3):

- The first step commenced with an assimilation of background and review material about Australian waterways and water quality, aquatic and freshwater-associated native wildlife assets, the ecology of carp in Australia, the biology and epidemiology of CyHV-3, botulism outbreaks in wildlife and the likely ecosystem-wide effects of a substantial carp die-off. The assimilation of background materials is described as a literature review, although was not undertaken under a formal search protocol as might be expected of some systematic literature reviews and meta-analyses. The review is documented in Part II of this report. Integrated into the findings of the review are key outcomes from companion projects undertaken under the NCCP, including the biomass project, the water quality project and the epidemiological modelling project.[5] The review was complemented by an expert elicitation study and a quantitative evaluation of the likelihood that other invasive species would rebound with the suppression or absence of carp. The approach to the expert elicitation study is summarised below in Section 2.4, while the study itself is described in Part II: Section 8.4. Likewise, the approach to the quantitative evaluation of the rebound of other invasive species is summarised below in Section 2.5, while the study itself is described in Part II: Section 9.
- The second step included the evaluation of outbreak scenarios, exposure pathways and a series of six case studies that investigated the likely impacts of CyHV-3 in a range of geographically-separated and physically-diverse settings. An overview of the approach taken to these steps is given below in Sections 2.3 and 2.6, respectively. The detailed evaluations of outbreak scenarios, exposure pathways and case studies are given in Part III: Sections 0, 11 and 12, respectively. A discussion that summarises outcomes from these parts of the ecological risk assessment is given in Part III: Section 13.
- The third step of the risk assessment is documented in Part IV of the report and focussed on an analysis of the risks posed by the release of CyHV-3 to the MNES listed under the EPBC Act. This

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[5] A draft report for the epidemiological modelling project was provided immediately prior to submitting the draft report for this ecological risk assessment. Some key findings have been carried through to the ecological risk assessment, to the extent that this was practically achievable within the timeline.

part of the risk assessment was informed by the evaluation of exposure pathways and case studies, and by a spatial risk assessment. The spatial risk assessment correlated the distribution of carp in Australia with the distribution of the threatened and migratory species, threatened ecological communities, Ramsar wetlands, World Heritage Properties, National Heritage Places, Commonwealth Marine Areas and Commonwealth Lands. An outline of this approach is given below in Section 2.7.

Integration of the components of the ecological risk assessment is shown schematically in Figure 4 overleaf.

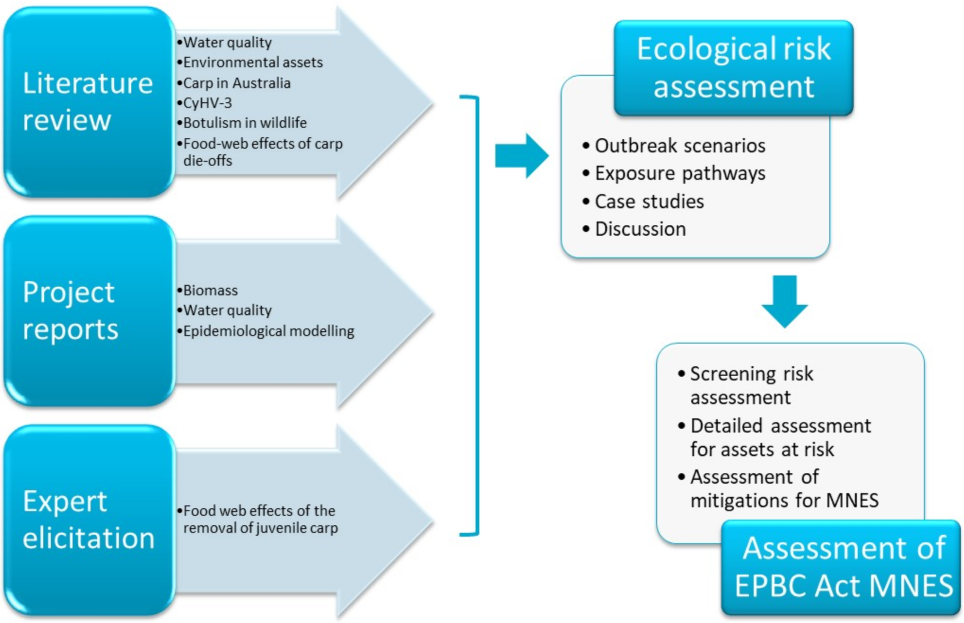

Figure 3 Approach to the ecological risk assessment

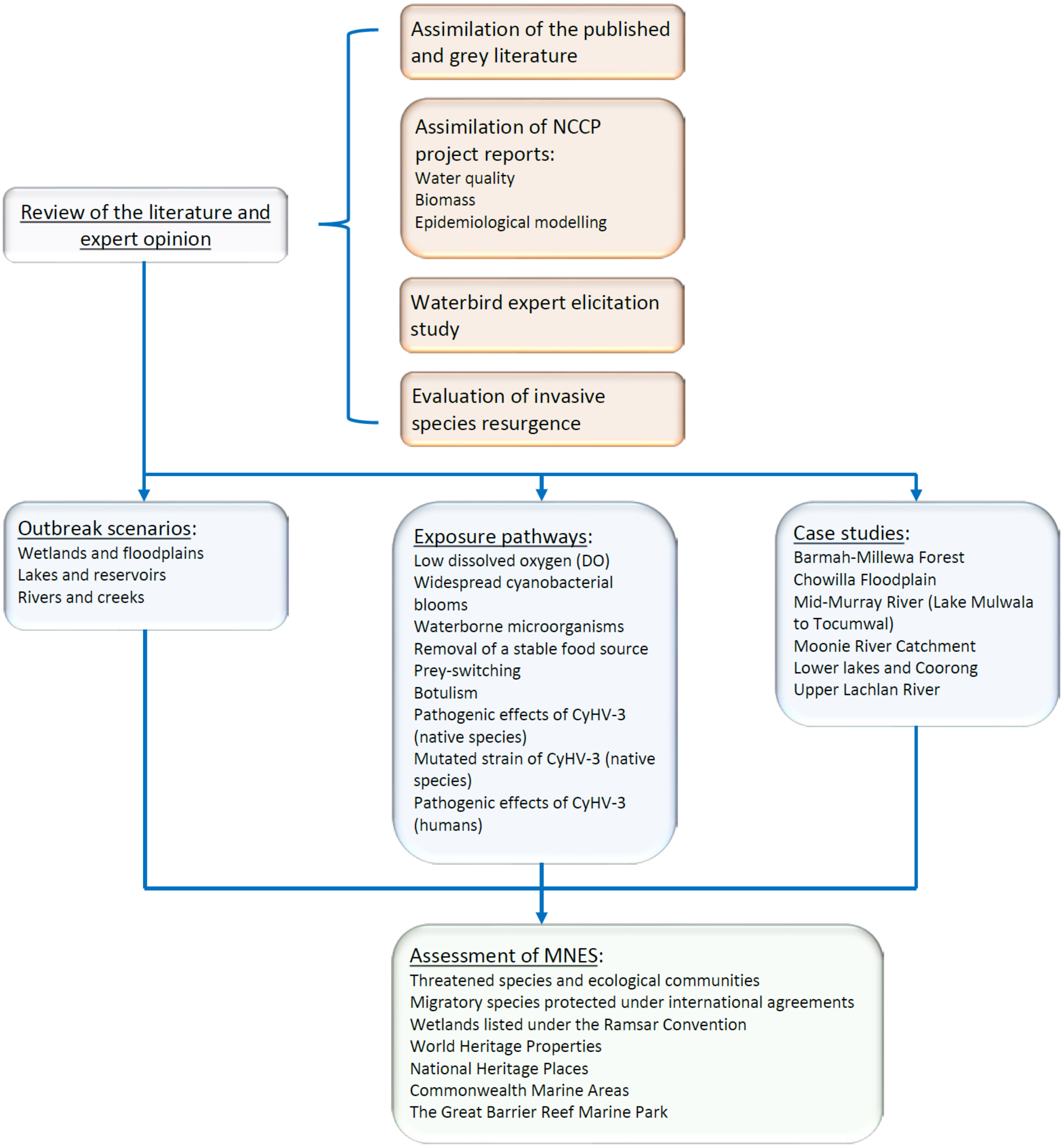

Figure 4 Integration of components of the ecological risk assessment

2.2 Risk assessment endpoints

Risk assessment endpoints represent the ways that harm to Australia's natural assets or values, or humans and livestock, might result from the release of CyHV-3 into the Australian feral carp population.

Endpoints for Australia's natural assets or values were termed here 'ecological endpoints' and were adapted from the Department of Environment and Energy's significant impact criteria. These criteria are set out in the Department's guidelines for determining whether an action is likely to cause harm to one or more of the MNES assets, and thus require Ministerial approval under the

EPBC Act.[6]

Separate criteria are given by the Department to assess the significance of possible impacts for each category of MNES asset. The detail of these criteria is given in Appendix A (page 405). The criteria for vulnerable, endangered or critically endangered species were collapsed into a single set of criteria. This single set of criteria was then applied equally to all non-migratory native species within the evaluation of exposure pathways and case studies. A separate set of criteria was retained for migratory species. A similar approach was taken in adapting the Department's criteria for critically endangered and endangered ecological communities to simplified criteria that could be applied to all ecological communities, and in adapting the Department's criteria for Ramsar-listed wetlands to all wetlands. The outcomes of this process are described below.

Ecological endpoints for **non-migratory native species**:

- Lead to a long-term decrease in the size of a population of a non-migratory native species
- Reduce the area of occupancy of a non-migratory native species
- Fragment an existing population into two or more populations of a non-migratory native species
- Adversely affect habitat critical to the survival of a non-migratory native species
- Disrupt the breeding cycle of a population of a non-migratory native species
- Modify, destroy, remove, isolate or decrease the availability or quality of habitat to the extent that a non-migratory native species is likely to decline
- Result in invasive species that becomes established in a non-migratory native species' habitat
- Introduce disease that may cause a non-migratory native species to decline
- Interfere with the recovery of a non-migratory native species.

In the context of non-migratory native species, a 'population' is defined as an occurrence of the species in a particular area. This may be a geographically-distinct regional population, or collection of local populations, or a population or collection of local populations that occurs within a particular bioregion.

Ecological endpoints for **migratory species**:

- Substantially modify (including by fragmenting, altering fire regimes, altering nutrient cycles or altering hydrological cycles), destroy or isolate an area of important habitat for a migratory species
- Result in an invasive species that is harmful to the migratory species becoming established in an area of important habitat for the migratory species
- Seriously disrupt the lifecycle (breeding, feeding, migration or resting behaviour) of an ecologically significant proportion of the population of a migratory species.

[6] Matters of National Environmental Significance, Significant Impact Guidelines (see: https://www.environment.gov.au/epbc/publications/significant-impact-guidelines-11-matters-national-environmental-significance)

Actions on, or impacting upon, Commonwealth land, and actions by Commonwealth agencies, Significant impact guidelines 1.2 (See: https://www.environment.gov.au/epbc/publications/significant-impact-guidelines-12-actions-or-impacting-upon-commonwealth-land-and-actions)

In the context of migratory species, 'important habitat' may include: (a) habitat utilised occasionally or periodically within a region that supports an ecologically-significant proportion of the population of the species; (b) habitat that is of critical importance to the species at particular life-cycle stages; (c) habitat utilised by a migratory species which is at the limit of the species range; or (d) habitat within an area where the species is declining. Similarly, in judging an 'ecologically significant proportion' with respect to a particular migratory species, consideration is given to population status, genetic distinctiveness and species-specific behavioural patterns (for example, site fidelity and dispersal rates). The population of a migratory species was taken to mean the entire population, or any geographically separate part of the population, a significant proportion of whose members cyclically and predictably cross one or more national jurisdictional boundaries (including boundaries within Australia).

Ecological endpoints for **ecological communities**:

- Reduce the extent of an ecological community
- Fragment or increase fragmentation of an ecological community, for example by clearing vegetation for roads or transmission lines
- Adversely affect habitat critical to the survival of an ecological community
- Modify or destroy abiotic (non-living) factors (such as water, nutrients, or soil) necessary for an ecological community's survival, including reduction of groundwater levels, or substantial alteration of surface water drainage patterns
- Cause a substantial change in the species composition of an occurrence of an ecological community, including causing a decline or loss of functionally important species, for example through regular burning or flora or fauna harvesting
- Cause a substantial reduction in the quality or integrity of an occurrence of an ecological community, including, but not limited to
  - Assisting invasive species, that are harmful to the ecological community, to become established, or
  - Causing regular mobilisation of fertilisers, herbicides or other chemicals or pollutants into the ecological community which kill or inhibit the growth of species in the ecological community, or interfere with the recovery of an ecological community.

Ecological endpoints for **wetland ecosystems**:

- Areas of the wetland being destroyed or substantially modified
- A substantial and measurable change in the hydrological regime of the wetland, for example, a substantial change to the volume, timing, duration and frequency of ground and surface water flows to and within the wetland
- The habitat or lifecycle of native species, including invertebrate fauna and fish species dependent upon the wetland being seriously affected
- A substantial and measurable change in the water quality of the wetland – for example, a substantial change in the level of salinity, pollutants, or nutrients in the wetland, or water temperature which may adversely impact on biodiversity, ecological integrity, social amenity or human health

- An invasive species that is harmful to the ecological character of the wetland being established (or an existing invasive species being spread) in the wetland.

Although the focus of the assessment was on the ecological impacts of CyHV-3, additional (non-ecological) endpoints were developed to capture the possible exposure of livestock or people to cyanobacterial toxins, waterborne microorganisms associated with the decomposition of carp carcasses and to *Clostridium botulinum* toxin as a result of a botulism outbreak.

Supplementary endpoints for **livestock**:

- Local exposure of livestock to the clinical effects of a pathogenic organism or its toxin
- Establishment of a pathogenic microorganism in livestock in areas not previously affected.

Supplementary endpoints for **public health**:

- Exposure of individuals to the clinical effects of a pathogenic organism or its toxin
- Exposure of groups of individuals or communities to the clinical effects of a pathogenic organism or its toxin.

2.3 Exposure pathways

The exposure pathways listed below represent the biological, ecological and physical scenarios that might follow from the release of CyHV-3 and result in the exposure of species, ecological communities, properties and places to the harmful effects of the virus, or to the harmful outcomes of its impacts on carp. In this way, the exposure pathways link the release of CyHV-3 to one or more of the risk assessment endpoints (above).

Nine exposure pathways were included in the assessment. A detailed explanation and evaluation of each pathway is provided in Part III: Section 11 of this report. The pathways with a non-negligible likelihood (termed throughout, a real chance or possibility) for one or more endpoints were taken forward to the individual case studies (Part II: Section 12).

- Exposure of native aquatic species to low dissolved oxygen (DO)
- Exposure of native species, livestock and humans to widespread cyanobacterial blooms
- Exposure of native species, livestock and humans to the microorganisms associated with decomposing fish carcasses
- Exposure of native piscivorous species to the removal of a dominant and stable food source
- Exposure of native species to increased predation as a result of prey-switching
- Exposure of native species, livestock and humans to an outbreak of botulism
- Exposure of native species to the direct pathogenic effects of CyHV-3
- Exposure of native species to the direct pathogenic effects of a mutated strain of CyHV-3 with altered species specificity
- Exposure of humans to the direct pathogenic effects of CyHV-3 in drinking water or consumed fish.

The assessment of exposure pathways focussed on events that may unfold under a maximally aggressive outbreak. In most settings, this will be the period immediately after release of the virus.

In disconnected riverine environments, however, outbreaks are more likely to be maximally aggressive during the dry season following from reconnection of the river system – that is, after affected fish have had an opportunity to be redistributed through the population.

2.4 Expert elicitation

The two exposure pathways concerned with the food web effects of removing juvenile carp were informed in part by a quantitative process of expert elicitation and analysis. Experts were identified in the areas of waterbird ecology and wetland ecosystems. A survey was sent by email, with follow-up webinars to help guide experts in providing reliable responses. This included discussion about the risk assessment and case studies, and the scenarios that they would be asked to evaluate. The questionnaires were analysed quantitatively. Further details of the approach are given in Part II: Section 8.4. The affiliation of identified experts, and preparatory materials, are given in Appendix B.

2.5 Resurgence of other invasive species

A quantitative analysis was undertaken to evaluate the correlation between the habitat requirements of carp and those of 11 key invasive fish species. The intent of this analysis was to inform a judgement as to the likelihood that one or more of these species would experience a resurgence in the absence of carp. An explanation of the approach and its outcomes is given in Part II: Section 9. A companion qualitative review was undertaken for invasive plant pest species.

2.6 Selection of case studies

Six case studies were selected for the assessment. The case studies were chosen to correlate as far as was practicable with the case studies undertaken by the NCCP project teams: (a) modelling the epidemiology of CyHV-3 outbreaks in an Australian context; and (b) evaluating the possible impacts of these outbreaks on water quality. In following this strategy, care was also taken to ensure that the case studies provided a broad geographical coverage and coverage of a wide range of ecosystems and ecological values. Each of the case studies represents a site where carp have colonised with negative consequences for the local ecosystem(s). To a variable extent, and with variable outcomes, the control of carp has also been active at each of the selected sites.

1	Barmah-Millewa Forest	This case study considers outbreak scenarios and possible impacts of CyHV-3 in a large regulated Ramsar wetland within the mid Murray River. The Barmah-Millewa Forest includes a range of threatened species and ecological communities and is also one of the places within the Murray-Darling Basin where carp recruit most successfully.
2	Chowilla Floodplain	This case study was included alongside the Barmah-Millewa Forest as it has a unique character arising from the placement of the Chowilla regulator and associated works. These structures enable the hydrology of the Chowilla Floodplain to be finely controlled,

		with environmental watering strategies targeted very specifically at particular physical or species assets. The Chowilla Floodplain has experienced a long-standing altered hydrology as a result of locks on the Murray River, and this has led to widespread salination and other environmental degradations. The regulator and associated works were commissioned in 2012 and seek to halt and remedy this degradation in the face of decreasing water flows within the river channel.
3	Mid-Murray River (Lake Mulwala to Tocumwal)	This case study considers the reach from Lake Mulwala (above Yarrawonga Weir) to Tocumwal (at the Newell Highway bridge). The reach includes exits to major irrigation channels to the north (Mulwala channel) and south (Yarrawonga main channel) of Lake Mulwala. The reach represents a highly-regulated riverine impoundment and release environment, and lotic downstream segment of the mid Murray River (mirroring many other lotic waterbodies downstream from locks), and includes some important environmental assets (for example, remnant breeding populations of the endangered trout cod: *Maccullochella macquariensis*).
4	Moonie River Catchment	This case study examines the Moonie River Catchment. The Moonie River is representative of a seasonally-disconnected and highly-turbid Queensland waterway. The characteristics of the ecosystem and ecological communities within this setting – and the stresses placed upon them by carp – are quite different from southern rivers.
5	Lower lakes and Coorong	The Lower Lakes and Coorong form a regulated and heterogeneous Ramsar wetland ecosystem that includes estuarine waters, coastal brackish or saline lagoons, permanent freshwater lakes and marshes and seasonally-flooded agricultural land. The Lower Lakes and Coorong also support a range of threatened species and significant ecological communities.
6	Upper Lachlan River	This case study represents a relatively fast-flowing riverine environment with lower winter and summer temperatures than those experienced in either mid-lower Murray River or the seasonally-disconnected waterways in western New South Wales and Queensland. The carp biomass density is also relatively lower in this reach, with a higher proportion of larger (older) fish.

A detailed description of each of the case study sites is given in Part III: Section 12 of this report. This description includes the physical characteristics of each site, as well as the hydrology and ecological values and the biomass and population dynamics of carp. With this as background, each case study then considers the importance of the identified exposure pathways to that site and provides an analysis of the risks to native aquatic and terrestrial species and natural ecosystems.

Cursory treatment of three additional case studies (the Macquarie Marshes, Kow Swamp and the upper Glenelg Catchment) is given in the Discussion in Part III: Section 13.

2.7 Assessment of Matters of National Environmental Significance

If the Australian Government determines that the release of CyHV-3 is a feasible proposition, it will be required to submit to the Department of Environment and Energy a Strategic Assessment. The assessment is a requirement of the EPBC Act and examines risks to MNES assets.

Part IV provides a systematic evaluation of the risks to the MNES assets encompassed by the EPBC Act and is designed to support the Strategic Assessment. The MNES assets considered include:

- Threatened species and ecological communities
- Migratory species protected under international agreements
- Wetlands listed under the Ramsar Convention
- World Heritage Properties
- National Heritage Places
- Commonwealth Marine Areas
- The Great Barrier Reef Marine Park.

Within each category, the specific natural assets and species were listed. Each item on these lists was then evaluated using the risk drivers identified through the evaluation of outbreak scenarios, exposure pathways and case studies. Measures to avoid, mitigate, offset and adaptively manage impacts were considered, and residual risk was assessed.

Further detail about the approach to the assessment of MNES assets is given in Volume 2 of this report.

Part II Review of the literature and expert opinion

A compilation of literature and expert opinion underpinning the ecological risk assessment for the release of cyprinid herpesvirus 3 (CyHV-3) for carp biocontrol in Australia

1 Introduction

This chapter provides an assimilation of background material about Australian waterways, aquatic and freshwater-associated native wildlife assets, the ecology of carp in Australia, the biology and epidemiology of CyHV-3, the possible impacts of fish kills on water quality, botulism outbreaks in wildlife and the likely ecosystem-wide effects of a substantial carp die-off. There is also discussion of a quantitative analysis undertaken to investigate the possible resurgence of other invasive species, given the suppression of carp.

The assimilation of background materials is described here as a review of the (published and grey) literature, although it was not undertaken under a formal search protocol as might be expected of some systematic literature reviews or meta-analyses. Existing creditable reviews of particular topics have also been included, and the research they have drawn upon has been noted. As they became available, the reports of other NCCP projects were also included. These included, in particular, the carp biomass modelling study (Stuart *et al*., 2019), and the water quality research (Walsh *et al.*, 2018) and modelling (Hipsey *et al*., 2019) studies. The report of the epidemiological modelling study (Durr *et al*., 2019a, 2019b and 2019c) was released in draft format immediately prior to the release of this risk assessment. An abstract from the epidemiological modelling study was included (Section 5.3.9) and key assumptions and conclusions were cross-checked for consistency with the outcomes of this risk assessment.

The outcomes of the review were used to inform the outbreak scenarios for CyHV-3 in Australian waterways (Section 10) as well as the analyses of exposure pathways (Section 11) and case studies (Section 12). The outcomes of the review also underpinned parts of the analysis of MNES, as detailed in Volume 2 of this report.

2 Australia’s freshwater waterways

2.1 Topographic drainage divisions and river regions

Australia is the world’s second driest continent, after Antarctica, with a long-term average rainfall of 430 mm and variations ranging across Australia from below 100 mm to above 3,000 mm per year. Most of this is lost through evapotranspiration, with the annual average run-off coefficient (a measure comparing the amount of run-off with the amount of precipitation) at about 12 percent. On average, some 383,000 GL remain after evapotranspiration to enter Australian freshwater environments, of which around 70,000 to 95,000 GL is used each year to meet Australia’s consumptive water needs (CSIRO, 2016).

Australia’s Topographic Drainage Divisions and River Regions (Figure 5) are used to depict where water flows and drains across the landscape and to provide a constant set of reporting boundaries for products such as the Australian Bureau of Meteorology’s Australian Water Resources

Assessment[7] (AWRA). The Topographic Drainage Divisions and River Regions were derived from the Australian Hydrological Geospatial Fabric[8] and provide a set of surface water reporting units based on drainage-enforced digital elevation models. The Topographic Drainage Divisions and River Regions build on earlier work of the Australian Water Resources Council (AWRC), which was established in 1963 to provide a national focus for the Australian water industry and a peak forum for consultation, cooperation and liaison for the development of water industry policy.

Within the context of this report, the Topographic Drainage Divisions and River Regions illustrate the connectedness and separation of individual river systems. This includes the Murray-Darling Basin, which is considered a contained drainage division (Figure 5) and is one of the key systems that has been invaded by the carp (Section 4).

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[7] See: http: //www.bom.gov.au/water/awra/

[8] See: http: //www.bom.gov.au/water/geofabric/

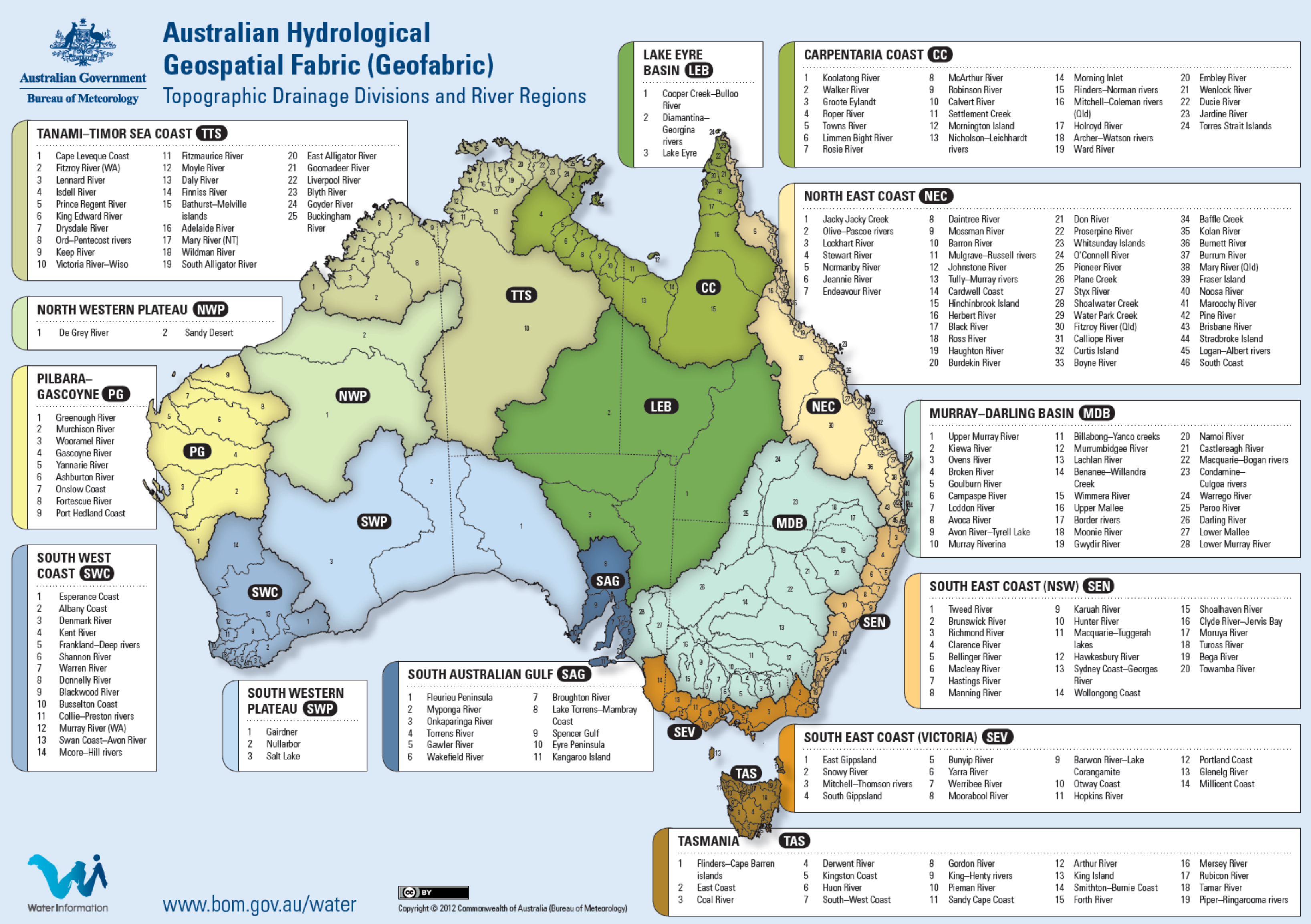

Figure 5 Australia's topographic drainage divisions and river regions

Source: Australian Bureau of Meteorology (2018)

2.2 Regulation of freshwater flows

The regulation of Australian freshwater waterways was reviewed by Piddocke (2018), and much of this section was taken directly from that work.

Regulation of river flows, through the construction of dams, weirs, locks, and other water-retaining structures – and the subsequent extraction of water for irrigated agriculture, stock use, and human consumption – has had major impacts on inland freshwater systems (Bice and Zampatti, 2011; Kingsford, 2011; Kingsford *et al.*, 2011; MacNally *et al.*, 2011; Catelotti *et al.*, 2015). Construction of water-retaining infrastructure began relatively early in Australia's colonial history. The Cooks River near Sydney, for example, was dammed in 1839 (Witton, 2013). The building of dams accelerated following construction of Hume Dam in 1936, and the Murray-Darling Basin now has a storage capacity of approximately 30,000 GL – or approximately 130% of the average annual discharge (Kingsford *et al.*, 2011).

Altered flow regimes can have important consequences for freshwater ecosystems, because many biological processes (including native fish lifecycles) have evolved to depend on the flood and drought cycles prevailing prior to river regulation (Humphries and Lake, 2000; Bice *et al.*, 2014; Doody *et al.*, 2015). If these cycles are disrupted by flow regulation, the ecosystem processes that rely on them may also be interrupted or altered. Conversely, the conditions occurring in altered flow regimes can be beneficial to invasive fish species, including carp (Driver *et al.*, 2005; Stuart and Jones, 2006a; Bice and Zampatti, 2011). Water releases from dams may also be hypolimnetic (that is, drawn from cold, oxygen-depleted bottom layers) and consequently suboptimal or directly harmful to native fish (Preece and Jones, 2002).

River regulation often reduces the connectivity between the main river channel and floodplains, reducing the frequency and extent of floodplain inundation (Balcombe *et al.*, 2011). Floodplains with reduced inundation frequencies consequently become drier, resulting in stress to river red gums (*Eucalyptus camaldulensis*) and other riparian trees and often favouring invasive weed growth (Catford *et al.*, 2011). River red gums are the dominant floodplain tree species in the Murray-Darling Basin and are a crucial component of floodplain ecology in south-eastern Australia (Catelotti *et al.*, 2015; Doody *et al.*, 2015). A decline in river red gum coverage can translate, for example, to reduced additions of coarse woody debris to waterways, potentially diminishing fish habitats (Koehn *et al.*, 2004).

The importance of flows and flooding to riverine ecology is now well-recognised. In 1994, the Council of Australian Governments (COAG) formally recognised the environment as a valid water user, enabling development of river management strategies that allocate 'environmental flows' to mimic a more natural river cycle (Bunn, 2017). Considerable research is now devoted to identifying optimal flow components for environmental flows intended to benefit various components of riverine and floodplain biota (Colloff *et al.*, 2018).

The Murray-Darling Basin is the largest and most complex river system in Australia, and covers one million square kilometres of south-eastern Australia, across New South Wales, Queensland, South

Australia, Victoria and the Australian Capital Territory. The Basin Plan[9] – including the Basin-wide environmental watering strategy – guides the use of water in the Murray-Darling Basin, with objectives that include protecting and restoring:

- A subset of all water-dependent ecosystems
- Ecological productivity
- Ecological dispersal
- Biodiversity (listed threatened species and support of their lifecycles)
- Representative populations and communities of native biota
- Connectivity, including longitudinal, lateral (between watercourses and floodplains/wetlands) and vertical and thus overcoming barriers to passage
- Diversity and dynamics of geomorphic structures, habitats, species and genes
- Ecosystem function, including recruitment, regeneration, dispersal, immigration and emigration.

By implementing these objectives, the intent of the Basin Plan is that water-dependent ecosystems:

- Support habitat diversity for biota at a range of scales
- Are not adversely affected by water quality
- Are resilient to climate change, climate variability and disturbances
- Protect refugia and allow for subsequent re-colonisation
- Mitigate human-induced threats
- Minimise habitat fragmentation.

The Commonwealth Environmental Water Office[10] within the Department of Environment and Energy provides annual watering plans to help ensure that the supply of available Commonwealth environmental water will help achieve the overall environmental objectives under The Basin Plan. The watering plans facilitate the scaling of actions across potential inflow scenarios and integration across specific sites within the Murray-Darling Basin. They provide flexibility so that water use can best accompany natural inflows and aim to support ongoing environmental recovery following the extended drought period. Annual watering plans have been developed for the river regions within the Murray-Darling Basin with mechanisms for environmental water delivery and watering strategies. Environmental water is also held and delivered by a range of other agencies (including the Murray-Darling Basin Authority and the state and territory Environmental Water Holders) and considerable effort is required to coordinate both plans and delivery to maximise environmental outcomes.

At the site scale, environmental water can be delivered actively to in-channel locations, low-lying floodplains and wetlands by manipulating weir-pool heights at locks and weirs. Manipulating these generally aims to mimic the pre-regulation conditions that native species are adapted to and, in turn, to achieve the greatest ecological benefits for a given volume of water. Environmental water

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[9] See: http: //www.mdba.gov.au/basin-plan/plan-murray-darling-basin

[10] See: http: //www.environment.gov.au/water/cewo

can be fed passively to pool-connected wetlands, regulated floodplain habitats (including the Chowilla Floodplain and the Barmah-Millewa Forest, both of which are case studies within this report – see Section 12) and irrigation systems by gravity and by controlling regulators. Above-pool wetlands (for example, the Hattah-Kulkyne lakes), and some irrigation systems, require the active delivery of environmental water through pumping against gravity. This activity, however, can be expensive and may result in the injury or death of entrained native fish (Baumgartner and Boys 2012; Baumgartner *et al.* 2014).

3 Environmental assets

This section provides brief notes on the ecological characteristics of key taxa, functional groups, species and ecological communities whose distribution and habitat broadly overlaps with that of carp. The information informs the discussion of exposure pathways (Part III: Section 11) and the case studies (Part III: Section 12). The information is considered baseline and well-established biographical and ecological data and is only lightly referenced to the literature.

3.1 Australian native freshwater fish

Although the terms 'large-bodied' and 'small-bodied' are not taxonomic, they are often used to delineate between two functional groups of Australian native freshwater fish. The large-bodied native freshwater fish are in general carnivorous, and the term encompasses a number of iconic Australian species, including the Murray cod (*Maccullochella peelii*), trout cod (*Maccullochella macquariensis*), silver perch (*Bidyanus bidyanus*), golden perch (*Macquaria ambigua*), Macquarie perch (*Macquaria australasica*) and freshwater (eel-tailed) catfish (*Tandanus tandanus*). Bony herring (*Nematalosa erebi*) are smaller, and are not carnivorous, but are generally included within this category. The unusual diadromous[11] Australian grayling (*Prototroctes maraena*) is less commonly included although has been added to the group for this assessment. The small-bodied native freshwater fish are a more heterogeneous group in respect of physical characteristics, habitat and spawning, although are in general considered to be either foraging generalists or wetland / floodplain specialists.

3.1.1 Large-bodied native freshwater fish

<u>Murray cod</u> were historically distributed throughout the Murray-Darling Basin, with the exception of the upper reaches of some tributaries. The species still occurs in most parts of this natural distribution up to approximately 1000m above sea level.[12] The Murray cod has undergone a substantial decline since European settlement, and particularly in the last 70 years. It is now listed under the EPBC Act as a vulnerable species. The Murray cod utilises a diverse range of habitats from clear rocky streams, such as those found in the upper western slopes of New South Wales and the Australian Capital Territory, to slower-flowing, turbid lowland rivers and billabongs. Murray cod are frequently found in the main channels of rivers and larger tributaries and the species is considered a main-channel specialist. Murray cod tend to occur in floodplain channels and anabranches when they are inundated, although the species' use of these floodplain habitats appears limited. Juveniles less than one year old have been found in main river channels where it appears they settle at a late larval stage. Preferred microhabitat consists of complex structural features in streams such as large rocks, snags, overhanging stream banks and vegetation, tree

[11] Spending part of its lifecycle in freshwater, and part in saltwater

[12] See: http: //www.environment.gov.au/cgi-bin/sprat/public/publicspecies.pl?taxon_id=66633

stumps, logs, branches and other woody structures. Such structures reduce or influence stream flows and provide shelter from fast-flowing water. They also serve as predatory ambush points for foraging, particularly during the day. The Murray cod is a long-lived species and both sexes reach sexual maturity at approximately 5 years of age. The reproductive cycle appears to culminate in a relatively short, well-defined spawning period of about 4 to 5 weeks. The Murray cod migrates upstream prior to spawning in late spring or early summer when the water attains a temperature of between 16 and 21C.[13] Increasing day length may also be a key trigger for spawning. The adhesive eggs are laid on solid substrates. Larvae then drift downstream before settling out in suitable protected habitat. Murray cod in both lakes and rivers have been shown to undertake substantial long-distance movements prior to spawning.[14] Some adult Murray cod have been tracked up to several hundred kilometres upstream, although there is also considerable variation between individuals. Adult Murray cod are considered solitary and highly-territorial, and will return to their territory following spawning.

Trout cod have also declined markedly in distribution and abundance since European settlement. The single naturally-occurring population is restricted to a small (approximately 120 km) stretch of the Murray River from Yarrawonga Weir to the Barmah-Millewa Forest, and occasionally beyond to Gunbower.[15] It is now listed under the EPBC Act as an endangered species.

The diet of the trout cod includes aquatic insects and crustaceans such as yabbies, crayfish and shrimps. The species may also leap from the water to take insects just above the surface. Larger trout cod will take Macquarie perch and other native or introduced fish and frogs. Trout cod occupy stream positions close to riverbanks in comparatively deep and rapidly moving water with a high abundance of large woody debris (snags). Trout cod are believed to form pairs and spawn annually from late September to late October, when water temperatures are between 14 and 22C. Spawning is triggered by increasing day length and water temperature and may precede that of Murray cod by about 3 weeks. Trout cod display high site fidelity, with a small home range, and do not routinely undertake migratory movements for spawning. As an exception to this, some individuals may move from the main channel to off-channel branches or to floodplains in the event of a significant flood.

Silver Perch were once widespread and abundant throughout most of the Murray-Darling river system. They have now declined to low numbers or disappeared from most of their former range.[16] Only one sizeable, self-sustaining population of silver perch is now found in the Murray-Darling Basin, in the middle reaches of the Murray River (including the Edwards/Wakool anabranches) from the base of Yarrawonga Weir to the Torrumbarry Weir, and then to the Euston Weir and downstream. Recent monitoring indicates this population now extends down to the South Australian border and up the lower Darling River. The silver perch is now listed under the EPBC Act as a critically endangered species.

[13] See: http: //www.environment.gov.au/biodiversity/threatened/conservation-advices/maccullochella-peelii-peelii

[14] See: http: //www.environment.gov.au/cgi-bin/sprat/public/publicspecies.pl?taxon_id=66633

[15] See: http: //www.environment.gov.au/cgi-bin/sprat/public/publicspecies.pl?taxon_id=26171

[16] See: http: //www.environment.gov.au/biodiversity/threatened/species/pubs/76155-conservation-advice.pdf

The reasons for the marked decline in the wild population of silver perch are not well understood, although may include the impacts of river regulation on eggs, larvae and adults; loss of juveniles and adult fish within non-viable irrigation channels; thermal pollution with cold-water pulses from weirs; widespread degradation of habitat, including de-snagging and siltation of the benthic environment. Invasive fish may compete for resources, predate upon eggs and juveniles and, in the case of carp, contribute actively to the ongoing degradation of habitat. The cumulative evidence indicates that silver perch reproduction is flexible in terms of flow conditions and temperature; reproduction can occur in both within-channel flows and floods and at relatively cool water temperatures, although a threshold of 23C is generally preferred. Spawning at lower temperatures (including as low as 17C) may be associated with lower larval survival. Spawning occurs in faster-flowing areas of the river, and generally over gravel or rock rubble substrates. Spawning is not flood-dependent although suitable flows appear to maximise spawning efforts and, presumably, recruitment. The former occurrence of silver perch in some cooler upper reach habitats with gravel and rock substrates suggest silver perch are also flexible in the type of river environments in which they can reproduce. Silver perch are a migratory freshwater fish and the extensive movements of adults, particularly during flooding, has long been considered a part of their spawning behaviour and a likely a strategy to offset the later downstream drift of eggs and larvae.

<u>Golden perch</u> is found primarily in the Murray-Darling Basin, although a subspecies is also found in the Lake Eyre / Cooper Creek system and another subspecies, suspected to be ancestral to all other populations, is found in the Fitzroy River system in Queensland. Evidence suggests that before European settlement, substantial shoals of golden perch roamed the entire lowland and slope reaches of the Murray-Darling Basin. The gradual loss of fish passage through the numerous dams, weirs, locks and other barriers has had severe impacts on the species. However, the golden perch is not currently listed under the EPBC Act as a threatened species.

Golden perch are predominantly found in lowland, warmer, turbid, slower-flowing rivers – often in sympatry with Murray cod – and are opportunistic carnivores. The diet of adult fish consists mainly of shrimps, yabbies, small fish and benthic aquatic insect larvae. Juvenile fish consume more of the smaller items such as aquatic insect larvae and micro-crustaceans. Golden perch have unusually broad temperature limits (from 4 to 37C) and unusually high salinity limits for a freshwater fish (up to 33 parts per thousand). Although temperatures close to 24C have traditionally been considered necessary for spawning, fertilised eggs have been identified in the Barmah-Millewa Forest in temperatures as low as 17C. The complete range is now considered to be 17 to 25C. Outside the breeding season, golden perch are nomadic, with schools of fish occupying home ranges of about 100 metres for weeks or months before relocating to another site where a new home range is established. Upstream movements by both immature and adult fish are stimulated by rises in streamflow, and most movement in the Murray occurs between October and April. Recent research in the Murray River has also suggested that some fish may move downstream to spawn. Water-hardened eggs are large (approximately 3 to 4 mm in diameter), semi-buoyant and drift downstream. Adult fish have been recorded migrating well over 1,000 km when flood conditions allow passage over weirs and other man-made obstructions.

<u>Macquarie perch</u> is closely related to golden perch (above), although a specialised upland species. While Macquarie perch used to inhabit the upland reaches of the southern Murray-Darling Basin, they have now been almost wholly displaced by introduced trout, large dams and associated

effects such as cold-water pollution and habitat degradation and modification. Of the Macquarie perch in the Murray-Darling Basin, only small discrete populations remain in the upper reaches of the Mitta Mitta, Ovens, Broken, Campaspe and Goulburn Rivers in northern Victoria and the upper reaches of the Lachlan and Murrumbidgee Rivers in southern New South Wales. A larger translocated population exists in the Yarra River and in Lake Eildon in the Goulburn River catchment. It is also found in low numbers in the Mongarlowe River, where the population is considered likely to be the result of a translocation from the Murray-Darling Basin.[17] The Macquarie perch is listed under the EPBC Act as an endangered species.

Macquarie perch is a riverine, schooling species that prefers clear deep water and rocky holes. Refuge is also important. As well as aquatic vegetation, refuge may include large boulders, debris and overhanging banks. Spawning occurs just above riffles (shallow running water), where rivers have a base of rubble (small boulders, pebbles and gravel). Although this species can tolerate temperatures less than 9C it requires a temperature of at least 16.5C for spawning. The eggs, which are adhesive, stick to the gravel, and newly-hatched yolk sac larvae shelter amongst pebbles. Some fish use the same river each year for spawning. Migrations are undertaken by fish resident in lakes, but not otherwise.

Freshwater catfish are a benthic species that prefers slower-flowing streams and lake habitats.[18] Freshwater catfish were widespread throughout the Murray-Darling Basin, in particular, in the lower and slower-flowing rivers. They were also found in coastal rivers from southern New South Wales to northern Queensland and have been stocked into some farm dams and lakes where breeding populations have established. Most riverine populations have declined significantly, and the species is no longer common in many areas where it was formerly abundant. However, the freshwater catfish is not currently listed under the EPBC Act as a threatened species.

Freshwater catfish spawn in spring and summer when water temperatures are between 20 and 24C. The nest is a circular to oval depression, 0.6 to 2 metre in diameter, constructed from pebbles and gravel and with coarser material in the centre. The eggs are comparatively large (approximately 3 mm) and non-adhesive and settle into the interstices of the coarse substrate. The freshwater catfish is a relatively sedentary species and adults show very limited tendency for movement or migration. They are predominantly an opportunistic carnivore, and the adult diet consists mainly of shrimps, freshwater prawns and yabbies, with aquatic insects, snails and small fishes also prominent. Aquatic insects are more important in the diet of juvenile fish.

Bony herring (sometimes called bony bream, hairback herring or pyberry)[19] is a medium sized, laterally-compressed and deep-bodied fish with a small head and mouth, large eyes and blunt snout. The maximum size is approximately 47 cm, although bony herring are more commonly 12 to 20 cm. Bony herring are one of the most widespread of Australia's native freshwater fish species. Most common in lowland river systems generally, in the Murray-Darling Basin they are known from the majority of lowland rivers. They are commercially fished in Lake Alexandrina, South Australia. They are largely absent from upland habitats, probably due to lower water

[17] See: http: //www.environment.gov.au/cgi-bin/sprat/public/publicspecies.pl?taxon_id=66632

[18] MDBA Factsheet on freshwater catfish (Jewfish, eel-tail catfish) – available at http: //www.mdba.gov.au/

[19] MDBA Factsheet on Bony herring (bony bream, hairback herring or pyberry) – available at http: //www.mdba.gov.au/

temperatures. River regulation (including barriers to fish passage and cold-water pollution) has reduced the abundance of the species, and it is now in lower abundance in the Murrumbidgee and Murray Rivers below Burrinjuck and Hume Dams. The bony herring is not listed as a threatened species under the EPBC Act.

Bony herring are a hardy fish, tolerating high temperatures (up to 38C), high turbidity, high salinity (up to at least 39 ppt) and low DO.[20] However, they are not tolerant of low water temperatures and, hence, are considered susceptible to the effects of cold-water pollution. The small eggs (0.83 mm diameter) are released in the still waters of shallow, sandy bays in October to February.[21] Daytime upstream movements have been recorded for juveniles and adults in the Murray and Murrumbidgee Rivers, and individuals as small as 22 mm have been recorded migrating. These movements are possibly related to the colonisation of new habitats by juveniles, as well as reproductive movements by adults. The species feeds predominantly during daylight hours. It is an algal detritivore, consuming large quantities of detritus, microalgae and micro-crustaceans. The proportion of algae consumed varies widely between studies. Bony herring are consumed by other fish such as Murray cod and golden perch, and also form a significant part of the diet of waterbirds such as cormorants and pelicans.

Australian grayling is a slender fish varying in colour from silvery with an olive-grey back and whitish belly, to olive green or brownish in the back with a darker mid-lateral streak and greyish fins.[22] It is considered to be omnivorous, with diet including both aquatic insects and plant material. The Australian grayling occurs in streams and rivers on the eastern and southern flanks of the Great Dividing Range, from Sydney southwards to the Otway Ranges of Victoria and in Tasmania. It is believed to be absent from the inland Murray-Darling system. The Australian grayling is listed under the EPBC Act as a vulnerable species. It is considered to be diadromous, spending part of its lifecycle in freshwater and at least part of the larval or juvenile stages in coastal seas. Adults (including pre-spawning and spawning adults) inhabit cool, clear, freshwater streams with gravel substrate and areas alternating between pools and riffle zones. Australian grayling have been found over 100 km upstream from the sea. It undergoes large, annual fluctuations in population numbers, depending on prevailing conditions. The species' high fecundity means that it is capable of explosive population increases when conditions are favourable. Spawning occurs in freshwater from late summer to early winter, with precise timing dependant primarily on water temperature and flow. Initiation of spawning appears to be caused by an increase in river flows from seasonal rains, coupled with a fall in water temperatures to 12 to 13C. Eggs are scattered over the substrate and newly hatched larvae drift downstream and out to sea, where they remain for approximately six months. Juveniles then return to the freshwater environment around November of their first year, where they remain for the remainder of their

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[20] This position was enunciated in the Fact Sheet for bony herring published by the MDBA. The MDBA Fact Sheets are adapted largely from the text, Fishes of the Murray-Darling Basin: An introductory guide (Lintermans, 2007). It was noted, however, that Fact Sheet is in this case at odds with the description provided by the Australian Museum, "*The Bony Bream* [herring] *can tolerate a wide range of temperatures and pH, although is **susceptible to low dissolved oxygen levels**. It is often the first species to die when ephemeral waters begin to evaporate*." This is discussed further in Part III: Section 2.1.

[21] The Character Description for the Barmah-Millewa Ramsar site, however, describes bony herring as a wetland specialist that spawns and recruits in floodplain wetlands and lakes, anabranches and billabongs during in-channel flows (Hale and Butcher, 2011). The disparity may reflect the widespread distribution of bony herring and their versatility within a range of habitats.

[22] See: http: //www.environment.gov.au/cgi-bin/sprat/public/publicspecies.pl?taxon_id=26179

lives. Most Australian Grayling die after their second year, soon after spawning, however a small proportion reach five years of age.

3.1.2 Small-bodied native freshwater fish

This is a mixed group including wetland or floodplain specialists, and foraging generalists.

The wetland or floodplain specialists within this group that are listed under the EPBC Act and whose distribution overlaps with that of carp include the critically endangered flathead galaxias (*Galaxias rostratus*), the endangered Murray hardyhead (*Craterocephalus fluviatilis*) and the vulnerable eastern dwarf galaxias (*Galaxiella pusila*). The listed foraging generalists whose distribution overlaps with that of carp include the endangered barred galaxias (*Galaxias fuscus*) and Oxleyan pygmy perch (*Nannoperca oxleyana*), and the vulnerable variegated pygmy perch (*Nannoperca variegate*) and Yarra pygmy perch (*Nannoperca obscura*). In addition to these is a wide range of small-bodied native freshwater species that are not listed under the EPBC Act, but occur within waters inhabited by carp. These include Australian smelt (*Retropinna semoni*), some of the freshwater galaxias (*Galaxias* spp), some of the rainbowfish (*Melanotaeniidae* spp), river blackfish (*Gadopsis marmoratus*), southern purple-spotted gudgeon (*Mogurnda adspersa*) and some other freshwater gudgeons (*Mogurnda* spp), some of the carp gudgeons (*Hypseleotris* spp), the southern pygmy perch (*Nannoperca australis*), the olive perchlet (or Agassiz's glass fish, *Ambassis agassizii*), and the unspecked hardyhead (*Craterocephalus stercusmuscarum*) and some other hardyheads (*Craterocephalus* spp).

Although individuals within this group have a range of adaptations and specialisations, and specialised requirements, lower-flow periods are generally conducive for recruitment while high-flows may render channel habitats unfavourable. A small pulse may nevertheless help to inundate spawning grounds, although this pulse would need to persist for 10 to 14 days to enable the sticky eggs to remain submerged through to the point of hatching. Lateral connection to off-channel habitats is likely to be important to most of these species, especially during higher flows. Many of these species have flexible spawning and recruitment strategies. Several species spawn multiple times over a protracted season (for example, Australian smelt), while others spawn once, or a few times, over a more-defined season (for example, Murray Rainbowfish: *Melanotaenia fluviatilis*). Because the small-bodied fish are also in general quite short-lived, and less likely to migrate substantial distances to escape poor conditions or locate better ones, local populations may be relatively exposed in the event of recruitment failures. Small-bodied native fish are threatened by habitat loss, particularly loss of aquatic vegetation, and by changes in hydrology and predation by introduced species such as redfin perch (*Perca fluviatilis*) and eastern gambusia (mosquito fish: *Gambusia holbrooki*). Both the wetland or floodplain specialists and the foraging generalists are relatively more tolerant of poor water quality (including to low DO) than are most of the large-bodied fish.

3.2 Australian and migratory waterbirds

Noting that there is a range of ways in which the term ‘waterbird’ has been used in the scientific and science-policy literature, the following was adopted as a framework for this assessment and report:

- Seabirds, including pelicans, cormorants and darters
- Shorebirds (order Charadriiformes), including waders, gulls, terns, dotterels and plovers
- Waterfowl, including ducks, geese and swans (order Anseriformes) as well as the grebes
- Large waders (order Ciconiiformes), including the storks, herons, egrets, ibises, spoonbills and others
- Cranes rails, crakes, coots, moorhens, swamphens and waterhens (order Gruiformes)
- Kingfishers (order Coraciiformes, family *Alcedinidae*)
- Songbirds (order Passeriformes, the passerine birds).

This structure is used widely in the context of conservation and builds on the principle that waterbirds are those that inhabit or depend on bodies of water or wetland areas. With this in mind, the raptors that are associated with waterbodies were included in this assessment as 'waterbirds'. A further group of 'bush-birds' was also included, insofar as these were birds such as kookaburras and galahs that may be linked to the ecology and ecological values of particular water-based ecological communities – or may themselves depend on a water-based ecosystem, and are otherwise listed as threatened species under the EPCB Act. Loons (divers) of the order Gaviiformes were removed from the list as they are not present in Australia. Brief notes on each category are provided below.

The seabirds (also termed 'piscivores' by some authors, although numerous species within other categories also consume fish) encompass the pelicans, cormorants and darters. Gulls and terns are sometimes included in this group, although most species are more correctly classed as shorebirds (below). Many seabirds are recognised for their long migrations – some crossing the equator or circumnavigating the earth. The seabirds can be highly-pelagic, coastal or, in some cases, may spend a part of the year away from the marine environment entirely. Most species nest in colonies, which can vary in size from a few dozen birds to millions. None of the seabirds whose distribution overlaps with that of carp are listed under the EPBC Act as threatened, nor listed under international agreements for the protection of migratory species to which Australia is a party.

The shorebirds (order Charadriiformes) are principally waders (sub orders Charadrii, Scolopaci and Thinocori) and feed by probing in the mud or picking items off the surface in both coastal and freshwater environments. Waders include stilts, snipes, sandpipers, plovers and oystercatchers. The shorebirds also, however, include the gulls and terns and their allies (suborder Lari) which take fish from the sea; and in the northern hemisphere, the auks (for example, the puffins – suborder Alcae), which are coastal species that nest on sea cliffs and 'fly' underwater to catch fish. Although the distinction is not always upheld, it is also useful to delineate the shorebirds from the 'large waders' of the order Ciconiiformes (see below). Most shorebirds nest individually within a loosely-defined area, although are not considered colonial nesters. Four of the shorebirds whose distribution overlaps with that of carp are listed under the EPBC Act as critically endangered: the northern Siberian bar-tailed godwit (*Limosa lapponica menzbieri*), the curlew sandpiper (*Calidris ferruginea*), the great knot (*Calidris tenuirostris*) and the eastern curlew / far eastern curlew (*Numenius madagascariensis*). The Australian painted snipe (*Rostratula australis*), the lesser sand plover / Mongolian plover (*Charadrius mongolus*) and the red knot (*Calidris canutus*) are endangered; while the bar-tailed godwit / western Alaskan bar-tailed godwit (*Limosa lapponica* *bauera*) is vulnerable. All of these except the northern Siberian bar-tailed godwit and the Australian painted snipe are also listed under international agreements for the protection of migratory species to which Australia is a party. This list contains the following 11 additional migratory shorebirds whose habitat may overlap meaningfully with carp: common sandpiper (*Actitis hypoleucos*); ruddy turnstone (*Arenaria interpres*); sharp-tailed sandpiper (*Calidris acuminate*); pectoral sandpiper (*Calidris melanotos*); red-necked stint (*Calidris ruficollis*); double-banded plover (*Charadrius bicinctus*); white-winged tern / white-winged black tern (*Chlidonias leucopterus*); Latham's snipe / Japanese snipe (*Gallinago hardwickii*); ruff / reeve, a sandpiper (*Philomachus pugnax*); common greenshank (*Tringa nebularia*); and marsh sandpiper / little greenshank (*Tringa stagnatilis*). Importantly, most of the migratory shorebirds listed here do not breed in Australia and are thus not exposed to risks associated with recruitment or the feeding and safety of chicks.

The waterfowl are principally of the order Anseriformes, which includes about 180 living species in three families: Anhimidae (the South American screamers), Anseranatidae (the Australian magpie goose), and Anatidae (the largest family, which includes over 170 species of waterfowl, among them the ducks, geese and swans). In addition to these, grebes (a widely distributed order of duck-like freshwater diving birds, some of which visit the sea when migrating) are often considered to be waterfowl. The group can be divided loosely into herbivores (including swans and geese) and ducks (which includes the dabbling and diving ducks and grebes). Dabbling ducks feed in shallow water and are more likely to have a diet with more aquatic plants and insects, while diving ducks feed deeper in the water and typically eat more fish or crustaceans. The diet of grebes consists mainly of small fish and aquatic invertebrates – prey is normally caught during deep underwater dives, but some is taken on the surface. Waterfowl may congregate to nest in loosely-defined areas, but do not form colonies. Although some waterfowl in the northern hemisphere migrate to escape harsh winters, the same does not occur in Australia. Waterfowl are, however, mobile and may move through a landscape to locate suitable habitat. None of the waterfowl whose distribution overlaps with that of carp are listed under the EPBC Act as threatened, nor listed under international agreements for the protection of migratory species to which Australia is a party.

The large waders are birds of the order Ciconiiformes, which includes the storks, herons, egrets, ibises and spoonbills. Although exotic to Australia, this category may also include flamingos of the order, Phoenicopteriformes. The large waders are carnivorous with a diet that may include fish, reptiles, amphibians, crustaceans, molluscs and aquatic insects. Many of the large waders are colonial nesters, and many are at least partially migratory. The endangered Australasian bittern (*Botaurus poiciloptilus*) is the single large wader to be listed under the EPBC Act. This bird favours permanent freshwater wetlands with tall, dense vegetation, particularly bulrushes and spike-rushes.[23] It hides during the day and feeds chiefly at night on frogs, fish, yabbies, spiders, insects and snails. Feeding platforms may be constructed over deeper water from reeds that have been trampled by the bird. These platforms are often littered with the remains of prey. Australasian bitterns breed from October to January, with nests built in secluded places on a similar platform of

[23] See: http: //www.environment.nsw.gov.au/threatenedSpeciesApp/profile.aspx?id=10105

reeds. They are not a colonial nester. None of the large waders was listed under international agreements for the protection of migratory species to which Australia is a party.

The Gruiformes include rails, crakes, coots, moorhens and waterhens, which, when associated with a wetland, may appear to be physically similar to some waterfowl. This order of waterbird is very large, with more than 160 recognised species, most of which are omnivorous (with a diet that may include molluscs, frogs, small fish and insects). Gruiformes are not migratory and are not colonial nesters, although may congregate in loosely defined areas. They are distinct from waders and waterfowl in that they are primarily land-based although may forage for food in marshes and shallow water. Some species use their pronounced beak to tear up ground in search of tubers and other plant materials, or insects. The order also includes cranes and brolgas, some of which are phenotypically similar to herons and other large waders and share some aspects of behaviour and preferred habitat. None of the birds in this category whose distribution overlaps with that of carp is listed under the EPBC Act as threatened, nor listed under international agreements for the protection of migratory species to which Australia is a party.

The kingfishers are birds of the order Coraciiformes and family Alcedinidae. Kingfishers are small to medium-sized birds with a cosmopolitan distribution – most species of which are found in the tropical regions of Africa, Asia and Oceania. The family contains 114 species globally and is divided into three subfamilies and 19 genera. All kingfishers have large heads, long, sharp, pointed bills, short legs and short tails. With the notable exception of the laughing kookaburra (*Dacelo novaeguineae*) kingfishers tend to have bright plumage. There are only slight differences between the sexes. While kingfishers are generally associated with waterbodies, and often regarded as piscivorous, many species live away from water and have a broader diet that includes small crustaceans and invertebrates. Like other members of their order, they nest in cavities, which are usually tunnels dug into natural or artificial banks in the ground. None of the kingfishers whose distribution overlaps with that of carp are listed under the EPBC Act as threatened, nor listed under international agreements for the protection of migratory species to which Australia is a party.

The songbirds are small, perching birds of the order Passeriformes (the passerine birds). With more than 110 families and more than 6,000 identified species worldwide, Passeriformes is the largest order of birds and among the most diverse orders of terrestrial vertebrates. The diet of passerine birds varies amongst species, and may include seeds, insects and small crustaceans. Some passerine birds, such as the clamorous warbler (*Acrocephalus stentoreus*), are true waterbirds. Others may be present in wetlands and other water-focussed ecosystems, whether permanently or as an opportunistic means by which to gain refuge from drought and other environmental stresses. A single species of songbird whose distribution overlaps with that of carp is listed under the EPBC Act as endangered (mallee emu wren, *Stipiturus mallee*) while another is listed as vulnerable (thick-billed grass wren, *Amytornis modestus*). In addition to this, the satin flycatcher (*Myiagra cyanoleuca*) and the rufous fantail (*Rhipidura rufifrons*) are listed under international agreements for the protection of migratory species to which Australia is a party.

The bush-birds are a heterogeneous and informally-defined catchall category that encompasses the range of species that may roost, forage and nest in riparian or floodplain forests (including river red gums) but are not generally considered to be true waterbirds. This group encompasses some of the psittacines (cockatoos, cockatiels, corellas, parrots, rosellas, and budgerigars) as well as some honeyeaters, frogmouths, curlews and others. The following three bush-birds whose distribution overlaps with that of carp are listed under the EPBC Act as critically endangered: the regent honeyeater (*Anthochaera phrygia*); swift parrot (*Lathamus discolour*); and the plains-wanderer (*Pedionomus torquatus*). A further three species are listed as endangered: the rufous scrub-bird (*Atrichornis rufescens*); red-tailed black cockatoo (*Calyptorhynchus banksii graptogyne*); and the black-eared miner (*Manorina melanotis*) – while six other species are listed as vulnerable: the painted honeyeater (*Grantiella picta*); malleefowl (*Leipoa ocellata*); red-lored whistler (*Pachycephala rufogularis*); regent parrot (eastern) (*Polytelis anthopeplus monarchoides*); superb parrot (*Polytelis swainsonii*); and the mallee western whipbird (*Psophodes leucogaster leucogaster*). In addition to these, the following six species are listed under international agreements for the protection of migratory species to which Australia is a party: the fork-tailed swift (*Apus pacificus*); oriental cuckoo / Horsfield's cuckoo (*Cuculus optatus*); white-throated needletail (*Hirundapus caudacutus*); grey wagtail (*Motacilla cinerea*); yellow wagtail (*Motacilla flava*); and the little curlew / little whimbrel (*Numenius minutus*).

The eastern osprey (*Pandion cristatus*) and white-bellied sea eagle (*Haliaeetus leucogaster*) are believed to be the only Australian raptors whose diet regularly includes fish. Both are considered to be aquatic raptors and are sometimes categorised as waterbirds. Ospreys feed primarily on live fish, while the white breasted sea eagle consumes both live fish and carrion as well as birds and other animals. The eastern osprey is listed under international agreements for the protection of migratory species to which Australia is a party. A wide range of other raptors may play a key role in wetlands and other water-focussed ecosystems, although are not considered to be waterbirds. These may include some of the Accipitriformes (hawks, goshawks, kites, eagles and ospreys) as well as the Falconiformes (falcons and kestrels) and Strigiformes (owls). Some of these are primarily hunters, with a diet that may include aquatic animals, birds, small mammals, crustaceans or insects. Other species will feed on carrion. The red goshawk (*Erythrotriorchis radiatus*), whose distribution overlaps with that of carp, is listed under the EPBC Act as vulnerable.

3.3 Australian native frogs

Two hundred and eight species of frog are currently recognised within Australia, although some others await description.[24] Of these, the southern corroboree frog (*Pseudophryne corroboree*) and northern corroboree frog (*Pseudophryne pengilleyi)* have a distribution that overlaps with that of carp and are listed under the EPBC Act as critically endangered. In addition, the following five species are endangered: the booroolong frog (*Litoria booroolongensis*); yellow-spotted tree frog / yellow-spotted bell frog (*Litoria castanea*); spotted tree frog (*Litoria spenceri*); Fleay's frog (*Mixophyes fleayi*); and the giant barred frog / southern barred frog (*Mixophyes iteratus*). A further five species are vulnerable: the giant burrowing frog (*Heleioporus australiacus*); green and golden bell frog (*Litoria aurea*); littlejohn's tree frog / heath frog (*Litoria littlejohni*); growling grass frog / southern bell frog / green and golden frog / warty swamp frog (*Litoria raniformis*); and the stuttering frog / southern barred frog (*Mixophyes balbus*). Overall, approximately 14 percent of the frog species within Australia are considered to be threatened.

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[24] See: http: //www.environment.gov.au/resource/australian-frogs-overview

Frogs occupy a wide variety of habitats, and many have developed highly-specialised adaptations to particular climates, ecological sites and communities. This degree of specialisation may in some cases be a contributing factor in the decline of a species. Other factors include the loss, degradation and fragmentation of habitats, land use practices, changes to hydrology, pollution and predation by introduced fish (in particular, carp and mosquito fish, both of which feed on tadpoles). Disease is also being evaluated as a cause for some declines in Queensland, and this may also have relevance elsewhere. It was not until 1989, at the First World Congress of Herpetology (Canterbury, England) that there was an established perception of a significant pattern of declines on a global basis and a recognition of the need to take special measures to ensure the survival of amphibians.

As most frog species are susceptible to dehydration, proximity to water is a general prerequisite. Preferred habitats can include rivers, lakes and wetlands. Because droughts and changes to water regulation impact directly on the patency and characteristics of aquatic habitats, both are likely also to impact on the persistence of many frog species.

3.4 Australian native freshwater turtles

Australia is home to 23 species of freshwater turtle.[25] Of these, the Bellinger River snapping turtle (George's snapping turtle or George's helmeted turtle: *Wollumbinia georgesi*) has a distribution that overlaps with that of carp and is listed under the EPBC Act as critically endangered; while the Bell's turtle (western saw-shelled turtle, Namoi River turtle or Bell's saw-shelled turtle: *Wollumbinia belli*) is vulnerable.

All but one of Australia's freshwater turtles belong to the family *Chelidae*, which is found only in Australasia and South America. These 'side-necked' turtles retract their head and neck beneath their shell by folding them to one side, rather than drawing their head backwards as most of the world's species of turtles and tortoises do.

Australia's freshwater turtles spend most of their time in rivers, lakes, wetlands, ponds and storage dams. However, they periodically come onto land to migrate between waterbodies or to nest in spring and summer. Some species can also survive for months in a dormant state (aestivation) buried in soil or dry lake beds. Turtles do not feed out of the water. Freshwater turtles are either carnivorous (including insects, tadpoles, small fish and crustaceans) or omnivorous, and some species may also feed on carrion. Nesting turtles dig a hole in the ground with their hind legs, lay their eggs in the hole then cover the eggs with earth. A clutch may comprise as many as 25 eggs, depending on the species of turtle and its size. After a few months the eggs hatch and the hatchling turtles make their way to the water, where they typically take around 10 years to grow to maturity. Little is known about the life span of Australian freshwater turtles, but they can likely live for 50 years or more.

Freshwater turtles face many threats. Introduced foxes and pigs rob their nests and in some areas consume over 90 percent of their eggs. Hatchlings then contend with turtle-eating fish, birds and other predators. Adult turtles are protected by their shells from most natural predators when they

[25] See: http: //www.environment.nsw.gov.au/topics/animals-and-plants/native-animals/native-animal-facts/freshwater-turtles

are in the water, but when they venture onto land they can be killed by dogs, foxes or pigs, or crushed by motor vehicles. Droughts also take a heavy toll on turtles by drying their habitats and depriving them of food. Habitat destruction and poor water quality are also key contributors to declining turtle numbers.

3.5 Australian native freshwater crustaceans

Australia has a wide range of native (macro) crustaceans, including crayfish, lobsters, prawns, shrimps and crabs, a subset of which inhabit freshwater ecosystems that may include carp. Of these, only the endangered Glenelg spiny freshwater crayfish (*Euastacus bispinosus*) is listed under the EPBC Act, although the population of Murray crayfish (*Euastacus armatus*) is thought to have declined markedly following the widespread and long-lasting blackwater event within the mid-lower Murray River in 2010-11 (Section 6.2.2).

- The Glenelg spiny crayfish occurs in a very limited area within the Glenelg River Basin and associated tributaries, and in several spring-fed coastal streams.[26] It is an omnivore, foraging on organic material as well as actively predating on fish and other macroinvertebrates. They are also known to strike at living prey, such as fish and other aquatic invertebrates. Adult Glenelg spiny crayfish can move significant amounts of riverbed substrate and organic matter during foraging, and this action is thought to play a role in nutrient recycling and structural dynamics in the streams where crayfish occur. They have a very limited dispersal and re-colonisation ability and are not able to move effectively over land without being subject to desiccation and predation. Glenelg spiny crayfish require flowing water throughout the year and prefer in-stream habitat that includes undercut banks, cobbles, rock boulders and woody debris as refuges. They also require relatively cool and well-oxygenated water.
- Murray crayfish are slow-growing, opportunistic detrivores, feeding principally on decaying animals and plants.[27] Murray crayfish become more active during the winter months. Mating generally occurs in May, most likely cued to a decline in water temperature. Eggs hatch during late spring and juveniles remain attached to the mother's pleopods until they have completed a series of moults. After this, they become self-sufficient. Murray crayfish can be found in the Murray River upstream of Mildura, in the Murrumbidgee River and in some dams, and are the only species in the *Euastacus* genus that live in both cold- and warm-water habitats. They prefer permanent rivers and large streams where the water flows moderately fast. In most settings, Murray crayfish burrow in amongst boulders and cobble, and use tree roots and snags for cover. Because they shred large woody and leafy debris while eating, they help the aquatic nutrient cycle. Murray crayfish are sensitive to water quality and the addition of sediment, thermal pollution, pollutant discharges and bushfire impacts.

A range of other native freshwater crustaceans whose distribution overlaps with carp, including the yabby (*Cherax destructor*) and freshwater shrimp (*Macrobrachium* spp.), are also sensitive to

[26] See: http: //www.environment.gov.au/cgi-bin/sprat/public/publicspecies.pl?taxon_id=81552

[27] See: https: //www.dpi.nsw.gov.au/fishing/fish-species/species-list/murray-crayfish

water quality – in particular, to low DO. These species were observed at the water's edge during the widespread and long-lasting blackwater event of 2010-11.

3.6 Ecological communities

An ecological community is a naturally occurring group of plants, animals and other organisms that are interacting within a unique habitat.[28] Its structure, composition and distribution are determined by environmental factors such as soil type, position in the landscape, altitude, climate and the availability and quality of water. An ecological community becomes threatened when its natural composition and function have been significantly depleted.

Listing of threatened ecological communities under the EPBC Act recognises that key natural assets are under pressure, and that a landscape (whole-of-system) approach to environmental protection is necessary. This means that additional protection is given to the threatened species which live within the community. Importantly, in the context of this risk assessment, **protection is also granted to species within the community that are not themselves listed under the EPBC Act as threatened** (but yet may be in decline).

A total of 82 threatened communities are currently listed by the Department of Environment and Energy. These include two communities that are vulnerable, 43 that are endangered and 34 that are critically endangered. There are also two communities that are listed as approval disallowed and one that is currently ineligible. Of these communities, 33 were ruled out of further consideration using a screening analysis. This considered whether each community overlapped with carp habitat (using the spatial dataset for carp habitat provided by the Department) and, for those that did, whether it was placed in a state or territory where the carp biomass density may be sufficient for an outbreak of CyHV-3 to result in a real possibility of harm. This excluded Western Australia, the Northern Territory, Tasmania and any Australian island territories. Communities were also excluded (if the spatial assessment had not already done so) if they related to alpine, desert, tropical, coastal (marine) or cave ecosystems.

The remaining 49 communities (see Volume 2 of this report) were grouped according to whether they were wetland, marsh or other freshwater-based communities (eight communities, two of which are listed as approval disallowed and one that is currently ineligible); or whether they were forest, woodland, grassland or other dryland communities (41 communities, all of which are classified as threatened).

The <u>wetland and marsh communities</u> include the assemblage of native flora, fauna and micro-organisms associated with and dependent upon the floodplain and river ecosystem. These are complex aquatic and terrestrial ecosystems of interconnected environmental subunits, and may include river channels, lakes and estuaries, tributaries and streams, floodplain wetlands and woodlands, and groundwater. In ecological terms, wetlands and marshes may best be considered landscape-scale ecological meta-communities whose hydrological connectivity is recognised as being essential to their long-term health. Wetland and marsh communities are fundamentally water-focussed, and those that occur within the distribution of carp could potentially be harmed

[28] See: http: //www.environment.gov.au/biodiversity/threatened/communities

through any of the exposure pathways considered in this assessment (Part III: Section 11). Although the possibility of indirect harm to forest, woodland and grassland communities was considered (as noted above), the impacts of CyHV-3 on both listed and unlisted wetland and marsh communities remained the principal focus of the component of the assessment that was concerned with ecological communities.

The forest, woodland, grassland or other dryland communities are listed under the EPBC Act with consideration to their floral (including canopy, mid-layer and ground-layer) and faunal (including terrestrial and aquatic animals) components. Importantly, the microflora and fauna are also considered to be part of the ecological community. Forest, woodland, grassland or other dryland communities are most likely to be exposed to harm as a result of the release of CyHV-3 through its indirect impacts on water quality – in particular, the contamination of water with cyanobacterial blooms, or with the organic products and microorganisms associated with carcass decomposition. The effect of contamination may be to cause stress to the faunal compartment of the community and, ultimately, either death of individuals or individual species or their migration to other communities. This cascade of impacts is likely to be exacerbated in coincidence with drought or significant dry periods when extant waterways are contracted, and alternative waterways may not be available to some species.

4 European carp in Australia

4.1 Background

Carp are members of the family *Cyprinidae*, the largest family of freshwater fish. Common carp (*Cyprinus carpio*) are native to Eastern Europe and Central Asia although they have successfully invaded parts of Europe, Asia, Africa, North, Central and South America, Oceania and Australia. There are no native cyprinid fishes in Australia but both the European (*Cyprinus carpio*) and East Asian sub-species (koi – *Cyprinus carpio haematopterus*) of European carp have been introduced and have established in Australian waterways.

Carp were first introduced into Australia in the mid-1800s, although they remained relatively contained until the release of the 'Boolarra' strain in the 1960s (NSW DPI, 2017). Carp are now widely established throughout the Murray-Darling Basin and can be found in all states and territories except the Northern Territory. Carp can now be found within waterways in most of the south-eastern Australian mainland, with isolated populations in Tasmania[29] and Western Australia. They occupy almost the entire Murray-Darling Basin apart from a small number of waterways upstream of barriers such as waterfalls or large dams (Driver *et al.*, 1997). The only substantial carp-free parts of the Murray-Darling Basin are in the New England tablelands region of New South Wales and some smaller upland areas in the southern catchments. Carp continue to expand their range, with the potential for future invasion of many other river systems within Australia (Koehn, 2004). Areas of concern for future invasion include the remaining upper reaches of the Murray-Darling Basin, remaining coastal river systems in south-eastern coastal and Indian Ocean regions, Tasmanian river systems and the Lake Eyre/Bulloo-Bancannia drainage division (Koehn, 2004). Carp now contributes more than 90 percent of fish biomass in many areas of south-east Australia (Koehn, 2004).

4.2 Carp as an invasive species

4.2.1 Attributes of an invasive species

Koehn *et al.* (2016) summarised the attributes of carp that are likely to have resulted in their widespread distribution within Australia and elsewhere. This information is reproduced in Table 1. The analysis shows that carp possess an unusually wide range of traits to favour their adaptability and competitiveness. Foremost, carp are a robust species, and within-catchment migration and downstream larval drift have proven to be effective methods of population dispersal (Stuart *et al.* 2001). Adult carp are also mobile, although the work of Stuart and Jones (2006b) showed that movements are quite variable. In studies initiated within the Barmah-Millewa Forest area, these authors found that approximately 80 percent of adult carp moved less than 5 km while more than

[29] At the time of writing, the population of carp in Tasmania may have been eradicated

7 percent moved more than 100 km. Males were more likely to move than females, who tended to remain close to the spawning grounds. The maximum distance that tagged carp were observed to have moved was 890 km. This work showed that carp have the ability to travel considerable distances. Human intervention is another major vehicle for the invasion of carp into new catchments, and transfer of carp between catchments by anglers (either accidentally or deliberately) occurs despite the illegality of keeping, transporting or releasing carp in most states of Australia (Koehn *et al.* 2000; Lintermans, 2004).

Table 1 Attributes of carp as an invasive species

Attribute	Details
Invasion history, wide distribution and abundance	Introduced and successfully established throughout Europe, Asia, Africa, North America, South America, Central America, Australia, New Zealand, Papua New Guinea and some islands of Oceania; likely further expansion
Wide environmental tolerances	Generally occur in most types of freshwater habitat and have high environmental tolerances: temperature ranges from 2 to 40.6C, salinity up to about 14 ppt (0.4 seawater), pH from 5.0 to 10.5 and oxygen levels as low as 7 percent saturation
High genetic variability	Multiple genetic strains in Australia
Early sexual maturity	Males at 1 year, females at 2 years
Short generation	2 to 4 years
Rapid growth	Hatching of eggs is rapid (2 days at 25C), and newly hatched Carp grow very rapidly
High reproductive capacity	They are highly-fecund broadcast spawners, with egg counts as high as 2 million per female
Broad diet	Omnivore/detritivore
Gregariousness	A schooling species
Natural mechanisms of dispersal	A mobile species, with fish moving between schools; dispersal can also occur with the downstream drift of larvae; rates of transfer can be affected by conditions such as flooding
Capacity for being commensal with human activity	Bred as an ornamental and aquaculture species; used as bait and sought by some anglers

Source: Koehn *et al.* (2016)

4.2.2 Population dynamics

The invasion of carp in south-eastern Australia illustrates how quickly an alien fish species can spread and dominate aquatic ecosystems. *Apropos* of this, Bajer and Sorensen (2010) noted that the population density of carp in United States and Australian waters was commonly in the order of 10-times that which is reached in most European settings. These authors observed that in parts of the world where carp exist in superabundance, they do so by making use of shallow ephemeral lakes, wetlands, marshes or floodplains as grounds for spawning (Figure 6).

Adults tend to aggregate in early spring, immediately prior to spawning. Carp prefer to spawn when the water temperature exceeds approximately 16 to 17C (Stuart and Jones, 2006a).

Although there is some regional variation, this is generally in mid-late spring. Carp eggs become sticky within minutes of contact with water, and this allows them to adhere to submerged vegetation in shallow water and thus resist drift (Figure 7) (Mansour *et al.*, 2009). In some settings, spawning may take place at the fringes of permanent off-channel still-waterbodies, with larvae then moving out into adjacent shallow and recently-inundated warm floodplains (Stuart and Jones, 2006a). Alternatively, spawning may take place on the floodplain. The recently-inundated floodplain is relatively free of predators and, whilst frequently low in DO and high in dissolved organic carbon (DOC), juvenile carp have been shown to be unusually tolerant of these conditions (Stuart and Jones, 2006a). Whether spawned within permanent off-channel still-waterbodies or on the floodplains, juveniles drift back toward tributaries and the river channel as floodwaters recede and, from there, they continue to drift downstream toward the river channel (Stuart and Jones, 2006a). Upon reaching a body size sufficient to swim actively against the water current, a proportion of the young-of-the-year carp move upstream within the channel and disperse into anabranches and tributaries (Brown *et al.*, 2005; Stuart and Jones, 2006a). Upstream migration ceases when the water temperature drops in late summer or early autumn (Mallen-Cooper 1999). The cycle then recommences in early spring.

Figure 6 Carp spawning amongst macrophytes in a shallow river wetland channel

Source: Meredith and Beesly (2009)

Figure 7 Carp eggs adhered to exposed vegetation

Source: Victorian Department of Environment, Land, Water and Planning (http: //www.dewlp.vic.gov.au)

Because carp are exceptionally fecund, with egg counts as high as two million per female (Koehn *et al.*, 2016), the floodplain spawning strategy described above can result in a very high rate of recruitment. The strategy also means that certain places within Australia's freshwater systems play a key role in the overall population strength of carp. Citing unpublished data, Koehn *et al.* (2016), for example, described the following twelve discrete carp recruitment 'hotspots' within the Murray-Darling Basin: Mid Darling, Lower Macquarie, Wimmera, Lower Gwydir, Koondrook-Perricoota-Gunbower, Lower Border Rivers, Lower Castlereagh, Great Cumbung Swamp, Upper Wakool, Barmah-Millewa Forest, Lower Murray River (between Lake Victoria and Chowilla) and Lake Brewster. These are places where large-scale recruitment can take place in seasons when the flow of water is sufficient to cause inundation of ephemeral wetlands, marshes and floodplains.

Importantly, recruitment from these hotspots does not occur every year as most ephemeral Australian lakes, wetlands, marshes and floodplains are only inundated with unusually heavy seasonal rains in the local area or in the upstream catchment. In the drier years between inundation and recruitment, the number of adult carp in river channels and other permanent waterways may slowly decline in some locations (Gilligan *et al.*, 2010). Spatial and temporal variability in recruitment can result in 'source-sink' population dynamics, with shallow ephemeral recruitment areas providing a 'source' of juveniles and other areas consisting of, for example, river channels, acting as a 'sink'. The source-sink model for carp populations in the United States was formalised by Dauphinais *et al.* (2018) who showed that whilst adult carp could be found across the entire United States watershed, reproductive success (the presence of young carp) was restricted to shallow ephemeral waterbodies. Similar conclusions had been reached by Stuart and Jones (2006a) after detailed field studies based on the Barmah-Millewa Forest wetland within the mid-Murray River, and through the Australian modelling work of Koehn *et al.* (2016). The latter deduced that control would be most effective when focussed on separating the source from the sink populations, or otherwise limiting spawning success in shallow ephemeral waters, than on culling large numbers of aggregated mature carp within the river bodies. This outcome has implications for the outbreak scenarios likely to follow from the strategic release of CyHV-3 (Part III: Section 10).

4.3 Impacts of carp on aquatic ecosystems

4.3.1 Carp as ecosystem engineers

The impacts of carp on Australian waterways were reviewed systematically by NSW DPI (2017) and much of this section has been adapted from that work. Carp are widely considered to be 'ecosystem engineers' that alter the fundamental characteristics of the ecosystems they invade. Their impacts result in establishment of a positive feedback mechanism whereby the degree of impacts escalates as the affected ecosystem deteriorates (Zambrano and Hinojosa, 1999; Zambrano *et al.*, 2001; Penne and Pierce, 2008). Within Australia, Harris *et al.* (1997) found that increasing carp numbers correlated with increasing environmental disturbance, noting that it is also important to consider the impacts of carp in the context of other interrelated anthropogenic changes to ecosystems (Hume *et al.*, 1983; Gilligan and Rayner, 2007).

The primary impacts of carp relate to interspecies competition for resources and to aquatic habitat alteration through benthic foraging by adult carp. Benthic foraging increases sediment resuspension, resulting in increased turbidity and the uprooting of aquatic vegetation (Fletcher *et al.*, 1985; Roberts *et al.*, 1995; King *et al.*, 1997; Robertson *et al.*, 1997; Schiller and Harris, 2001; Zambrano *et al.*, 2001). Carp predation upon micro- and macro-invertebrates has been shown to change the composition of aquatic invertebrate communities (Wilcox and Hornbach, 1991; Robertson *et al.*, 1997; Weber and Traunspurger, 2014). Other direct impacts include potential predation on the eggs and larvae of native fishes (Lachner *et al.*, 1970, Page and Burr, 1997, Schiller and Harris, 2001) and amphibians (Leslie, 1995, Healey *et al.*, 1997; Gillespie and Hero, 1999; Hunter *et al.*, 2011). The eggs of bottom-nesting fish, such as the eel-tailed catfish and Macquarie perch, are particularly vulnerable to predation by carp (Schiller and Harris, 2001). Adult carp also compete with native benthic foraging fish, and both adult and juvenile carp compete for zooplankton with planktivorous fish and the planktivorous larvae of most native fish species (Fletcher, 1986; Schiller and Harris, 2001). Further, if the density of zooplankton is reduced then grazing on phytoplankton (including algae and cyanobacteria) will also be reduced and the incidence and severity of blooms may increase (Gehrke and Harris, 1994; Lougheed *et al.*, 1998; Khan, 2003; Khan *et al.*, 2003; Parkos III *et al.*, 2003).

The indirect impacts of increased turbidity associated with the foraging behaviour of carp include the effects on photosynthesis of aquatic plants and biofilms, the inhibition of feeding by visual aquatic predators (Newcombe and MacDonald, 1991; Lougheed *et al.*, 1998; Schiller and Harris, 2001; Zambrano *et al.*, 2001) and sediment deposition and the resultant smothering of demersal native fish eggs (Schiller and Harris, 2001). Indirect impacts of the destruction of aquatic plants include reduced populations of invertebrates (Lougheed *et al.*, 1998), less habitat for juvenile fish (Hume *et al.*, 1983), amphibians (Gillespie and Hero, 1999) and waterfowl (King and Hunt, 1967; Hume *et al.*, 1983; Haas, 2007), and the instability of bottom sediments (Lougheed and Chow-Fraser, 2001).

The ecological impacts of carp were summarised in diagrammatic form by Weber and Brown (2009) as reproduced in Figure 8 below.

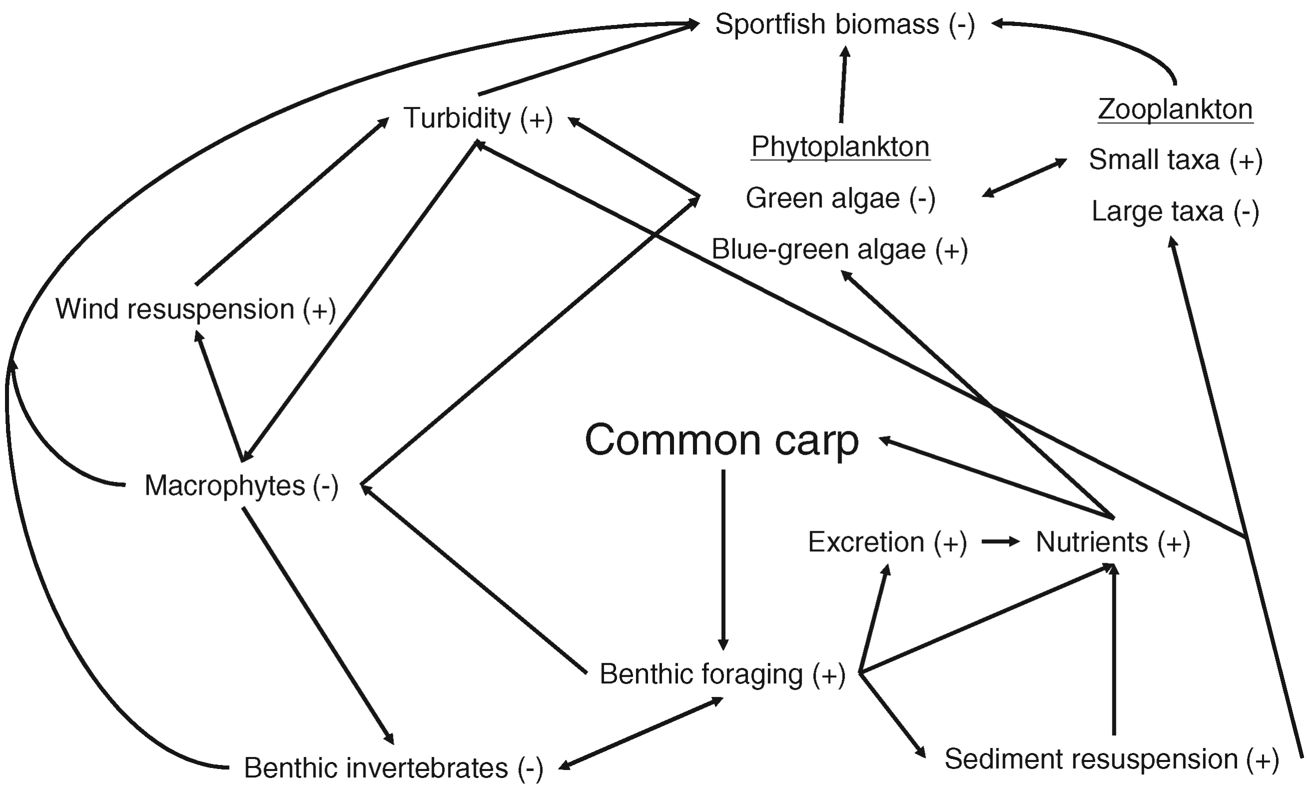

Figure 8 Ecological impacts of carp (arrowheads represent the direction of the interaction)

Source: Weber and Brown (2009)

Within Australia, carp have also been associated with the introduction or spread of a number of aquatic parasites. The external parasitic anchor worm (*Lernaea cyprinacea*), *Tetrahymena* spp., *Dactylogyrus anchoratus* and the Asian fish tapeworm (*Bothriocephalus acheilognathi*) may have been introduced with carp and are found on or in native fish species (Rowland and Ingram, 1991; Hindmarsh, 1994; Dove *et al.*, 1997; Dove and Ernst, 1998; Hassan *et al.*, 2008; Read and Wales, 2010). The distribution of the Asian fish tapeworm was correlated with that of its principal host, carp, and did not occur at sites where carp were not present. The parasite was recorded from all native fish species that were collected with carp (Dove *et al.*, 1997; Dove and Fletcher, 2000). Species of the Trichodinids (including *Trichodina heterodentata*, *T. reticulata* and *T. mutabilis*) were introduced with carp (Dove and O'donoghue, 2005), while *Argulus japonicus* spread throughout the world with trade in carp (Catalano and Hutson, 2010). *Ichthyophthirius multifiliis* was introduced into Victorian waters through carp (Butcher, 1947; Forwood, 2015). These parasites have the potential to have serious pathogenic effects on native fish species (Rowland and Ingram, 1991; Hassan *et al.*, 2008; Read and Wales, 2010).

4.3.2 Threshold density for ecosystem impacts

In Australia and elsewhere, the impacts of carp may not be observed until carp abundance exceeds a threshold density. Koehn *et al.* (2016) noted that turbidity increases significantly at carp densities of 50 to 75 kg/ha (Zambrano and Hinojosa 1999; Vilizzi *et al.* 2014), with noticeable shifts from a clear to a turbid water state at 200 to 300 kg/ha (Williams *et al.* 2002; Parkos *et al.* 2003; Haas *et al.* 2007; Matsuzaki *et al.* 2009). Decline in aquatic vegetation cover and detrimental effects on aquatic macrophytes have been observed at carp densities ranging from 68 to 450 kg/ha (Hume *et al.* 1983; Fletcher *et al.* 1985; Osborne *et al.* 2005; Pinto *et al.* 2005; Bajer *et al.* 2009; Vilizzi *et al.* 2014) and a decline in the use of wetlands by native waterfowl when carp density exceeds approximately 100 kg/ha (Bajer *et al.* 2009). These impacts stem largely from the carp’s benthic feeding behaviour (Sibbing *et al.* 1986) and are most commonly reported in shallow off-stream habitats (Parkos *et al.* 2003) where carp congregate.

Managing an invasive species to a predetermined density threshold, below which its impacts on environmental values are acceptable, is a key component of many integrated pest management strategies (Koehn *et al.*, 2016). Citing a range of primary and review works, Koehn suggested that an appropriate threshold density for carp should be 100 to 174 kg/ha or lower (Haas *et al.* 2007; Bajer *et al.* 2009; Matsuzaki *et al.* 2009), noting that this is substantially less than historic density estimates of carp in Australian waterways of 450 kg/ha (Fletcher *et al.* 1985). The ecological impacts of carp were also reviewed by Brown and Gilligan (2014). These authors summarised threshold densities from the literature below which carp did not appear to be impacting on the aquatic environment, including 100 kg/ha (Bajer *et al.*, 2009), 120 kg/ha (Haas *et al.*, 2007), 161 kg/ha (Matsuzaki *et al.*, 2009), 174 kg/ha (Parkos *et al.*, 2003), 450 kg/ha (Fletcher *et al.*, 1985), and 500 kg/ha (Miller and Crowl, 2006). From their modelling studies, Brown and Gilligan (2014) maintained that a density of 88 kg/ha was required to abate environmental damage in the Lachlan river catchment. Koehn *et al.* (2016) noted the densities of carp in situations where environmental damage had occurred, including Moira Lake: 190 kg/ha (Brown *et al.*, 2003); a range of billabongs: 150 to 690 kg/ha (Hume *et al.*, 1983); Bogan River: 690 kg/ha (Reid and Harris, 1997); and the Campaspe irrigation channels: up to 619 kg/ha (Brown *et al.*, 2003).

4.3.3 Predators and competitors of carp in Australian waterways

Predators: NSW DPI (2017) reviewed the aquatic and terrestrial animals that currently prey on carp in Australian waterways, noting as a caveat that the rapid expansion of carp within Australia may have been assisted by a lack of predatory pressure. Koehn (2004) suggested that with few effective predators of carp in the ecosystem, detrital carbon that is sequestered in the bodies of carp, rather than passing up through subsequent trophic levels of macro-invertebrates and smaller fish (Bunn and Davies, 1999), may become 'locked away' from the trophic chain for the lifespan of a carp (up to 50 years).

Given the large gape of Murray cod, and that species' ability to attain up to 180 cm in length (Lintermans, 2007), Murray cod are likely to be the only predator of adult carp. Carp up to 410 mm total length have been recorded in the stomachs of large Murray cod (NSW DPI, 2017[30]). A dietary study by Ebner (2006) found that 35 percent of Murray cod sampled (all cod >500 mm total length) contained carp. Similarly, Baumgartner (2005) found carp and goldfish constituted up to 25 percent of prey occurrence in Murray cod sampled from the Murrumbidgee River. By contrast, examination of Murray cod stomachs from rivers of the southern Murray-Darling Basin found that <7 percent contained carp (Doyle *et al.*, 2012). These authors attributed the low occurrence of carp in the diet of Murray cod to the variation in habitat utilisation between early life stages of carp (which primarily inhabit shallow floodplains) and Murray cod (which prefer main channels). This observation may be true *in situ* but does not explain the disparity of findings when compared with Ebner (2006) or Baumgartner (2005). Golden perch and Australian bass may also consume small carp, although their gape size is limited (Ebner, 2006; Doyle *et al.*, 2012). The critically endangered trout cod has also been shown to eat carp, although carp made up <1 percent of their prey (Baumgartner, 2005).

NSW DPI (2017) noted that feral cats (Jones, 1981), the eastern water rat (Woollard *et al.*, 1978) and cormorants (Miller, 1979) have also been shown to eat carp – albeit as a small proportion of their diet. Hume *et al.* (1983) suggested that carp within billabongs may provide a reliable food source for Australian pelicans.

Competitors: NSW DPI (2017) noted that the diet of small carp is generally dominated by microcrustacea, including cladocerans and copepods (Khan, 2003). There is then a shift with ontogenetic development to a broader diet with increased proportions of macro-invertebrates. The diet of larger carp is dominated by gastropods, ostracods, amphipods and detritus (Vilizzi, 1998; Khan, 2003). Hume *et al.* (1983) found that plant matter did not make up a large part of the diet of carp in Australian waters.

The specialist feeding strategy of adult carp, which involves sieving the substrate for detrital material, allows carp to take advantage of a potentially underutilised resource – given that true detritivorous fish are lacking from most Australian freshwater fish communities (Koehn, 2004). In addition to this, however, carp may compete with native fish, including bony herring, freshwater catfish, silver perch, Australian smelt, carp gudgeon and flathead galaxias (Reynolds, 1976; Khan, 2003; Cadwallader, 1978; Fletcher, 1986). Carp may also compete with decapods, such as yabbies,

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[30] Citing a personal communication with Jerom Stocks, NSW Department of Primary Industries

that primarily feed on detritus (Reynolds, 1976). The extent to which dietary overlap (above) has led to competitive exclusion or detriment of native fish or crustaceans is unknown and is also likely to vary amongst locations.

Citing the work of Khan *et al.* (2003), NSW DPI pointed to a decline in the abundance of zooplankton coinciding with a high abundance of larval carp, which suggested that predation by carp larvae played a role in regulating the population of zooplankton and, potentially, caused a trophic cascade. Gehrke *et al.* (2010) reported that large zooplankton, such as *Boeckella* and *Daphnia*, increased by more than 10-fold following a 50 percent reduction in carp within a number of experimental billabongs, and the number of aquatic insects and crustacean species also increased strongly. Similarly, Robertson *et al.* (1997) illustrated the potential of carp predation to alter aquatic micro- and macro-invertebrate communities. Citing the work of Schiller and Harris (2001) NSW DPI also noted that carp at all stages of ontogenetic development eat microcrustacea, which are also the primary food source of all larval and most juvenile native freshwater fish species. Khan (2003) proposed that an ontogenetic and competitive bottleneck could profoundly affect the recruitment of species with zooplanktivorous larvae (Werner and Gilliam, 1984). Further, the plasticity in carp's spawning timing gives them a competitive advantage over other native species. When carp are able to spawn early in a breeding season, their larvae and juveniles gain access to food earlier than native species, which are generally constrained by temperature to spawn later (Roberts and Ebner, 1997; Khan, 2003).

4.4 Methods for carp control

4.4.1 Traditional methods for carp control

A wide range of strategies and tools is currently available for the control of carp in Australian waterways. These were reviewed by Brown and Gilligan (2014) and by Koehn *et al.* (2016). A brief summary is given below. Both Brown and Gilligan (20014) and Koehn *et al.* (2016) stressed that while these tools and strategies have merit in isolation, local or widespread success in the management carp can only be achieved using an integrated pest management approach.

Fishing, traps and barriers

- Commercial or targeted harvesting: this approach will not catch all carp, but will aid in reducing numbers. The approach can also be used to remove large tonnages of carp during annual spawning migrations. Depending on the level of effort required to achieve a satisfactory reduction in the biomass of carp, this may be a comparatively expensive option and may incur some native bycatch.
- Promotion of recreational fishing targeting carp: this approach has limited impact on populations but may increase public awareness of the problem.
- Use of the 'Judas carp' approach to maximise the commercial harvest and efficiency of removal exercises by targeting spawning or winter aggregations of carp (Inland Fisheries Service, 2008; Bajer *et al.*, 2011). This approach was shown to be very effective in Lake Crescent (Tasmania), but may be less effective in the Murray-Darling Basin.
- Fish exclusion screens or barriers in wetland flow control structures to restrict access to spawning sites or to limit colonisation (or re-colonisation) by carp (Verrill and Berry, 1995;

French *et al.*, 1999; Hillyard *et al.*, 2010). Restricting access of adult carp to wetland spawning grounds can be effective. However, without active screen management (that is, opening/closing) or periodic wetland drying there is potential to compress larger carp into wetlands. Flow control structures are required, and these can be expensive. The strategy will restrict the access of large-bodied native fish to wetlands, although will not restrict juveniles – which may then grow to adult size in wetlands.

- Installation of Williams carp separation cages in fishways (Stuart *et al.*, 2006; Thwaites, 2011). These can remove large tonnages of carp during annual spawning migrations. Williams cages are most cost-effective in river fishways. They require expensive infrastructure to mechanically lift and empty captured fish and can restrict the passage of native fish. Fishways may also become blocked by carp during migration periods. Williams cages are only deployable within engineered fishways – they are not suited to 'natural' fishways such as rock ramps.
- Installation of push-style traps in inlet and outlet channels of floodplain wetlands that capitalise on carp's innate behaviour in preferentially accessing these locations (Thwaites *et al.*, 2010). Field trials have shown push-style traps to work in combination with separation cages (jumping traps). When fitted as an exit gate within an exclusion screen, push-style traps may allow large carp to exit a wetland.
- Electrical barriers for restricting movements (Verrill and Berry, 1995; Moy *et al.*, 2011). These are used to restrict movements of fish (usually upstream) by establishing an electrical field between two electrodes. Fish are shocked, following which they either turn around or are briefly paralysed and flow downstream before recovery. The approach is generally unsuitable due to its high cost, and the potential risks to both the general public and native species.
- Barrier netting and liming to sabotage spawning (Inland Fisheries Service, 2008). Fine-mesh netting is deployed to restrict access of fish to preferred spawning habitat. The approach has been effective in Tasmania at reducing spawning success of carp. However, the amount of fine-mesh netting required to net-off all fringing habitat is substantial and the cost may be prohibitive.
- Maximising the cost-efficiency of physical removal efforts, by targeting refugia during drought or lower-flow periods (Roberts *et al.*, 1997).

Manipulation of water levels

- Strategic delivery of water to disadvantage carp by providing a non-preferred inundation regime or mosaics of fast- and slower-flowing habitats has been proposed, but is yet to be fully evaluated (Stuart *et al.*, 2011).
- Strategic non-consumptive use of environmental water allocations or other flow deliveries to induce carp spawning activity, followed by a rapid short-term reduction in water level and subsequent carp-recruitment sabotage (Shields, 1958; Verrill and Berry, 1995; Summerfelt, 1999; Yamamoto *et al.*, 2006). This approach can be used to expose and desiccate eggs deposited on fringing vegetation. The approach requires flow and water level control structures and the timing of manipulations is critical as there is otherwise the potential to impact negatively on the spawning of native species.
- Strategic consumptive use of environmental water to trap and eradicate adult carp on inundated floodplains (Stuart and Jones, 2006a). Draining and drying can be extremely effective in

eradicating carp, although the approach is not species-specific and will affect any native fish that are also present. If the wetland cannot be fully drained, then there is the additional option of destroying any fish remaining in residual pools with rotenone.

Chemical methods

- Pheromone attractant traps (Sorensen and Stacey, 2004).
- Chemical piscicides such as rotenone (Sanger and Koehn, 1997; Clearwater *et al.*, 2008). These can be effective at locally eradicating carp, although are not species-specific and will kill native fish. Large quantities of chemical may be needed in multiple applications, and this can be expensive. Wetlands will need to be isolated, and residual chemical treated, to avoid downstream mortalities. The approach requires specialised training and permits.
- Hormone implants to produce 'lure fish' to maximise trapping catch rates of carp in and around spawning sites (Sorensen and Stacey, 2004; Lim and Sorensen, 2010).

Targeting carp recruitment hot spots

- Identification and targeting of carp-recruitment hotspot locations (Driver *et al.*, 2006) to achieve catchment-wide population control (Gilligan, 2012).

Modelling studies

- Use of simulation models to evaluate alternative population control or eradication strategies (Brown and Walker, 2004; Brown and Robertson, 2007; Brown and Gilligan, 2014; Koehn *et al.*, 2016).

4.4.2 Genetic biocontrol

Genetic biocontrol (also termed autocidal control) is based on the intentional environmental release of genetically-modified organisms that are designed to disrupt the survival or reproduction of a targeted invasive species (Kapuscinski and Sharpe, 2014). These methods involve: (a) manipulation of the chromosomes of a target species in order to skew sex ratios of the target species; (b) recombinant DNA techniques to insert a deleterious gene construct into the target species' genome, in order to disrupt its lifecycle; or (c) a combination of both techniques. Genetic biocontrol has the potential to better target a specific invasive species than conventional control methods, such as culling or physical removal (Kapuscinski and Sharpe, 2014). The impact that genetic control has may be specific and relatively simple to evaluate – for example, a reduced reproductive efficiency within the target population. However, because these methods require alteration to the species' genome there may be other more subtle effects such as a change in the fitness of the species or its resistance to pathogens.

Two genetic biocontrol methods are currently under consideration for the eradication of carp: the Trojan Y chromosome and daughterless carp.

- The Trojan Y chromosome strategy (Gutierrez and Teem, 2006) makes use of a fish with multiple Y chromosomes to change the sex ratio of an invasive fish population, potentially causing its extinction. Under this approach, an autocidal female fish with two Y chromosomes (Trojan Y) is added to a target population (Teem *et al.*, 2014). Mating of the autocidal Trojan Y fish with males of the target population results in male progeny, half of which are so-called 'supermales'

– that is, males with two Y chromosomes. Supermales also produce exclusively male progeny in matings with normal females. Continuous addition of the autocidal Trojan Y fish to the target population leads to a progressive decline in females and a corresponding increase in both males and supermales. Under certain assumptions, the females are eliminated leading to the possible extinction of the target population.

- The daughterless carp strategy (Bax and Thresher, 2009) is similar to the Trojan Y chromosome approach that also shifts the sex ratio of a population. Under this approach, fish containing multiple copies of a genetically engineered aromatase inhibitor gene (D) are added to an invasive fish population (Teem *et al.*, 2014). The D gene causes all fish that inherit it to develop as a male, regardless of sex chromosome composition. Mating of the daughterless autocidal fish to females in the population results in only male progeny, which will subsequently pass the D gene on to their own progeny. Over time, the number of females in the population will be reduced relative to the number of males. Continuous addition of the autocidal daughterless fish to the target population eventually eliminates females from the population, thus leading to extinction.

Bax and Thresher (2009) compared eight genetic biocontrol strategies to investigate their potential to drive the extinction of an invasive population. They modelled the daughterless carp strategy with eight gene copies (DC8) and found it to be the most robust approach. The Trojan Y chromosome strategy was not included in this evaluation as the authors anticipated that it would produce an outcome similar to that expected for a daughterless strategy (DC2), in which the introduced fish contained two copies of the aromatase inhibitor gene.

Teem *et al.* (2014) used a mathematical modelling approach based on ordinary differential equations that allowed the kinetics of female decline to be assessed under identical modelling conditions. Using this approach, Teem *et al.* (2014) found that the Trojan Y chromosome strategy to result in female extinction more rapidly than the daughterless carp strategy. These authors also found that the Trojan Y chromosome strategy required the introduction of fewer autocidal fish to the target population to achieve local extinction of females, when compared with the daughterless carp strategy. The authors concluded that the relatively lower efficiency of female reduction associated with the daughterless carp strategy was a consequence of a greater capacity to produce females and a reduced capacity to produce males.

4.4.3 Biocontrol using infectious agents

Di Giallonardo and Holmes (2015) provided a superficial review of the biocontrol of invasive species. These authors noted that, to date, there have only been three successful viral biocontrols of vertebrates: the release of feline panleukopenia virus (parvovirus) to eliminate cats on Marion Island and the well-documented cases of myxoma virus (MYXV) and rabbit haemorrhagic disease virus (RHDV) in Australia and New Zealand to control the feral rabbit population. Two years after the successful use of MYXV in Australia, the virus was privately released as a biocontrol in France. The virus spread rapidly through France and England, reducing the size of the rabbit population by more than 90 percent. Thirty years later, the highly-virulent RHDV naturally spread through European rabbit populations. However, the overall impact of RHDV on rabbit numbers in Europe was smaller than in Australia and New Zealand. In the 1950s, classical swine fever virus was released on small islands in California in an attempt to reduce the wild pig population, although the virus did not establish or result in persistent transmission in those settings. It is important that in each of these examples, the target organism was mammalian. The immune system of the fish is very different to that of mammals. No infectious biocontrol agents to control pest fish species have been released to-date.

To be effective as a biocontrol, a virus should be species-specific, readily transmissible and highly-virulent. Ideally, the virus should also spread quickly and kill rapidly, but retain the ability to transmit readily to new hosts. MYXV and RHDV are both highly-virulent in European rabbits, killing within a few days. For reasons that are currently unclear, these viruses have experienced strikingly different trajectories of virulence evolution – that is, the mean virulence of field strains of MYXV declined (although high virulence strains are still sampled), while there is no evidence that RHDV has attenuated in the field.

5 Cyprinid herpesvirus 3

5.1 Taxonomy, classification and pathobiology

The taxonomy and classification of cyprinid herpesvirus 3 (CyHV-3) was reviewed by NSW DPI (2017). CyHV-3 is the third cyprinid herpesvirus to be identified and one of four viruses in the genus *Cyprinivirus* (family Alloherpesviridae within the order Herpesvirales – the fish and amphibian herpesviruses). Waltzek *et al.* (2009) described the family Alloherpesviridae as comprising 13 fish and amphibian herpesviruses. Two major clades are present within the family: the first contains cyprinid and anguillid taxa, while the second contains ictalurid, salmonid, acipenserid and ranid taxa (King *et al.*, 2012). The clade comprising cyprinid and anguillid taxa is shared with carp pox virus (CyHV-1), which is carp specific but not particularly virulent and only lethal to small juvenile fish (Sano *et al.*, 1985a; Sano *et al.*, 1985b; Sano *et al.*, 1991; Gilad *et al.*, 2003; Gilad *et al.*, 2004), goldfish hematopoietic necrosis herpesvirus (CyHV-2) which only affects goldfish (*Carassius auratus*) (Hedrick, 2000; Gilad *et al.*, 2004; Hedrick *et al.*, 2006) and anguillid (eel) herpesviruses 1 (AngHV-1). Both CyHV-1 and CyHV-2 are already present in Australia (Stephens *et al.*, 2004). Three genetically similar strains and nine distinct genotypes of CyHV-3 have been characterised to date. Whole-genome sequencing confirmed that CyHV-3 clusters into two major lineages, European and Asian, with the Indonesian isolate relating more closely to the Japanese isolate within the Asian lineage (Figure 9).

The **Indonesian** isolate **KHV C07** is the strain of CyHV-3 that is being investigated for release in Australia.

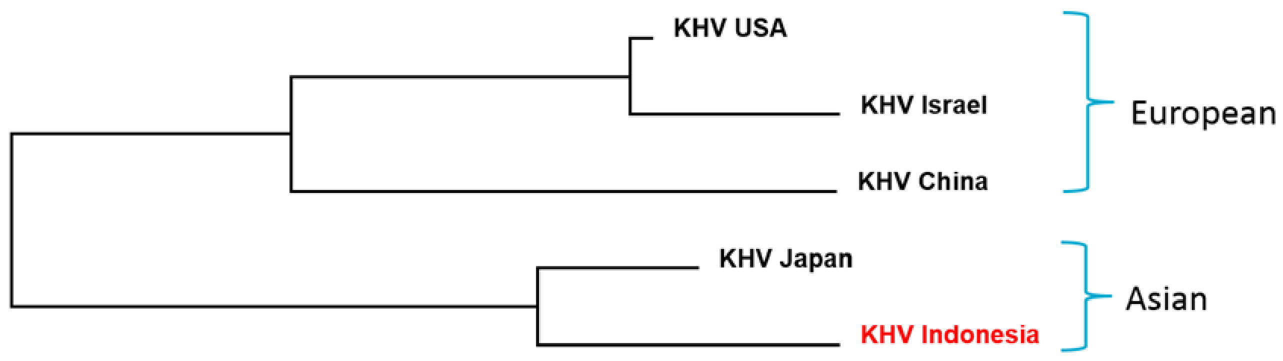

Figure 9 Phylogenetic tree of CyHV-3 inferred from the whole-genome sequences

Source: NSW DPI (2017)

Members of the order Herpesvirales share the following key biological properties (Boutier *et al.*, 2015):

- Lyse host cells upon production of progeny virions
- Establish lifelong latent infection, characterised by maintenance of the viral genome as a non-integrated episome and expression of a limited number of viral genes and microRNAs – at the time of reactivation, latency is replaced by lytic replication

- Establish persistent infection in immunocompetent hosts, as a consequence of immune evasion.

In addition to these biological properties common to the order Herpesvirales, fish alloherpesviruses exhibit four key specific biological properties (Boutier *et al.*, 2015).

1. While herpesviruses generally show only modest pathogenicity in their natural immunocompetent hosts, fish herpesviruses can cause outbreaks associated with mortality reaching 100 percent (within aquaculture environments). The markedly higher virulence of fish herpesviruses could reveal a lower adaptation level of these viruses to their hosts. However, it could also be explained by other factors such as the high-density rearing conditions and inbreeding present in many intensive aquaculture settings.
2. The tropism of members of the family Herpesviridae is generally restricted to their natural host species or closely related species. In contrast, whereas some alloherpesviruses induce severe disease in only one or few closely related members of the same genus, others are able to establish subclinical infections in a broader range of hosts.
3. An age-dependent pathogenesis has been described for several fish herpesviruses – in particular, a predilection for juvenile fish (more than 13 days).
4. A marked difference in the outcome of herpesvirus infection in poikilothermic hosts is related to their temperature dependency. In general, fish herpesvirus-induced infection is less severe or even asymptomatic if the ambient water temperature is suboptimal for virus replication.

The relationship between temperature and the outcome of infection (point 4 above) is considered one of the drivers for the seasonality of CyHV-3 outbreaks (Boutier *et al.*, 2015). Outbreaks occur naturally when the water temperature is between 18 and 28C (Boutier *et al.*, 2015). Virus propagation and virus gene transcription are turned off when cells are moved to a non-permissive temperature of 30C or higher (Dishon *et al.*, 2007). However, despite the absence of detectable replication, the virus itself remains viable within cells maintained for 30 days at 30C and may recommence replication when these cells are returned to permissive temperatures (Dishon *et al.*, 2007).

Michel *et al.* (2010) also noted that because fish are poikilotherms, CyHV-3 only causes clinical disease when the water temperature is within the range of approximately 18 to 28C. The dependence of CyHV-3 on water temperature was examined further by Uchii *et al.* (2014). These authors found that replication-related genes were transcribed only during the spring when water temperatures were permissive to CyHV-3 replication. However, genes that may convey latency were transcribed under non-permissive temperatures. Uchii *et al.* (2014) concluded that CyHV-3 may persist in carriers by establishing latent infection and then reactivating with the spring temperature increase when carp aggregate for mating.

Michel *et al.* (2010) identified literature that showed that CyHV-3 may also persist in infected fish through successive cycles of permissive water temperatures. For example, CyHV-3 DNA has been detected by PCR at 65 days post infection in clinically healthy fish (Gilad *et al.*, 2004) and has persisted in a wild population of carp for at least 2 years after the initial outbreak (Uchii *et al.*, 2009). Michel *et al.* (2010) questioned whether long-term persistence reflects classical gene-based latency – as seen in herpesviruses generally, and as described by Uchii *et al.* (2014) – or a form of low-grade chronic infection.

5.2 Global emergence of CyHV-3 in farmed and wild carp

The emergence of CyHV-3 as a pathogen of farmed and wild carp was summarised by Boutier *et al.* (2015). Citing a range of published sources, these authors noted that the geographical range of CyHV-3 has become extensive since the first outbreaks in Germany in 1997 and in the USA and Israel in 1998. Worldwide trade in common and koi carp is generally held responsible for the spread of the virus before methods of detection were available and implemented (OIE, 2012).

In Europe, CyHV-3 has been found in carp farms and fisheries in Germany, Poland, and the UK (Bergmann *et al.*, 2006; Taylor *et al.*, 2010; Gotesman *et al.*, 2013), and is also known to occur in, or has been recorded in fish imported into, Austria, Belgium, Czech Republic, Denmark, France, Hungary, Italy, Luxembourg, The Netherlands, Republic of Ireland, and Switzerland (Haenen *et al.*, 2004; Pokorova *et al.*, 2010; McCleary *et al.*, 2011; Pretto *et al.*, 2013). Outbreaks have also been reported to the OIE from Romania, Slovenia, Spain, and Sweden (OIE, 2012). Three novel CyHV-3-like viruses were also identified by PCR in The Netherlands, UK, Austria and Italy, sharing only 95 to 98 percent nucleotide identity with the CyHV-3 J, CyHV-3 I, and CyHV-3 U strains. Carp carrying the CyHV-3 variants did not show clinical signs consistent with CyHV-3 infection and originated from locations with no actual CyHV-3 outbreaks. These strains might represent low- or non-pathogenic variants of CyHV-3 (Engelsma *et al.*, 2013).

The first outbreaks in the Middle East (Israel) were seen in 1998, and in the following 3 years the virus had spread to 90 percent of all carp farms. In Southeast Asia, the first outbreaks occurred in cultured koi carp in Indonesia in 2002 and were associated with an importation of koi from Hong Kong (Haenen *et al.*, 2004; Sunarto *et al.*, 2011). Later in 2002, CyHV-3 was reported in koi carp in Taiwan. In 2003, CyHV-3 was found in Japan following mass mortalities of cage-cultured carp in the Ibaraki prefecture (Sano *et al.*, 2004). Since then, the virus has been confirmed in 90 percent of the 109 class-A natural rivers in Japan and in 45 of the 47 prefectures (Lio-Po, 2011; Minamoto *et al.*, 2012). Similarly, CyHV-3 spread rapidly in Indonesia with disease outbreaks reported on most of the major islands by 2006 (Lio-Po, 2011). CyHV-3 has also been detected in China (Dong *et al.*, 2011), South Korea (Gomez *et al.*, 2011), Singapore (Lio-Po, 2011), Malaysia (Musa *et al.*, 2005) and Thailand (Pikulkaew *et al.*, 2009; Lio-Po, 2011).

In North America, the first reports of CyHV-3 outbreaks were in 1998 and associated with koi dealers (Hedrick *et al.*, 2000; Gray *et al.*, 2002). In 2004, CyHV-3 was confirmed from mass mortalities of wild carp in South Carolina and New York states (Terhune *et al.*, 2004; Grimmett *et al.*, 2006). In Canada, CyHV-3 was first detected during disease outbreaks in wild carp in Ontario in 2007 and further outbreaks were reported in Ontario and Manitoba in 2008 (Garver *et al.*, 2010). More recently, mass mortalities of carp have been reported along the United States-Canadian border in Michigan and Wisconsin (Gotesman *et al.*, 2013).

There are no reports of CyHV-3 from South America or Australasia, and the only reports from the African continent are from South Africa (OIE, 2012).

5.3 Epidemiology of CyHV-3

5.3.1 Species specificity

Authors of primary research and reviews agree that the clinical disease associated with CyHV-3 is only seen in European and koi carp. There appears to be some divergence, however, as to whether CyHV-3 is able to infect other fish species – albeit at a low level and without clinical disease. The chief implication of the infection of other species is the supposition that these might then play an active role in the spread or persistence of the disease in the wild.

Boutier *et al.* (2015) notes that hybrids of koi and goldfish, and koi and crucian carp, can be infected by CyHV-3 with mortality rates of 35 percent and 91 percent, respectively (Bergmann *et al.*, 2010). Hybrids of carp and goldfish have also been reported to show some susceptibility to CyHV-3 infection, albeit with a mortality of just 5 percent (Hedrick *et al.*, 2006). CyHV-3 DNA has been detected by PCR in a range of other non-cyprinid fish, freshwater mussels and crustaceans (Kempter and Bergmann, 2007; Matbouli *et al.*, 2007; Kempter *et al.*, 2009, 2012; Kielpinski *et al.*, 2010; El-Matbouli and Soliman, 2011; Radosavljevic *et al.*, 2012; Fabian *et al.*, 2013). Similarly, cohabitation experiments suggest that some of these fish species (including goldfish, tench, vimba, common bream, common roach, European perch, ruffe, gudgeon, rudd, northern pike, Prussian carp, silver carp and grass carp) might carry CyHV-3 asymptomatically and transmit it to carp (Bergmann *et al.*, 2010; El-Matbouli and Soliman, 2011; Kempter *et al.*, 2012; Radosavljevic *et al.*, 2012; Fabian *et al.*, 2013). Fabian *et al.* (2013) gathered wild fish of a range of species from carp ponds during acute outbreaks of CyHV-3 mortality. They found that CyHV-3 DNA could be isolated from these wild fish, and that the disease could be induced in naive carp after a period of cohabitation with the wild fish. *In vitro* studies have shown that CyHV-3 can replicate in cell cultures derived not only from European and koi carp but also from silver carp and goldfish (Davidovich *et al.*, 2007). Finally, OIE (the World Organisation for Animal Health) lists one CyHV-3 susceptible species (*C. carpio* and its hybrids) and several suspected carrier fish species – including in this context, goldfish, grass carp, ide, catfish, Russian sturgeon and Atlantic sturgeon (OIE, 2012).

McColl *et al.* (2017) acknowledge this body of work, although use a diagnostic tool based on messenger RNA (mRNA) to evaluate the hypothesis that the DNA detected in aquatic animals other than European and koi carp (as summarised by Boutier *et al.*, 2015, and paraphrased above) is present in passive form only – that is, that these species may be exposed to CyHV-3 DNA and subsequently contaminated but that active infection does not occur. To test this hypothesis, McColl *et al.* (2017) undertook tank studies using rainbow trout (*Oncorhynchus mykiss*); 13 species of native Australian teleost fish comprising silver perch, Murray cod, golden perch, common galaxias (*Galaxias maculatus*), short-finned eel (*Anguilla australis*), salmon catfish (*Neoarius graeffei*), freshwater catfish, Australian smelt, crimson-spotted rainbowfish, sea mullet (*Mugil cephalus*), carp gudgeon, olive perchlet and bony herring; short-headed lamprey (*Mordacia mordax*); two amphibian species comprising tadpoles and mature stages of Peron's tree frog (*Litoria peronii*) and mature spotted marsh frogs (*Lymnodynastes tasmaniensis*); two reptilian species comprising the Eastern water dragon (*Intellagama lesueurii*) and Macquarie short-necked turtle (*Emydura macquarii*); a freshwater yabby (*Cherax destructor*); chickens (*Gallus gallus domesticus*); and laboratory mice (*Mus musculus*). Carp were challenged as positive controls.

The results of McColl *et al.* (2017) were complicated by the fact that CyHV-3 DNA was identified in almost 30 percent of the negative controls. This was explained by contamination, but nevertheless made it difficult to infer with confidence that exposed animals that were also DNA-positive – but mRNA negative – were only carrying the CyHV-3 DNA passively and were not infected. It seems entirely possible that some or all of these DNA-positive exposed animals were exposed through contamination, in the same way as 30 percent of the unexposed appeared to have been – that is, that these animals might otherwise have been both DNA and mRNA negative and therefore unable to demonstrate the strength of the mRNA method.

A further point of issue with the conclusions of McColl *et al.* (2017) concerns the view that an absence of mRNA shows unequivocally that transmission has not occurred. The authors' view is that fish with a very low grade or latent infection would have experienced an acute and active phase in the transmission process, prior to the development of latency. This view does not appear to fit well with the results of Uchii *et al.* (2014) who used the mRNA method to identify latently-infected carp (which were mRNA negative) and to delineate between these fish and those in which replication of the virus was active (mRNA positive). It seems biologically plausible that non-target species with a very low level of infection might present a similar profile to latently-infected carp, and may not have experienced an acute phase of active infection.

The conservative view at this point in the rapidly changing landscape of CyHV-3 research will be to accept the OIE's position that aquatic animals other than European and koi carp may act as passive carriers of the virus. This notwithstanding, it does seem unlikely that such animals contribute substantially (if at all) to the epidemiology of CyHV-3 outbreaks in wild carp. In support of this, Thresher *et al.* (2018) noted that reports of mass die-offs of carp due to CyHV-3 almost invariably cited carp as the only species affected, even in species-rich environments.

5.3.2 Susceptibility of humans to CyHV-3

In a systematic review of the literature, Roper and Ford (2018) concluded that there is no published evidence to indicate that the virus has or will develop zoonotic capability. In support of this, the authors also pointed out that the permissive temperature range for the transmission of CyHV-3 is approximately 18 to 28C (Section 5.3.5) while human body temperature is approximately 36.1 to 37.2C.

5.3.3 Clinical presentation

The 2017 OIE Manual of Diagnostic Tests for Aquatic Animals describes the clinical presentation of the disease caused by CyHV-3 as follows:

> *All age groups of fish appear to be susceptible to KHVD, although, under experimental infection, younger fish up to 1 year old are more susceptible to the disease. On closer examination of individual fish, typical clinical signs include pale discolouration or reddening of the skin, which may also have a rough (sandpaper-like) texture, focal or total loss of epidermis, over- or under-production of mucus on the skin and gills, and pale discolouration of the gills. Other gross signs include enophthalmia (sunken eyes) and haemorrhages on the skin and base of the fins, and fin erosion. Fish become lethargic, separate from the shoal and gather at the water inlet or sides of a pond and gasp at the surface of the water. Some*

fish may experience loss of equilibrium and disorientation but they may also show signs of hyperactivity.

McColl *et al.* (2017) observed the development of lesions in carp that had been challenged by submersion in a bath containing cell culture supernatant or by intra-peritoneal injection. These authors found that, in general, small (4 to 10 cm) carp responded to CyHV-3 challenge more uniformly than larger carp. Similarly, Ito *et al.* (2007) found that juvenile carp (13 days to 3 months, or 2.5 to 6 g) were most affected, with mortality rates between 69 and 100 percent; while Perelberg *et al.* (2003) found that juveniles (1 to 3 months, or 2.5 to 6 g) had a mortality rate of approximately 85 percent while mature fish (over a year, or 230 g) had a mortality rate of approximately 56 percent.

Carp larvae (up to 3 days post hatching) are not believed to be susceptible to the virus, although become so when they mature regardless of whether or not they had been exposed to the virus as larvae (Ito *et al.*, 2007). Regardless of the route of infection, smaller carp became quiet, ceased to feed and developed excess skin mucus followed by sandpaper-like focal skin lesions within 5 to 7 days post challenge. In larger carp, diffuse reddening, or a generalised increase in pigmentation, of the skin, was frequently the earliest clinical signs of disease followed by excess skin mucus, pale patches and a sandpaper-like appearance of the skin. In the gills, excess mucus was often the only consistent sign of infection, regardless of the size of the carp.

A broadly similar clinical picture was described by Boutier *et al.* (2015), who included a caution that the course of infection and the clinical signs observed are variable between individual fish, even after simultaneous and controlled experimental inoculation.

5.3.4 Infection, pathogenesis, transmission and immunity

Infection: until approximately 2009, the commonly held view was that CyHV-3 enters the host through the gills and (to a lesser extent) the intestine (Michel *et al.*, 2010; Boutier *et al.*, 2015). This view reflected the observation that gills undergo histopathological lesions early after inoculation and that viral DNA is detectable at that stage (Boutier *et al.*, 2015). However, in 2009 Costes *et al.* (2009) used bioluminescent imaging and a system for performing percutaneous infection restricted to the posterior part of the fish to show that CyHV-3 entered carp through the skin covering the fins and body. The skin of teleost fish[31] is a stratified squamous epithelium that, unlike its mammalian counterpart, is living and capable of mitotic division at all levels, even the outermost squamous layer – noting that the scales are dermal structures (Michel *et al.*, 2010). This finding was supported by independent reports that showed the expression of CyHV-3 RNA in the skin as early as 12 hours post infection (Adamek *et al.*, 2013) and detection of viral DNA in infected cells by *in situ* hybridisation in the fin epithelium as early as 2 days post infection (the earliest positive organ) (Miwa *et al.*, 2015). Further substantiation of the importance of epidermal infection was provided through the development and evaluation of a recombinant live vaccine for CyHV-3 (Boutier *et al.*, 2015). The above notwithstanding, the epidermal route is not the only

[31] The teleosts, or Teleostei, are the largest infraclass in the class Actinopterygii, the ray-finned fishes, and make up 96% of all extant species of fish

means by which carp can be infected with CyHV-3. Further bioluminescence trials (Fournier *et al.*, 2012) showed that CyHV-3 can enter through the pharyngeal periodontal mucosa, and that the pathogenesis and outcome of infection by this route is essentially the same as for epidermal infection (Boutier *et al.*, 2015).

Pathogenesis: after initial replication in the epidermis or the pharyngeal periodontal mucosa, the virus spreads rapidly. As early as 24 hours post-infection, CyHV-3 DNA was recovered from almost all internal tissues (including liver, kidney, gut, spleen and brain) (Gilad *et al.*, 2004), where viral replication occurs at later stages of infection and causes lesions. One hypothesis regarding the rapid and systemic dissemination of CyHV-3 is that the virus secondarily infects blood cells (Michel *et al.*, 2010). Virus replication in organs such as the gills, skin, and gut at the later stages of infection, may then provide a source for the excretion of virus into the environment (Michel *et al.*, 2010). After natural infection under permissive temperatures (18 to 28C), the highest mortality rates occur at 8 to 12 days post-infection (Perelberg *et al.*, 2003). Gilad *et al.* (2004) suggest that death is due to loss of the osmoregulatory functions of the gills, kidneys and gut.

Transmission: horizontal transmission of CyHV-3 can occur either by direct contact between fish or by indirect transmission (Boutier *et al.*, 2015). Direct contact can occur with skin-to-skin contact between an actively infected or carrier fish and a naive one, or from the cannibalistic and necrophagous behaviours of carp (Raj *et al.*, 2011; Fournier *et al.*, 2012). Transmission appears to be accelerated in places where carp gather, as at spawning or mating (Boutier *et al.*, 2015). Indirect transmission may occur through contact with, or ingestion of infectious material in, fish droppings (Dishon *et al.*, 2005), plankton (Minamoto *et al.*, 2011), sediments (Honjo *et al.*, 2012) or aquatic invertebrates feeding by water filtration (Kielpinski *et al.*, 2010). Free water in contact with skin, pharyngeal mucosa or filtered through gills may also provide a means of infection (Minamoto *et al.*, 2009). Virus replication in organs such as the gills, skin and gut may result in virus excretion into the water (Boutier *et al*, 2015). Citing their own primary research, Boutier *et al.* (2015) noted that although the spread of CyHV-3 through physical contact was more efficient than through free water, the latter was nevertheless viable. To-date, there is no evidence for the vertical transmission of CyHV-3 from parent to offspring.

Immunity: environmental temperature has an important effect on the immune system of fish (Michel *et al.*, 2010). In carp, for example, at <14C, adaptive immunity is inhibited, but the innate immune response remains functional (Bly and Clem, 1992). This means that carp kept below 14C are less immunocompetent than carp kept above 14C, regardless of the nature of the challenge. Host temperature also has an effect on the replication of CyHV-3, which can occur only within the range of 18 to 28C. In carp that are infected and maintained at 24C, antibody titres begin to rise at about 10 days and plateau at 20 to 40 days post infection (Perelberg *et al.*, 2008). In the absence of antigenic re-exposure, the specific antibodies gradually decrease over approximately 6 months to a level slightly above, or comparable to, that of unexposed fish. Although protection against CyHV-3 is proportional to the titre of specific antibodies during primary infection, immunised fish, even those in which antibodies are no longer detectable, are resistant to a lethal challenge – possibly because of the subsequent rapid response of B and T memory cells to antigen re-stimulation (Perelberg *et al.*, 2008).

5.3.5 Observed characteristics of CyHV-3 outbreaks

The mortality from CyHV-3 can approach 90 to 100 percent in caged (that is, farmed) carp (Haenen *et al.*, 2004). In Japan, mortality in caged carp was estimated at 'over 60 percent in the most severe cases' (Sano *et al.* 2004). Sunarto *et al.* (2005) reported the mortality in Indonesian cage cultures and aquaculture ponds to be 80 to 95 percent. More recently, Indonesian aquaculturists observed an 80 percent mortality across all age classes in the first year of an outbreak in pond cultures, 50 percent in the second year and 30 percent in the third year (Thresher *et al.*, 2018).

Thresher *et al.* (2018) examined the characteristics of outbreaks of CyHV-3 in wild carp and questioned whether the disease was likely to be as devastating in that setting. These authors reviewed 17 reported North American outbreaks in wild carp that had occurred since 2004. The outbreaks spanned 10 states within the United States and two Canadian provinces. Four of the identified outbreaks involved multiple die-offs in interconnected waterbodies, the most extensive being that in Ontario, Canada, which spanned 24 lakes. Outbreaks appeared to span approximately 20 to 40 days. This period was not driven by the window of permissive temperature for CyHV-3 in North American waters, which remains within range for considerably longer than is likely to be the case within Australia. Sano *et al.* (2004) reported a similar duration (early October to mid-November) in Lake Kasumigaura, Japan. Outbreaks in North America only occurred in spring and summer (May through August) and appeared to be triggered by spring spawning activity which created opportunities through the close proximity of fish.

Thresher *et al.* (2018) found that in all but one location, CyHV-3 did not have a substantial or persistent impact on the populations of carp in the longer term. This was despite significant initial kills. The single exception was an artificial impoundment in northwest Missouri, close to a major population centre and extensively used for recreational purposes. In this setting, carp aggregated in very large numbers close to a dock, where they were fed. Carp were also observed to be spawning immediately prior to the outbreak. The impact of the die-off on the carp population in this lake was substantial and persistent, with electrofishing catch rates falling from over 200 carp per hour prior to the event to 78 per hour a week after the die-off, 69 per hour a year following (2013) and 35 per hour in 2017. About 25 percent of the carp caught in 2013 and 2017 had lesions similar to those observed in 2012. However, although small numbers of dead carp have been seen in this lake every year since 2012, there have been no subsequent mass die-offs. This single exception to the outbreaks studied by Thresher *et al.* (2018) may have resulted from the artificial aggregation caused by feeding at the dock and the fact that this population of carp is closed to emigration from outside populations.

The results of Thresher *et al*. (2018) were addressed by Durr *et al*. (2019a) whose modelling showed that, despite the virus continuing to suppress the population, noticeable fish die-offs are only likely in an Australian context during the first epidemic. Subsequent seasonal epidemics will occur in all age classes, although are at least one order of magnitude smaller in the adult population. Durr *et al*. (2019a) maintained that the observations of Thresher *et al*. (2018) were for this reason consistent with the modelling undertaken in an Australian context and did not suggest that CyHV-3 would not be an effective biocontrol for carp in Australian waters.

In support of this, Matsui *et al.* (2008) suggested as much as 70 percent of wild carp (more than 100,000 fish) in Lake Biwa, Japan, died during an outbreak of CyHV-3. The virus was introduced into Japanese carp farms in 2003 and then spilled over into Japanese wild European carp populations (Haramoto *et al.*, 2007). Although no second mass mortality has been reported in Lake Biwa, CyHV-3 appears to have persisted in the host population (Uchii *et al.*, 2009, 2011).

5.3.6 Latency and recrudescence

It was pointed out in Section 5.1 that one of the defining characteristics of members of the order Herpesvirales is the ability to establish a life-long latent state in their host. This is characterised by maintenance of the viral genome as a non-integrated episome, and expression of a limited number of viral genes and microRNAs. At the time of reactivation, latency is replaced by lytic replication. Uchii *et al.* (2014, as discussed in Section 5.3.1) examined the dependence of CyHV-3 on water temperature and found that replication-related genes were transcribed only during the spring when water temperatures were permissive to the replication of CyHV-3. However, genes that may convey latency were transcribed under non-permissive temperatures. Uchii *et al.* (2014) concluded that CyHV-3 may persist in carriers by establishing a latent infection, which then reactivates with the spring temperature increase at a time when carp aggregate for mating. Michel *et al.* (2010) questioned whether long-term persistence reflects classical gene-based latency – as seen in herpesviruses generally, and as described by Uchii *et al.* (2014) – or a form of low-grade chronic infection. The balance of opinion at this point would be in favour of classical latency consistent with other members of the order Herpesvirales.

Boutier *et al.* (2015) explored research to identify the tissue(s) in which CyHV-3 persisted in a latent state. The work of Uchii *et al.* (2014) had indicated that the brain was likely, although when Boutier *et al.* (2015) considered this in the light of other work they appeared to conclude that CyHV-3 was most likely to persist within white blood cells – specifically IGM+ B lymphocytes.

5.3.7 Persistence in the environment

Shimzu *et al.* (2006) showed that CyHV-3 survives for approximately 3 days in lake or river water held within the permissible range (18 to 28C). Survival can be extended significantly with autoclaving, suggesting that bacteria may be responsible for degradation of the virus. Supporting this hypothesis, a recent report showed that bacteria isolated from carp habitat waters and carp intestinal contents possessed some anti-CyHV-3 activity (Yoshida *et al.*, 2013). Studies of carp spawning areas in Lake Biwa reported the detection of CyHV-3 DNA in plankton samples and in particular the *Rotifera* species (Minamoto *et al.*, 2011), and it is likely that other invertebrate or vertebrate species could play a significant role in CyHV-3 persistence in aquatic environments (Boutier *et al.*, 2015).

5.3.8 Vaccination

Attenuated live vaccines are the most appropriate for mass vaccination of carp (Michel *et al.*, 2010). Attenuated vaccine candidates have been produced by successive passages in cell culture (Ronen *et al.*, 2003). The vaccine strain candidate was further attenuated by UV irradiation to increase the mutation rate of the viral genome (Perelberg *et al.*, 2008). A vaccine strain obtained by this process has been produced by KoVax Ltd. (Jerusalem, Israel) and has been shown to confer protection against a challenge (Michel *et al.*, 2010). However, this vaccine is only available in Israel and has two key constraints: (a) the molecular basis for the reduced virulence is unknown and, consequently, reversion to a pathogenic phenotype cannot be excluded; and (b) under certain conditions, the attenuated strain could retain residual virulence that could be lethal for a portion of the vaccinated fish (Perelberg *et al.*, 2008). An inactivated vaccine candidate was described by Yasumoto *et al.* (2006). This consists of formalin-inactivated CyHV-3 trapped within a liposomal compartment. This vaccine can be used for oral immunisation in fish food. Protection efficacy for carp is approximately 70 percent (Yasumoto *et al.*, 2006).

McColl *et al.* (2016) suggest that vaccination of farmed carp and koi carp may result in the genesis of a more pathogenic field strain, and the consequent need for a more effective vaccine – in an ongoing cycle. It is true that some viruses have behaved and evolved in this way, and it is possible that the same may turn out to be true for CyHV-3. That said, there are many other considerations to the evolution of viruses within complex populations and it would seem (at this point in time) prudent to withhold judgement on this matter.

5.3.9 Modelling the epidemiology of CyHV-3

A joint project undertaken by CSIRO and RMIT University for the NCCP was concluded and reported against immediately prior to the submission of this ecological risk assessment. An excerpt from the draft report for the epidemiology component of this work (Durr *et al.*, 2019a) is given in the text below. The modelling supported a number of the key assumptions that underpinned the ecological risk assessment, including the importance of contact transmission (both prior to and during spawning), the likelihood of secondary outbreaks amongst juvenile carp and the role of seasonally-trigged latency and recrudescence.

Abstract: *common carp (*Cyprinus carpio*) now dominate much of the Murray-Darling Basin in South-eastern Australia and are implicated in the degradation of Australia's waterways. The need to reduce carp abundance has led the Australian government to fund research into* Cyprinid herpesvirus 3 *(CyHV-3) as a possible biocontrol agent, with the intention to release within the next 5 years. We developed a series of integrated habitat quality, demographic and epidemic models and applied these to five representative catchments to predict the impact of releasing CyHV-3 on carp abundance. The dynamics of transmission and infection were captured by a set of differential equations embedded in a discrete demographic metapopulation model wherein ageing, movement, natural mortality and recruitment of carp occurred on a weekly time-scale. To study the potential of the virus under realistic population dynamics the model was run using historical time series of hydrological conditions recorded from the mid-1990s to the end of 2016 as available for each of the five catchments. These time series include the dramatic fluctuations in flows and conditions associated with the Millennial Drought and its ending that affected much of Australia. Whether CyHV-3 caused a meaningful reduction in carp abundance critically depended on whether seasonal reactivation of the virus, and onward infection, occurs in chronically infected carp. In the absence of reactivation, the virus was predicted to cause a single mortality event from which carp populations rapidly recovered from. When seasonal reactivation was allowed for the model predicted similar initial mortalities but also sustained reductions in carp abundance driven by seasonal outbreaks mostly amongst immature carp. The subsequent outbreaks were predicted to be at least an order of magnitude smaller than the initial outbreak following release. With seasonal reactivation and favourable contact rates the model results were insensitive to the frequency that reactivation occurs and the year of release, with suppression of carp abundance*

consistently being 50-70% of that expected if there was no virus. The results were sensitive to the survival rate of carp following first infection with CyHV-3 underlining the importance that Australian carp are naïve to the virus.

6 Impacts of fish kills on water quality

6.1 Condition of Australian freshwater waterways

The condition of Australian freshwater waterways was reviewed by Piddocke (2018), and much of this section was taken directly from that work.

Globally, aquatic ecosystems support natural processes essential for human health, such as nutrient cycling and toxin absorption and breakdown, and provide food, drinking water, recreation and cultural meaning. Despite this importance, both inland and coastal freshwater habitats worldwide have undergone greater human-induced change than any other ecosystem type, and usually in ways that impair ecosystem function and delivery of ecological goods and services (Meybeck, 2003; Vorosmarty *et al.*, 2010).

Aside from direct, extractive use of water and biota, there are several reasons for the rapid changes many freshwater systems have experienced. Freshwater systems, whether rivers, creeks, lakes, or wetlands, tend to occur at the lowest points in the landscape and consequently act as a focus or final receptacle for potentially damaging processes and products (Jackson *et al.*, 2016). The generally linear nature and unidirectional flow of many freshwater ecosystems also renders them vulnerable to human impacts (Koehn and Lintermans, 2012). Consequently, the ecological condition of freshwater ecosystems globally is now influenced primarily by societal, economic, and technological forces (including urbanisation, industrialisation, population growth, water engineering and environmental regulation), rather than variables such as vegetation, landform, climate and topography (Meybeck, 2003).

These global trends have been mirrored in Australia. During the (approximately) 230 years since European colonisation, many Australian freshwater ecosystems have experienced major changes to the processes that regulate their function – and to the abundance and diversity of species inhabiting them. These changes have been greatest in areas where human use is most intense. The Murray-Darling Basin has been particularly affected, with native fish abundance estimated to be at approximately 10% of pre-European levels (Balcombe *et al.*, 2011), although many coastal systems have also been altered substantially. In general, coastal rivers in catchments with relatively low levels of human use have changed least although some remote river valleys in the north-western Murray-Darling Basin have also been relatively less affected (Davies *et al.*, 2010).

Changes to Australian freshwater ecosystems reflect the cumulative, interlinked impacts of many stressors. Nineteenth and early 20th century land and water management included many practices now known to have negative effects on biodiversity and ecological processes, including:

- Clearing riparian (riverside) vegetation, which resulted in silt and pollutants flowing into rivers
- Removing coarse woody debris (snags) from rivers to improve navigation, resulting in the removal of important habitat for many native fish species and having an impact on flood pulses
- Stocking sheep and cattle at unsustainably high densities, leading to the depletion of native grasses that bind and stabilise topsoil and the consequent transport of soil and nutrients into waterways

- Uncontrolled use of wetlands by livestock
- Introduction, whether deliberate or unintentional, of invasive plant and animal species, including non-native fish – some of which have become established as pests and may have changed the ecological character of freshwater systems
- Channelisation and construction of in-stream barriers (including dams and weirs) and the consequent disruption of fish movement and migration and degradation of native fish habitat
- Intensive harvesting of fish and other aquatic biota.

6.2 Blackwater events

6.2.1 Causation of blackwater events

The causation and ramifications of blackwater events in Australian waterways were reviewed by Whitworth *et al.* (2012). Blackwater events are characterised by high concentrations of DOC sufficient to produce a dark colour similar to black tea in the water column of waterbodies that do not usually carry elevated concentrations of dissolved organic matter. Where the blackwater is also associated with reduced concentrations of DO[32] in the water column, either within the main river channel or on the floodplain, this is described as an hypoxic blackwater event. Whitworth *et al.* (2012) qualify this by noting that there are rivers in other parts of the world that are dark or black in colour and have a persistently high DOC, but that the ecology of these 'blackwater rivers' is such that DO is not compromised. Examples of this include the Siak River in Indonesia, the Ogeechee River in Georgia (USA) and some of the rivers within the Amazon Basin in South America.

Blackwater events are generally associated with low gradient river systems with forested floodplains or extensive wetlands (Whitworth *et al.*, 2012). Between flood events, plant detritus accumulates on floodplains and in dry channels. Upon inundation, organic carbon compounds are leached from this material – thus adding DOC to the water column. In-and-of-itself, DOC is an important substrate for riverine food webs and flooding greatly enhances both river and floodplain productivity. However, when conditions favour rapid microbial metabolism of the solubilised carbon, and re-aeration rates are relatively low, hypoxia may occur. In some situations, the chemicals that constitute DOC may also be harmful to aquatic life (Section 6.2.3).

Whitworth *et al.* (2012) based their analysis on a particularly significant and long-lasting blackwater event that occurred in the mid-Murray River in 2010-11. Floodplains within this part of the Murray River are dependent on overbank flows, usually derived from winter and spring rainfall and from snow melt in the upper catchment. The majority of these floodplains had been subjected to severe moisture stress during the preceding 10 years, due to a combination of drought and upstream water use. In late 2010, near-record spring and summer rainfall resulted in widespread

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[32] Oxygen saturation (SO2) is a related measure that appears in some of the literature. This the ratio of observed DO to the maximum amount of oxygen that will dissolve in the water at that temperature and pressure under stable equilibrium. Well-aerated water (such as a fast-moving stream) without oxygen producers or consumers will generally be 100% saturated. As the temperature of water increases, the volume of dissolved gas it will hold at equilibrium decreases. Thus, warmed water can become supersaturated. This effect can be exacerbated by phytoplankton. Supersaturated water can be toxic to fish and can result in 'gas bubble' disease.

flood events, with inundation of the floodplains and the mobilisation of organic materials. Hypoxic blackwater was first observed downstream of forested Koondrook-Perricoota Forest floodplain in September 2010. Oxygen concentrations as low as 2 mg/L were observed as far downstream as the South Australian border, and values in the 2 to 4 mg/L range persisted down to the entrance to the Lower Lakes at the end of the freshwater region of the Murray-Darling Basin. Hypoxia was also observed within the lowland reaches of all major tributaries of the southern Murray-Darling Basin. By the end of April 2011, DO exceeded 6 mg/L at virtually all monitoring sites within the mid-Murray River and the hypoxic blackwater event was considered to have resolved. At the peak of this event, the plume of hypoxic blackwater extended for approximately 1,800 km along the Murray River channel.

6.2.2 Impacts of low dissolved oxygen on aquatic biota

De-oxygenation caused by heterotrophic metabolism (microbial degradation) of natural organic matter leached from flooded plant material is the primary cause of hypoxia or anoxia in blackwater events. Additional factors that influence the extent and duration of oxygen drawdown during blackwater events include temperature, water quality (for example, salinity and toxic or inhibitory compounds), water column dilution or exchange, rate of aeration through the air-water interface, extent of wind-driven mixing, and the strength and persistence of thermal stratification (Wallace and Lenon, 2010).

Citing a range of primary and review literature, the MDBA (2014a) noted that hypoxia results in fish kills as well as the disruption of endocrine systems and embryonic development, decreased hatch rates and survival of fish, and the degradation of aquatic macroinvertebrate communities in streams and wetlands. Anoxia may lead to the release of sediment-bound materials such as manganese, iron, ammonium and phosphorus; the conversion of dissolved organic nitrogen to ammonia and nitrate; and accumulation of redox-sensitive compounds from anoxic sediments – some of which (for example, ammonium and sulphide) are toxic to many aquatic organisms.

The Basin Plan provides a target of greater than or equal to 50 percent saturation. At 20C, in freshwater, this equates to a DO of approximately 4.5 mg/L (MDBA, 2014a). The ANZECC Water Quality Guidelines[33] provide guideline 'trigger values' for monitoring the quality if water in local and regional aquatic ecosystems. The ANZECC guideline value for DO in lowland rivers in southeast Australia is 85 to 110 percent, with the qualifier that DO "*should not normally be permitted to fall below 6 mg/L or 80−90 percent saturation, determined over at least one diurnal cycle*". A level of 85 percent saturation is equivalent to approximately 9.1 mg/L and 7.6 mg/L for freshwater at 20C and 30C, respectively (MDBA, 2014a). The ANZECC guideline value is likely unrealistically high for recently-flooded lowland floodplains and wetlands where the inundation of organic material is anticipated as a normal and desirable biogeochemical process (MDBA, 2014a). Research evaluating the DO needs of Australian native fish is evaluated below. As a general rule, MDBA (2014a) note that native fish and other large aquatic organisms require a DO level of at least 2 mg/L to survive, although may begin to suffer at levels below 4 to 5 mg/L. Few species can tolerate conditions of less than 3 mg/L for prolonged periods. Larvae and young-of-year juveniles may

[33] Australian and New Zealand Environment and Conservation Council (October, 2000)

survive at DO as low as 20 percent saturation (for example, 1.8 mg/L at 20C), but growth is likely to be restricted. With these broad parameters in mind, the Basin Plan targets of ≥50 percent saturation and DO of 4.5 mg/L (at approximately 20C) are widely regarded as the critical values for river channels and anabranch creeks. A DO of 6 mg/L may then provide a useful trigger value for enhanced monitoring and instigation of management plans.

Small *et al.* (2014) examined the sensitivity of juvenile predatory native fish species to low DO. These authors found that juvenile Murray cod experienced 50 percent mortality (LC50) when DO in freshwater was held at 1.58 mg/L for 48 hours at 25 to 26C. Under the same conditions, the LC50 for juvenile silver perch was 1.04, for golden perch was 0.85 and for freshwater catfish was 0.25 mg/L. When the experiments were repeated using simulated blackwater (including leachate from river red gum leaves – see below), the tolerances were reduced to 2.11 mg/L for Murray cod, 1.75 mg/L for silver perch, 1.70 mg/L for golden perch and 1.11 mg/L for freshwater catfish. This analysis was extended by Gilmore *et al.* (2018), who measured the tolerance of juvenile silver and golden perch to low DO before and after 10 months of exposure to either normoxic (6 to 8 mg/L) or hypoxic (3 to 4 mg/L) conditions. These authors found that golden perch had a higher tolerance to hypoxia than silver perch, and that this species also adapted more effectively to persistently hypoxic conditions. These experiments suggested that golden perch could acclimate to water with a DO of approximately 3 mg/L.

McNeil and Closs (2007) examined the behavioural responses of a range of native and introduced freshwater fish to hypoxia. These authors found that Australian smelt, redfin perch and flathead galaxias were the first three species to rise and engage in ASR (Figure 10), with 10 percent of individuals using ASR by 2.55, 2.29 and 2.21 mg/L DO, respectively. Goldfish and common carp were the last two species to rise to ASR, with 10 percent of individuals using ASR by 0.84 and 0.75 mg/L DO, respectively. In contrast to other species, oriental weatherloach largely ceased gill ventilation and used air-gulping as their primary means of respiration during severe hypoxia and anoxia.

Figure 10 Carp engaging in aquatic surface respiration (ASR) within the Murrumbidgee River

Source: P. Caley (January 2019)

King *et al.* (2012) undertook an analysis of the short-term effects of the prolonged 2010-11 hypoxic blackwater event within the Murray River. These authors noted that although substantial fish kills were not reported, the event was nevertheless likely to have had significant and complex effects on the fish community. In support of this, they found a reduced abundance of large-bodied fish, reduced recruitment and abundance of small-bodied fish and an increase in the abundance of alien species – in particular, young-of-year carp – in blackwater-affected reaches, when compared with unaffected reaches. These differences resulted in a substantial immediate change in the composition of the fish community and may also have resulted in longer-lasting effects.

King *et al.* (2012) also discussed the impact of the 2010-11 hypoxic blackwater event on crayfish and other crustaceans. Emerged Murray crayfish were found in higher numbers at sites with low DO concentrations, which were closer to the source of the blackwater. Emerged crayfish ranged from very small to large individuals and were frequently observed in groups under exposed tree roots or crevices in the riverbank. The crayfish were very lethargic, always close to the water's edge and returned to the water when disturbed but re-emerged within minutes. Large numbers (estimated to be hundreds to thousands) of both live and dead shrimp (mainly *Macrobrachium* spp.) and smaller numbers of yabbies were also observed at the water's edge at two of the sampled blackwater-affected sites. McCarthy *et al.* (2014) investigated the population-level impact of the 2010-11 blackwater event on the Murray crayfish. These authors surveyed 10 sites that had been affected by backwater within a 1,100 km reach of the mid Murray River, and a further six sites that were unaffected. They found that the abundance of Murray crayfish at affected sites was 81 percent lower in 2012 than it had been prior to the flood event, while the population at unaffected sites remained stable. The hypoxic blackwater event had affected crayfish of both sexes and all size-classes, and these effects were considered likely to be long-lived given the very slow growth rate of Murray crayfish. Emergence occurred at approximately 2 mg/L with juveniles having a LC50 of 2.2 mg/L (McCarthy *et al.*, 2014). Strategic restocking of some reaches of the Murray River with is being undertaken by NSW Department of Primary Industries, with encouraging interim results.[34]

Hypoxic blackwater events may also have an impact on zooplankton and macroinvertebrates.

Zooplankton occupy a central position in river-floodplain food webs, as consumers of phytoplankton, fungi and bacteria and as potential prey items for fish, waterbirds, amphibians and macroinvertebrates. Ning *et al.* (2015) showed that hypoxic conditions (less than about 2 mg/L) significantly reduced the taxon richness and abundance of zooplankton emerging from the sediments of two wetlands within the Murray River. The concentration of DOC, however, had no consistent effect. These authors found that effects of hypoxia on zooplankton were partially reversed when oxygen concentrations were returned to normal values within 3 weeks. Overall, the findings suggested that hypoxic blackwater events could reduce the availability of food resources to planktivorous biota, and subsequently through the aquatic food web.

Connolly *et al.* (2004) investigated the impact of hypoxia on macroinvertebrates. These authors found that macroinvertebrate assemblages from two Australian tropical streams (one upland and the other lowland) tolerated all but very low DO (<0.87 mg/L), although a reduction in emergence

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[34] See: https://www.dpi.nsw.gov.au/about-us/media-centre/releases/2018/murray-crayfish-population-clawing-back

of insect taxa at intermediate levels (2.02 mg/L) was also observed. Mayflies showed the highest sensitivity to low oxygen conditions, and lethal effects were observed at DO levels of 1.60 mg/L for several upland and lowland species. For other taxa, including several *Chironomidae*, mortality was observed when oxygen concentrations were below 0.74 mg/L. A drift response (downstream movement in stream currents) was observed only when oxygen concentrations reached near lethal levels (0.78 mg/L). The lack of a drift response at DO concentrations above this indicated that, in moderately poor oxygen conditions, macroinvertebrates will remain at a location and, hence, will experience sub-lethal effects such as suppressed emergence that may nevertheless impact on their population.

As a final note on the topic of hypoxia, it is important to recognise that healthy Australian freshwater systems are frequently subject to marked diurnal variation in DO. High temperatures, nutrient enrichment and light availability, especially in the absence of riparian shade, facilitate abundant growth of aquatic plants (including macrophytes) and phytoplankton (including algae and cyanobacteria). During daylight, aquatic plants and phytoplankton can release through the process of photosynthesis around six times the oxygen they consume (Flint, 2005). However, during the night, or on cloudy days, requirements for respiration outweigh oxygen production and aquatic plants and phytoplankton become net consumers of oxygen (Flint *et al.*, 2015). This results in a generally high DO in the late afternoon that drops during the night to a minimum near dawn (Brady *et al.*, 2009; Bunch *et al.*, 2010). In some circumstances, the overnight minimum DO that is reached in waterways with a marked diurnal variation (including many tropical freshwater waterways) is lower than the threshold that would characterise an hypoxic blackwater event. For example, in Lagoon Creek in the Herbert catchment of the wet tropics of northern Queensland, DO can fluctuate between approximately 0.2 mg/L and 6.5 mg/L daily over several weeks (Pearson *et al.*, 2003; as cited by Flint *et al.*, 2015).

Likewise Walsh *et al.* (2018) in a study undertaken for the NCCP to examine the impacts of decaying carp on water quality found that although the daytime maximum DO concentration within their *control* mesocosm (that is, without any carp carcasses) generally lay in the range of 20 to 30 mg/L, the overnight minimum was frequently below 2 mg/L – and on three occasions was very close to complete anoxia (minimum 0.308 mg/L). The magnitude of this diurnal variance was attributed to a luxuriant growth of phytoplankton that had established within the control and treatment mesocosms prior to commencement of the experiment. A similarly striking diurnal range was observed from the wetland experiment undertaken by the same authors. Here, although the DO concentration at the test site closest to the deposited carp carcasses remained low for several days after placement of the carcasses, other test sites showed a marked fluctuation between high daytime and very low overnight DO concentration. This experiment did not include a control. The authors explained that the wetland was due for a managed drawdown, with the intent to completely drain and dry it out. Although the reasons for this planned course of action were not provided, it seemed possible that the wetland was in poor environmental health at the commencement of the experiment and that this contributed to the very marked diurnal fluctuation in DO. The work of Walsh *et al.* (2018) is discussed further in Section 6.3.

6.2.3 Other harmful effects of blackwater events

Whitworth *et al.* (2012) provided a systematic analysis of the processes underpinning the 2010-11 blackwater hypoxic event within the mid-Murray River. Leaf litter from river red gum forest had accumulated on the floodplain during the preceding years of drought and was the key source of DOC following mobilisation in the 2010-11 flood event. Although the half-life of leaf litter is thought to be approximately 6 to 12 months under normal seasonal conditions, the prolonged dry period may have created an environment where decomposition on the forest floor was slowed. This supposition was supported by an apparent variance in the reactivity of the DOC entering the river system with progressive flood events. At the start of the blackwater event, approximately 60 percent of DOC was available to microorganisms – almost twice the amount that would usually be metabolised – and it seemed plausible that this was largely the top layer of undecomposed leaf litter. Later in the event, the proportion of DOC available to microorganisms dropped to approximately 30 percent as lower layers of partly-decomposed litter were removed. In addition to leaf litter from riparian river red gum forest, DOC was supplied in water runoff from agricultural land on and adjacent to the floodplains and higher in the catchment. This runoff contained significant amounts of livestock manure, plant debris and topsoil. The blackwater event occurred during spring and summer, when water temperatures were high. This was particularly the case for the slower-moving and shallower waters of the floodplain. Higher water temperature is associated with a reduced solubility of oxygen, an increased rate of microbial growth and a higher biological oxygen demand (BOD). River regulation may also have played a role in the major 2010-11 blackwater event, as key storage dams had filled to capacity with the early rains and thereafter lost the ability to mitigate the downstream flow of repeated inundations.

The release of hypoxic hypolimnetic water (water from the basal layers of water storage reservoirs) may have exacerbated the event, although re-oxygenation of this water generally occurs over a relatively short span of the river channel (approximately 25 km). An example of this is shown in the data from downstream of Redbank weir on the Murrumbidgee River (Figure 11, data accessed from Water New South Wales (WaterNSW) on 18-01-2019). A discharge from the weir took place on the 13th January. Because water is released from the base of the weir, it is markedly hypoxic, and the DO in water immediately downstream was reduced to close to zero for a period of 5 days. It is not known how far downstream this plume of hypoxic water persisted, but the effect is nevertheless striking.

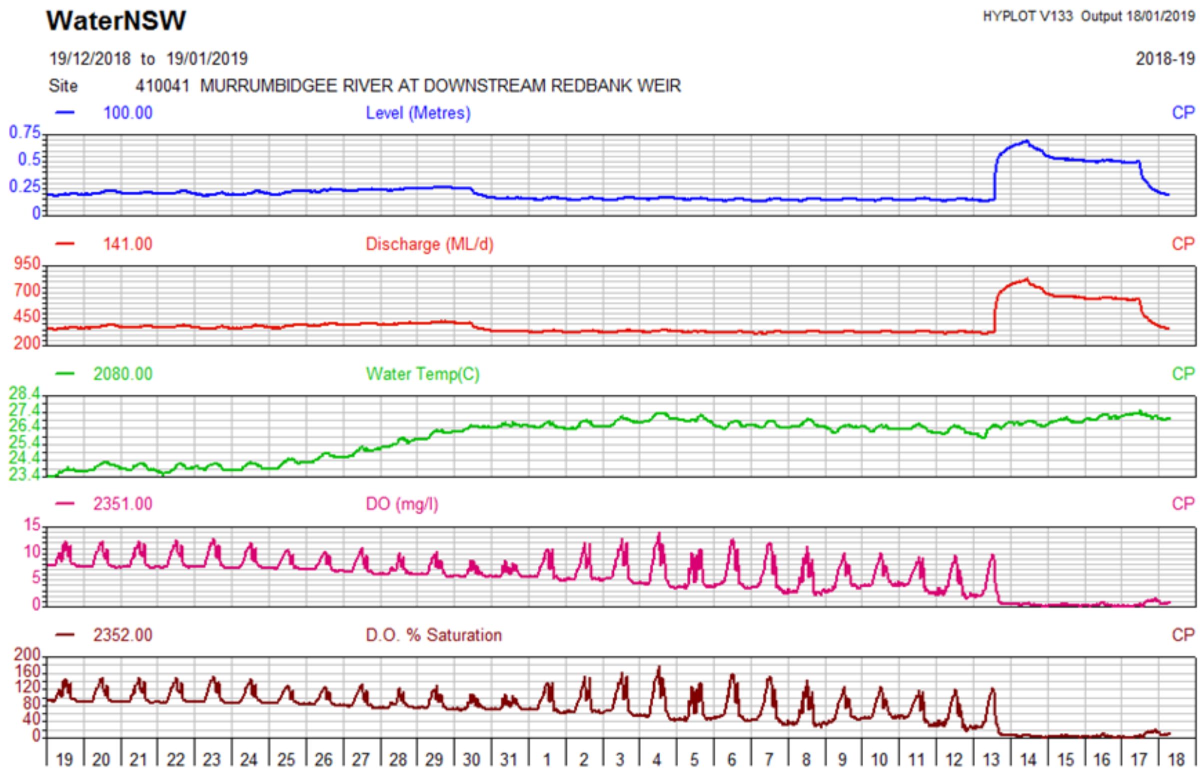

Figure 11 Water data for the Murrumbidgee River (downstream from Redbank weir)

Source: WaterNSW (https: //realtimedata.waternsw.com.au/)

DOC plays an important role in the development of hypoxia and anoxia, although may also be directly toxic to aquatic fauna. This is particularly the case of leachate from river red gum leaves, which contain toxic polyphenols (McMaster and Bond, 2008). Pathological changes in the gills were observed by Temmink *et al.* (1989) when common carp were exposed to bark extracts containing high polyphenol concentrations. Unoxidised tannins were shown to cause hypertrophy and hyperplasmia of the gill epithelium, fusion of the secondary lamellae and detachment of the respiratory epithelium from the underlying pillar cells. In a series of experiments, Gehrke *et al.* (1993) found that leachate from river red gum leaves – in combination with low DO – led to altered behaviour, gill structure and survival of golden perch larvae and juveniles. McMaster and Bond (2008) identified an interaction between the pathogenic effect of leachate from river red gum leaves and low oxygen concentration. Under high DOC concentrations (50, 70 and 80 mg/L), fish exposed to low-oxygenated tanks exhibited substantially reduced resistance to DOC when compared with fish in aquaria with continual aeration. These authors also observed an acute toxic effect when DOC from leached river red gum leaves exceeded about 100 mg/L and found that this effect overrode the effects of low DO.

Blooms of cyanobacteria also commonly occur in conjunction with blackwater events. Cyanobacteria such as *Anabaena*, *Aphanizomenon*, *Dolichospermum* and *Microcystis* are highly-productive in warm, turbid waters and may form blooms in conditions of high DOC (Walsh *et al.*, 2018). Different types of cyanobacteria favour different kinds of water conditions. Some prefer flowing water, while others prefer still water. Buoyant species such as *Anabaena* do well when the water column settles into layers (stratifies) with warmer water on top and cooler water below (MDBA, 2014c). Stratification can also result from saltier, denser water at the bottom remaining unmixed with the fresher surface water above (Wallace and Lenon, 2010). When water velocity is low (0.04 to 0.06 m/s) and solar radiation is high, wind speed is the dominant factor limiting the development and persistence of thermal stratification (MDBA, 2014c). Stratification that persists for 2 days is sufficient for cells of a buoyant species such as *Anabaena circinalis* to accumulate in the surface layer. The magnitude of the problem increases markedly if the water column remains stratified for more than 7 days (MDBA, 2014c).

Blooms of cyanobacteria may be associated with the release of cyanotoxins that are hazardous to fish and other aquatic life (Chorus and Bartram, 1999). The toxins produced by cyanobacteria include both hepatotoxins (liver damaging) and neurotoxins (nerve damaging) (MDBA, 2014c). Cyanobacterial poisoning can occur by two routes: through consumption of cyanobacterial cells from the water; or indirectly through consumption of other animals that have themselves fed on cyanobacteria and accumulated cyanotoxins. Cyanotoxins may also bioaccumulate in aquatic vertebrates and invertebrates, including fish, mussels and zooplankton. Consequently, there is considerable potential for toxic effects to be magnified in aquatic food chains. Cyanobacteria may also affect the taste and smell of water (MDBA, 2014c). The National Health and Medical Research Council *Guidelines for Managing Risks in Recreational Water* (NHMRC, 2008) state that freshwater that is used for recreational purposes should not contain:

- More than 10 µg/L total microcystins; or ≥50,000 cells/ mL toxic *Microcystis aeroginosa*; or biovolume equivalent of ≥4mm^3/L for the combined total of all cyanobacteria where a known toxin producer is dominant in the total biovolume, or
- More than 10mm^3/L for total biovolume of all cyanobacterial material where known toxins are not present, or
- Cyanobacterial scum.

A situation assessment and alert levels framework for the management of algae/cyanobacteria in recreational waters has been developed that allows for a staged response to the presence and development of blooms (NHMRC, 2008).

The taste and odour compounds produced by cyanobacteria are Geosmin (trans-1, 10-dimethyl-trans-9-decalol) and MIB (2-methylisoborneol). These compounds are difficult to remove with conventional water treatment and require expensive activated carbon for adequate removal. When conditions are no longer optimal for growth, cyanobacterial blooms subside and decay. This can occur in response to a sudden change in temperature, or as a result of the depletion of substrate. The aerobic decomposition of algal blooms can exacerbate an already-high BOD and, thus, reduce DO. Decomposing algal mats may also provide suitable conditions for an outbreak of botulism (Galvin *et al.*, 1985) (Section 7).

These considerations notwithstanding, it is also important to recognise that phytoplankton represent a critical component of functioning aquatic food webs and that algal blooms are not indicative of an unhealthy system. The desired situation is for increased nutrient concentrations to promote both phytoplankton and macrophyte growth, and for these to be combined with sufficient water movement to inhibit the development of stratification and anoxia and thus avoid a shift towards cyanobacterial dominance (MDBA, 2014c). Because phytoplankton are a poor substitute for zooplankton as a substrate for higher orders, cyanobacterial dominance can result in a compromised flow of carbon through aquatic food webs (Lampert, 1987).

6.3 Carcass decomposition and water quality

6.3.1 Carcass decomposition and the recycling of nutrients

Fish kills can be caused by toxic processes, infectious diseases and stranding, but in slow-moving Australian freshwater waterways are most commonly associated with hypoxia (Section 6.2.2) or other water quality ramifications of flood events (Section 6.2.3). Despite the frequency of fish kills in Australian and overseas freshwater waterways – a number of which have been very substantial – there is a relative paucity of literature concerned with the subsequent decomposition of fish carcasses and the effect that this may have on water quality and aquatic ecosystems.

One of the valuable references in this field is a relatively small-scale field study by Parmenter and Lamarra (1991) who examined the decomposition of fish and bird carcasses over summer within a wetland in Wyoming. These authors were concerned with the time required for the decomposition of fish and bird carcasses and their relative contributions to nutrient recycling in aquatic ecosystems. Reviewing earlier works, Parmenter and Lamarra (1991) noted that Minshall *et al.* (1991) had reported that the breakdown of rainbow trout carcasses in Mink Creek, Idaho, took 50 days in summer and more than 120 days in winter. Corroborating this, Parmenter and Lamarra (1991) found that most of the decomposition activity took place in the first 60 days. Both fish and bird carcasses exhibited rapid initial mass loss, followed by a prolonged period of much slower loss of mass. Fish carrion lost more of its initial mass than did waterfowl. Fish carcasses generated less gas than bird carcasses and had a greater tendency to sink without bloating. This limited the extent to which terrestrial invertebrates could feed on the fish carcases. No aquatic invertebrates were observed feeding on the submerged fish carcasses. The rates and sequences of nutrient losses from both fish and bird carcasses were dependent on the tissues in which the various nutrients were incorporated. The most rapidly lost nutrients were K, Na and N. Potassium and Na, both common constituents of blood and interstitial fluids, exhibited extremely rapid loss rates, presumably due to leaching activity. Nitrogen is found predominantly in soft-tissue proteins and also left the carcass quickly. Sulphur, a more widely-distributed element in animal bodies but commonly found in muscle tissue, was lost rapidly from the fish carcasses. In contrast, elements principally associated with bone materials (P, Ca and Mg) were much slower in leaving the carcasses. In fish samples, only 60 percent of the original P was lost during the study, while practically none of the Ca and Mg was lost from bones and scales, which were incorporated into the benthic sediments. In this way, vertebrate wildlife may act as a sink for P, Ca and Mg and some other elements.

In a similar vein, Walsh *et al.* (2018) undertook a series of experiments for the NCCP in order to evaluate the impacts of decaying carp on water quality. These experiments are of pivotal importance to the assessment of risks associated with the release of CyHV-3. Given this, some discussion of their design – and the inference that may be drawn from them – is warranted. Four groups of experiments were undertaken: (a) cold and warm pond[35] experiments that focussed on DO; (b) laboratory-based bucket experiments that focussed on P flux; (c) mesocosm experiments within a wetland in the Murray River near Swan Reach, South Australia, that focussed on DO; and

[35] The ponds in this experiment were plastic containers filled with 712.65 L of tap water.

(d) a wetland experiment that focussed on DO and nutrient recycling at Little Duck Lagoon, a 2.5 ha (10 ML) managed wetland approximately 4 km south of the township of Berri in South Australia (Figure 12).

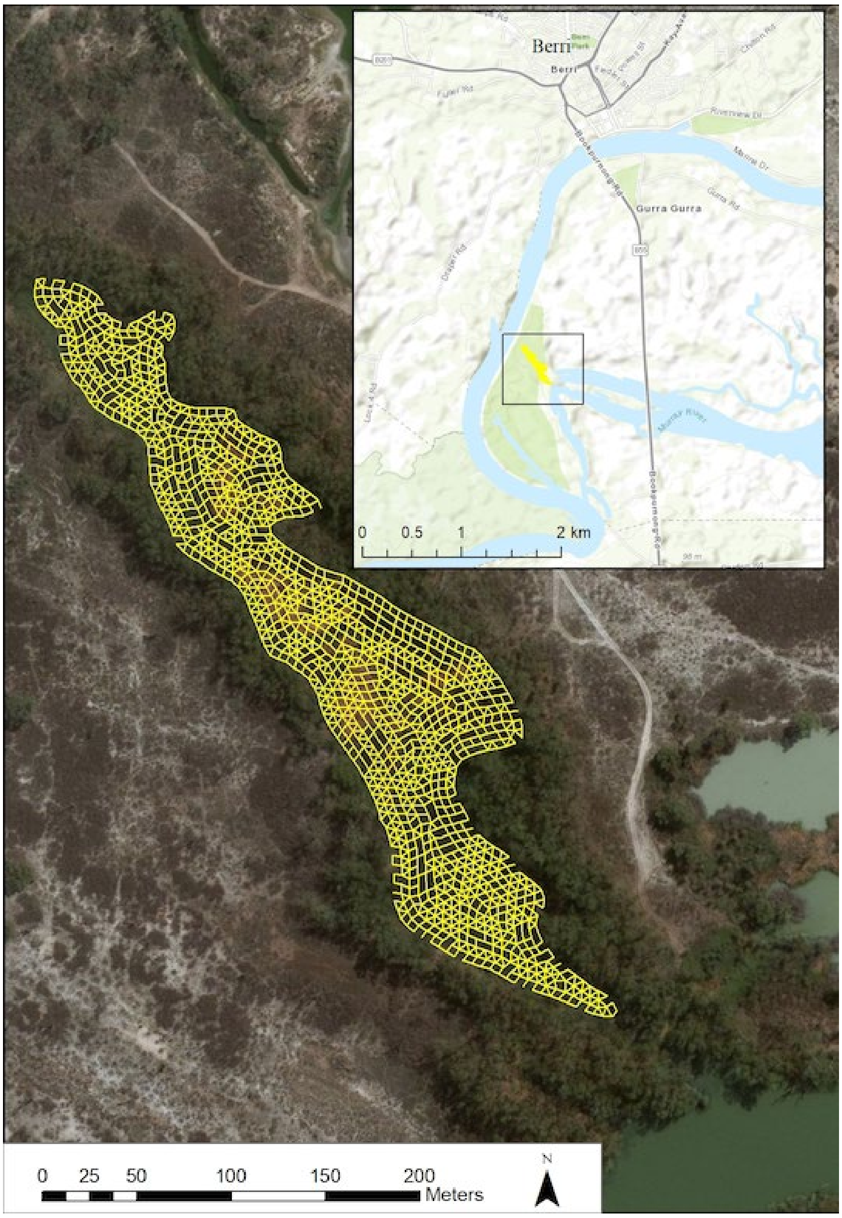

Figure 12 Little Duck Lagoon study site

Source: Hipsey *et al.* (2019)

Table 2 Biomass density estimates adopted by Walsh *et al.* (2018)

Experiment	Biomass densities
Cold and warm pond experiments	1 carp carcass (mean weight 2.75 to 3.38 kg) in approximately 700 L water
Laboratory-based bucket experiments	Low – 265 kg/ha[36] Moderate – 696 kg/ha High – 3,144 kg/ha
Mesocosm experiments	Three controls Three low – 1,282 kg/ha Three high – 2,564 kg/ha
Wetland experiment	1,200 kg/ha when calculated across the wetland, although the 6 tonnes of carp carcasses were deposited at a single site

Source: Walsh *et al.* (2018)

Table 2 shows the biomass densities for each of the experiments. The concerns with this choice of biomass densities are twofold.

First the estimates themselves are significantly higher than the biomass densities reported by most authors.[37] The figure of 3,144 kg/ha used in the laboratory-based experiments focussed on nutrient recycling is in fact the highest density that we were able to locate within the literature. The reference provided to support this estimate (as cited by Walsh *et al.*, 2018) was not from a direct measurement but extrapolated in the 1990s from a catch mass in the Lachlan River in New South Wales and an estimate for catch efficiency within the Bogan River in Queensland (Driver *et al.*, 1997). By way of comparison, Koehn *et al.* (2016) cites numerous estimates for carp biomass density, most of which lie in the range of approximately 150 to 650 kg/ha. One instance of a particularly high carp biomass density within a wetland was given as approximately 1,200 kg/ha (SARDI, unpublished data cited in Koehn *et al.*, 2016). This is close to one third of the 'high' estimate used by Walsh *et al.* (2018) for their laboratory-based experiment, and half that used for the wetland experiment or the 'high' estimate used in the mesocosm experiment.

The second challenge relates to predicting water quality outcomes that are consistent with the expected spatial and temporal course of CyHV-3 epidemics and the associated generation of dead carp. In particular, it seems unlikely that CyHV-3 would kill all carp in any given location and do this at a single point in time. This makes the estimates used by Walsh *et al.* (2018) likely overestimates of fish kill biomass. The characteristics of outbreaks caused by this virus are discussed in Section 5.3.5, where it is explained that outbreaks in wild carp may kill up to about 70 percent of a local population over a period of 3 to 6 weeks. This means that the biomass of carp carcasses would, at most, be 70 percent of the biomass at that location, and probably less, as the carp themselves would be unlikely to die and accumulate in a single place and at a single point in time. With these considerations in mind, a 'high' estimate for the biomass of carp carcasses likely to be present at a

[36] Although a number of metrics have been used to estimate and describe carp biomass density, the most common is kg/ha. In an applied setting, this is the weight of carp in kg that is present in 1 ha of water. The measure is two-dimensional, in the sense that it does not consider the depth of the water.

[37] The estimates of Walsh *et al.* (2018) are also significantly higher than the (currently draft) distribution-wide estimates obtained from the NCCP biomass project. The outcomes of the biomass project will be carried through to this review when the project has been finalised.

single place and point in time seems more probably to lie in the region of 500 kg/ha. Estimates for 'moderate' and 'low' biomass of carp carcasses should then be adjusted downwards accordingly.[38]

Additionally, Walsh *et al.* (2018) found that total phosphorus (TP) and filtered reactive phosphorus (FRP) liberated from carp carcasses commenced almost immediately upon their placement within a series of plastic ponds held at 20C and increased over time until day 14. After day 14, the concentration of TP and FRP was relatively stable. It seems likely that substantial P, Ca and Mg remained bound within the more resilient carcass components – including some bones and scales. It was also interesting that the pattern of TP was quite different within the wetland experiment that was undertaken by the same researchers. Here TP peaked at around 7 days at measurement sites close to the location where carp carcasses were deposited within the wetland, and then declined markedly. A secondary peak appeared to occur at around day 28 at some sites while the TP at others (in particular, the sites furthest from the carp carcasses) appeared to rise through to the cessation of the experiment at 42 days. These results are not inconsistent with the progressive movement of P (and other decomposition products) through the wetland and away from the site at which the carp carcasses were deposited. They are however, markedly different to the plateau effect observed from the plastic ponds.

Walsh *et al.* (2018) extended the outcomes of the pond experiment (above) to calculate TP load and the potential concentration of chlorophyll-α[39] at a range of biomasses (265, 696 and 3,144 kg/ha) and depths (1, 2 and 3 metres) (Table 3). While N was not measured in this study, it was estimated from Schoenebeck *et al.* (2012) who reported a P and N flux of 0.5 kg/ha and 4.3 kg/ha, respectively, from a carp biomass of 177 kg/ha. Extrapolating these findings out to 3,144 kg/ha gives an estimated P and N loading of 8.9 kg/ha and 76.7 kg/ha, respectively. Using the proportional difference between the P concentrations reported in this experiment (6.67 kg/ha) and the previous study (8.9 kg/ha) Walsh *et al.* (2018) estimated an N flux of 57.5 kg/ha.

[38] This may be addressed through the water quality modelling that is currently being undertaken for the NCCP. The outcomes of the water quality modelling project will be carried through to this review when the project has been finalised.

[39] The concentration of chlorophyll-α is an indicator for the amount of photosynthetic plankton, or phytoplankton. This may include true algae, as well as cyanobacteria.

Table 3 Total phosphorus (TP) loading and chlorophyll-α concentration for three biomasses

Biomass (kg/ha)	TP Load (kg/ha)	TP Load (mg/m^2)	Assumed Depth (m)	TP Load (mg/L)	Chlorophyll-α (mg/L)
265	0.56	56.21	1	0.056	0.013
			2	0.028	0.007
			3	0.019	0.005
696	1.48	147.63	1	0.148	0.030
			2	0.074	0.016
			3	0.049	0.011
3,144	6.67	666.87	1	0.667	0.131
			2	0.333	0.066
			3	0.222	0.045

Source: Walsh *et al.* (2018)

Walsh *et al.* (2018) also compared their results for TP, N and chlorophyll-α (as well as for P, Ammonia, Nitrate, Nitrite, DOC, BOD and lipid) with the 'trigger values' for lowland river systems of south-central Australia provided in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality[40]. These are the values below which concerns can be raised as to the health of a waterway. The authors analysed the maximum values obtained from the wetland experiment against these trigger values. These maximum values, however, were obtained from a site close to the point at which all 6 tonnes of fish carcasses were deposited within the wetland. Mean values for lower carcass densities (150 to 500 kg/ha) that were obtained mathematically are likely to be more realistic. While these values exceeded the trigger values for N, P and TP for healthy lowland waterways, the difference was much less marked. As the carcasses were effectively decomposed in this setting within about 14 days, it is also likely that the raised levels would not persist for an extended period.

In a modelling study undertaken for the NCCP, Hipsey *et al.* (2019) found that phosphate, nitrate and ammonia levels all increased with carcass decomposition, although marked elevations were only observed in shallow and poorly connected lakes and wetlands when the carp biomass density was two to five times higher than anticipated. These artificially high levels are similar to those created by Walsh *et al.* (2018) in the wetland experiment described above. Hipsey *et al.* (2019) observed similar trends for the risk of cyanobacterial blooms, although noted that, unlike oxygen, nutrients persist within the ecosystem and are not 'reset' by reaeration. This means that the risk of a cyanobacteria bloom can persist after the risk of low DO have been resolved.

6.3.2 Effect of carcass decomposition on dissolved oxygen

Walsh *et al.* (2018) examined the effect of carcass decomposition on DO using: (a) cold and warm pond experiments; (b) mesocosm experiments within a wetland in the Murray River near Swan Reach, South Australia; and (c) a wetland experiment within Little Duck Lagoon near the township

[40] Australian and New Zealand Environment and Conservation Council (October, 2000)

of Berri, South Australia. The initial conditions for the mesocosm experiment were marked by the presence of a luxuriant growth of algae, prior to adding the carp carcasses. The effect of the algae was to produce a marked diurnal fluctuation in DO, to the extent that the control mesocosm reached overnight lows that were very close to anoxia. Against this backdrop, the quantitative effect of the carcass treatments was difficult to evaluate. The wetland experiment appeared to have similar characteristics. Although there were no control sites (the wetland being quite small and contained) marked diurnal fluctuations were again evident from the commencement of the experiment. The wetland was due to be drained, and it is possible that the wetland was in poor environmental condition at the time of the experiment. The biomass loading component of the experimental trial was narrow inasmuch as the full 6 tonnes of carp carcasses (equating to approximately 2,400 kg/ha across the wetland) were deposited at a single location. A much more widespread distribution of carcasses would be expected, even if the outbreak coincided with a dense aggregation event. These observations notwithstanding, the experiment did demonstrate that the effect of the decomposing carp was to reduce DO to the extent that it reached anoxia at the site closest to the place where the carp carcasses were deposited. Anoxia appeared at this site on about day 2 and persisted until day 12. A shorter secondary period of anoxia appeared at the same site on about day 26 and persisted until day 30. A similar pattern was observed at the second closest site to the place where carp were deposited, where anoxia was recorded on (approximately) days 6 to 8 and again on days 28 to 31. The secondary period of anoxia at both sites may have been caused by an algal bloom, or by a die-off of carp within the wetland that was observed between about days 14 to 21. At other sites within the wetland, the DO fell overnight throughout the experiment (in some readings, to zero) but rose again to above 20 mg/L each day.

The experiments undertaken in plastic ponds using cold and warmed water were less complicated, and here the addition of carcasses produced the results shown in Figure 13. The carp biomass density adopted for the pond experiments was not given, although it was explained that one carcass (mean weight 2.75 to 3.38 kg) was placed in approximately 700 L of either warm or cold water. The experiment showed that under these conditions, anoxia occurred twice as quickly in warm ponds as cold ponds, and that the mean oxygen demand of carp carcasses in warm and cold ponds was 1.022 and 0.496 mg/kg/min, respectively. This appeared to be the only study within the literature that has successfully made direct quantifications of the effect of decomposing fish carcasses on DO.

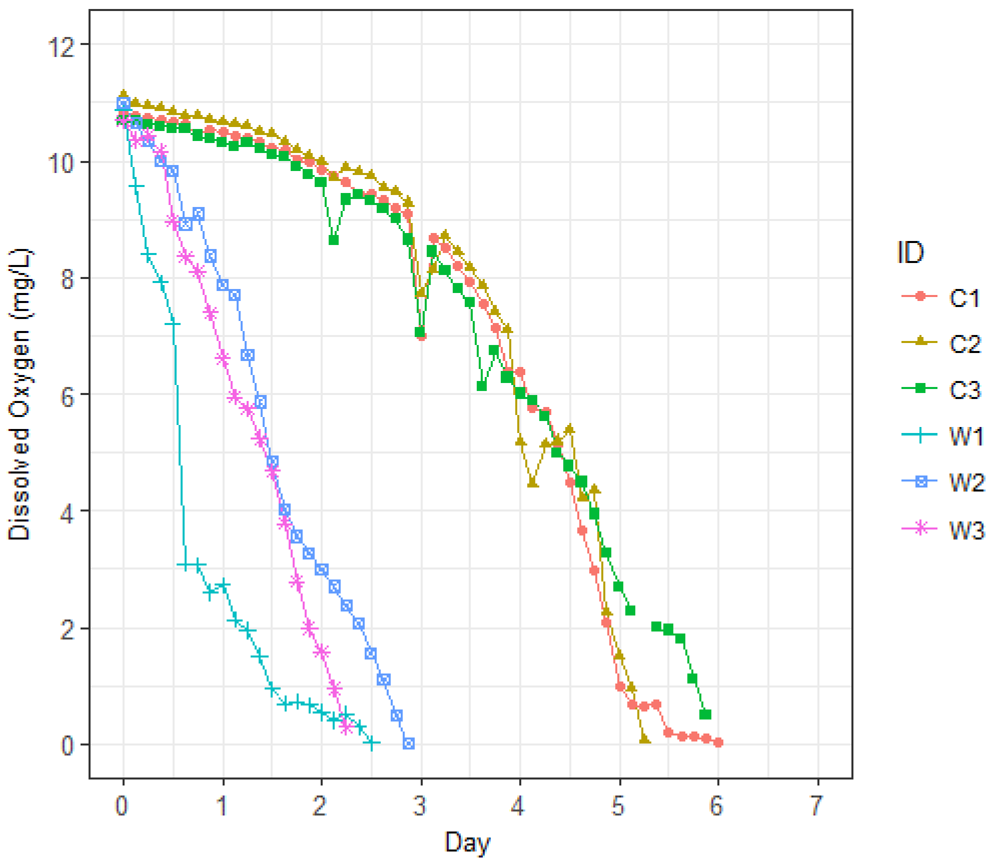

Figure 13 Dissolved oxygen for warm (W1 - W3) and cold pond replicates (C1 - C3)

Source: Walsh *et al.* (2018)

In a modelling study undertaken for the NCCP, Hipsey *et al.* (2019) found that the decomposition of carp carcasses did not lead to excessive water column deoxygenation when the carp biomass density remained at forecast levels. There were some exceptions to this, such as large, shallow areas with poor levels of hydrologic connectivity with the main flow (for example, off-channel wetlands and lake systems), although the decrease in DO in these places returned rapidly to normal levels with the reaeration associated with wind or water flows. When the carp biomass density was artificially elevated to up to five times the forecast level, deoxygenation occurred and persisted for a period of weeks. This scenario was similar to the wetland experiment created by Walsh *et al.* (2018).

6.3.3 Effect of carcass decomposition on dissolved organic carbon

In Section 6.2.3 it was explained that McMaster and Bond (2008) identified an interaction between the antagonistic effects of DOC and DO. Fish maintained under low DO exhibited less resistance to high DOC (50, 70 and 80 mg/L) than fish in aerated conditions. These authors also observed an acute toxic effect when the concentration of DOC exceeded 99 mg/L, and found that this effect overrode the effects of low DO. The caveat to these observations is that here and in most other evaluations of the effect of DOC on freshwater aquatic fauna, the DOC in question is derived from the leachate of the leaves of trees – in particular, the leaves of river red gums. This leachate contains chemicals (including phenols) that have been shown to be toxic to aquatic fauna (Temmink *et al.*, 1989; Gehrke *et al.*, 1993; McMaster and Bond, 2008).

Walsh *et al.* (2018) examined the effect of carcass decomposition on DOC within a wetland. The wetland experiment was confounded by the extremely large loading of carp carcasses at a single place (6 tonnes, equating to 2,400 kg/ha across the area of the wetland), and by marked diurnal fluctuation in DO at all monitoring sites. The DOC in this experiment peaked at between 7 and 14 days at the four sites closest to the place where the carcasses were deposited, and thereafter fell sharply to levels slightly above those at the start of the experiment. At the two remaining sites, DOC rose linearly throughout the study period. At three sites, DOC peaked at close to 200mg/ml. At the remaining three sites, the peak DOC was around 120 mg/L. At all sites, BOD increased until about day 28, and thereafter either plateaued or declined slightly. It was noted in Section 6.3.2 that a secondary fish kill (of carp) occurred within the wetland on about days 14 to 21. This coincided approximately with peak DOC at four of the sites. Whether the fish kill can be attributed in part to the very high levels of DOC is unknown, as in this case DOC was not derived from the toxic leachate of river red gum leaves but from the decomposition of carp carcasses.

6.3.4 Other effects of carcass decomposition

One of the most consistently recorded outcomes associated with increasing the DOC in warm and slow-moving waterways is an elevation in the growth of phytoplankton. This may include true algae, as well as cyanobacteria such as *Anabaena*, *Aphanizomenon*, *Dolichospermum* and *Microcystis* (Walsh *et al.*, 2018). Further, if the density of zooplankton is reduced through low DO or turbidity (such as accompanies the presence of higher densities of carp) (Ning *et al.*, 2015) then the extent to which zooplankton graze on phytoplankton will also be reduced and the incidence and severity of blooms may rise (Gehrke and Harris, 1994; Lougheed *et al.*, 1998; Khan, 2003; Khan *et al.*, 2003; Parkos *et al.*, 2003). Even with grazing, cyanobacteria are a poor substrate for zooplankton and therefore a poor pathway for carbon flow through aquatic food webs (Lampert, 1987). Blooms of cyanobacteria may also be associated with the release of cyanotoxins that are hazardous to fish and other aquatic life (Chorus and Bartram, 1999; Ferrao-Filho and Kozlowsky-Suzuki, 2011). Importantly, this includes frogs, turtles, crustaceans and zooplankton (Ferrao-Filho and Kozlowsky-Suzuki, 2011). Cyanobacterial poisoning can occur by two routes: through consumption of cyanobacterial cells from the water, or indirectly through consumption of other animals that have themselves fed on cyanobacteria and accumulated cyanotoxins. Cyanotoxins may also bioaccumulate in common aquatic vertebrates and invertebrates, including fish, mussels and zooplankton. Consequently, there is considerable potential for toxic effects to be magnified in aquatic food chains. When conditions are no longer optimal for growth, the bloom subsides and decays. This can occur in response to a sudden change in ambient and water temperatures, or as a result of the depletion of substrate. The aerobic decomposition of algal blooms can exacerbate an already-high BOD and, thus, reduce DO. Decomposing algal mats may also provide suitable conditions for an outbreak of botulism (Galvin *et al.*, 1985) (Section 7).

The concentration of chlorophyll-α is an indicator of the amount of photosynthetic phytoplankton and is generally regarded as a measure for the growth of cyanobacteria. Walsh *et al.* (2018) evaluated changes in chlorophyll-α when 6 tonnes of carp carcasses (2,400 kg/ha) were placed in a shallow wetland. Chlorophyll-α peaked on about day 21, approximately 7 days following a peak in DOC, and then declined. At sites close to where the carcasses were deposited, chlorophyll-α remained in decline. At sites further from the carcasses, chlorophyll-α increased and remained on an upward gradient until the end of the experiment. This coincided with a similarly linear rise in

DOC at these more distant sites. The continued rise in DOC and chlorophyll-α may have resulted from the progressive diffusion of putrefaction products from the place within the wetland where the carcasses were deposited. There was also a fish kill of unknown magnitude on about day 14 to day 21, and this is likely to have led to further DOC within the wetland. The fish kill may have been precipitated by an algal bloom (with rising levels of chlorophyll-α) quite early in the experiment – alternatively, the fish kill may have resulted directly from low DO.

The experiment of Walsh *et al.* (2018) highlighted the complexity of events likely to follow from the deposition of a large mass of carcasses in a wetland, or a shallow and slow-moving waterway, and the high probability that these events will include a bloom of toxic cyanobacteria. The longevity of the bloom, and the events that follow from it, will depend on the continuance of a source of DOC and other conditions within the waterway.

While the ecology of cyanobacterial blooms (often termed 'harmful algal blooms', or HABs) has been described extensively in the literature, far less attention has been given to the release of other harmful microorganisms into aquatic ecosystems as a result of decomposition of large numbers of fish carcasses. This is despite documented evidence of frequent and substantive fish kills in both freshwater and saltwater environments.

Walsh *et al.* (2018) did not undertake an analysis of the bacteria released into the wetland as a result of the decomposition of a large mass of carp carcasses. Similarly, authors evaluating either the cause of major freshwater fish or waterbird kills or their ecological consequences have not included an analysis of the microorganisms released into aquatic ecosystems. This includes both Australian reports (Woodall, 1982; Galvin *et al.*, 1985; McComb and Davis, 1993; Townsend and Edwards, 2003; Grillo *et al.*, 2013; McCarthy *et al.*, 2014; Thiem *et al.*, 2017) and reports of fish kills in other countries (Murphy *et al.*, 1999; Gilbert *et al.*, 2002; Diego-McGlone *et al.*, 2008; Ruuhijärvi *et al.*, 2010; Schoenebeck *et al.*, 2012). A number of authors have examined nutrient recycling and other matters associated with the death of salmon after spawning in northern waters (Kline *et al* 1997; Yanai and Kochi, 2005; Bilotta and Brazier, 2008; Chidami and Amyot, 2008; Premke *et al.*, 2010), but these reports have not included an analysis of harmful microorganisms released into aquatic ecosystems. Public health authorities in Australia[41] and elsewhere[42] warn of the importance of removing carcasses (and other foreign materials) from drinking water, but very little detail is provided as to the particular pathogens that are likely to be important.

Relevant microorganisms might include carp gut flora and enteric pathogens, as well as spoilage organisms. With suitable conditions on the carcass or in the water, coliforms (including *Escherichia coli*) and opportunistic pathogens (including *Pseudomonas spp*) may also grow (Monis *pers. comm.*, 2019).[43] Shiga toxin-producing *E. coli* (STEC) has been isolated from bodies of water (such as ponds and streams), wells and water troughs, and has been found to survive for months in

[41] See: http: //www.health.gov.au/

[42] See: http: //www.aphis.usda.gov/

[43] Dr Paul Monis, Adjunct Associate Professor, School of Natural and Built Environments, University of Adelaide and Manager, Research Stakeholders and Planning, South Australia Water

manure and water-trough sediments.[44] STEC is notifiable throughout Australia, and waterborne transmission has been reported both from contaminated drinking-water and from recreational waters. *Aeromonas spp* are aquatic bacteria that occur in irrigation water, rivers, springs, groundwater, estuaries and oceans (in particular where water has been contaminated by sewerage) and are of public health concern (Kelly *et al.*, 1993; Kivanc *et al.*, 2011). These and other microorganisms may be constrained to waterways affected by decomposing carp or might persist in unaffected waters or in waters that have returned to a healthy state following a fish kill.

In 2019, WaterNSW documented an experiment undertaken in Prospect Reservoir to investigate the impacts of decomposing carp carcases on a range of water quality parameters (Pera *et al.*, 2019). The study was submitted to CSIRO as an extended abstract, with a range of figures and tabulated results. The study was undertaken to investigate the possible impact of an outbreak of CyHV-3 on the quality of drinking water in New South Wales. For this reason, the study focussed on the proliferation of microorganisms, and their correlation with Geosmin and Methyl-Isoborneol (MIB). Geosmin and MIB are naturally-occurring compounds that have a very strong, earthy taste and odour.[45] Both compounds are generally present in drinking water, although not at noticeable levels. Geosmin is produced by the gram-positive bacteria *Streptomyces*, and by various cyanobacteria, and is released when these microorganisms die. Generally, Geosmin becomes an aesthetic issue when levels are in the range of 20 to 30 nanograms per litre, although some people who are particularly sensitive may notice it at levels above 10.

Pera *et al.* (2019) added carp carcasses to the mesocosms at biomass densities of approximately 250, 500, 1,000, 1,200, 2,300 and 6,000 kg/ha. Comparisons included a control mesocosm, and water from the reservoir itself. These authors found that coliforms increased in all mesocosms that contained carp carcasses (Figure 14). Counts fell to around zero after about a week, although increased again at around 25 days in the mesocosms containing lower carp biomass densities. The authors also assessed bacterial counts, including those of: (a) signature lake bacteria; (b) environmental copiotroph bacteria; and (c) fish gut signature bacteria (Figure 16). The signature lake bacteria decreased in mesocosms that included the decaying fish. Both environmental copiotroph bacteria and fish gut bacteria increased, although in some cases levels were higher or highest within mesocosm with a lower carp biomass density. The authors also undertook the network analysis shown in Figure 15 to investigate associations between bacterial species and both Geosmin and MIB.

Collectively, the results show that the bacterial flora within closed aquatic systems will be altered by the presence of carp carcasses. Although a valuable addition to the sparse research in this area, the results are not unexpected. The results may also be clouded to some extent by the fact that the mesocosms were small (200 L) closed systems without water exchange. Although this scenario might be approximated in some similarly small and disconnected pools or waterholes, decomposing carp carcasses will in most settings be flushed or otherwise diluted and it is less likely that bacteria released under those conditions would either accumulate in significant numbers or survive ongoing exposure to environmental stresses and competing flora.

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[44] See: http: //www.who.int/news-room/fact-sheets/detail/e-coli

[45] See: https://www.hunterwater.com.au/Water-and-Sewer/Water-Supply/Water-Quality/Geosmin-and-MIB.aspx

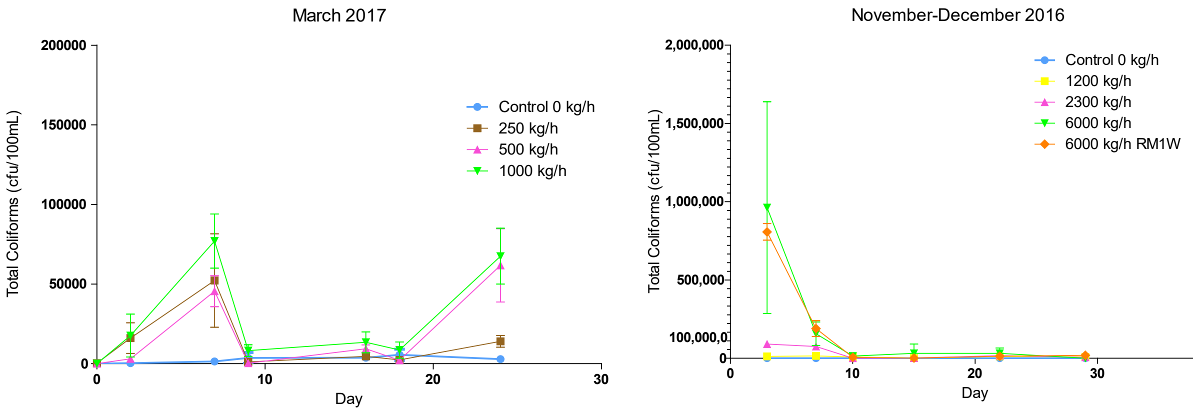

Figure 14 Coliform counts from mesocosm experiments undertaken in Prospect Reservoir

Source: Pera *at al*. (2019)

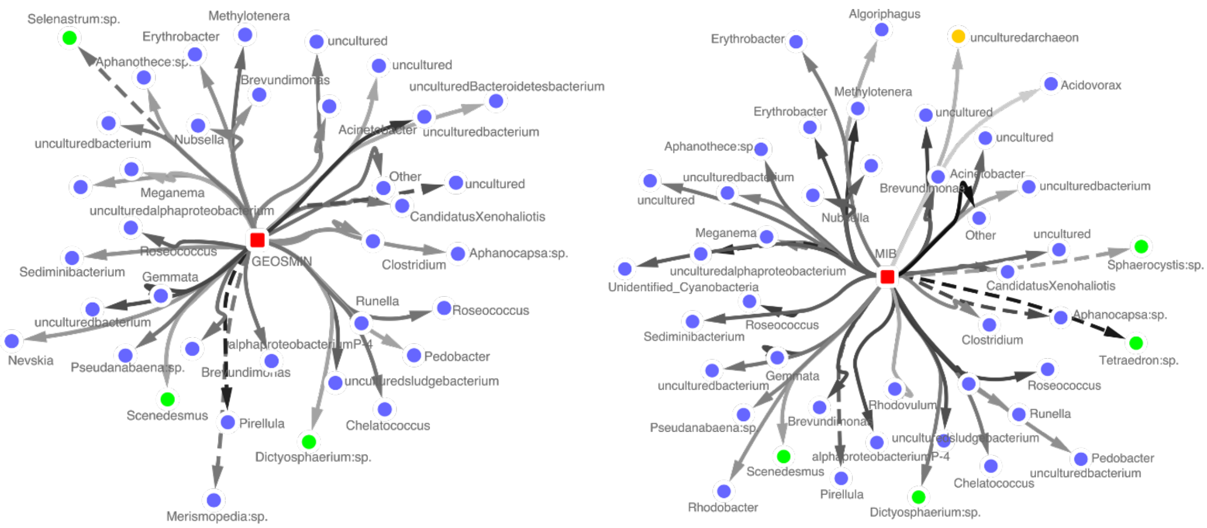

Figure 15 Correlation between waterborne bacteria and metabolites geosmin and MIB

Source: network analysis showing the correlation between the indicator metabolites Geosmin and MIB and a range of identified waterborne bacteria Pera *at al*. (2019).

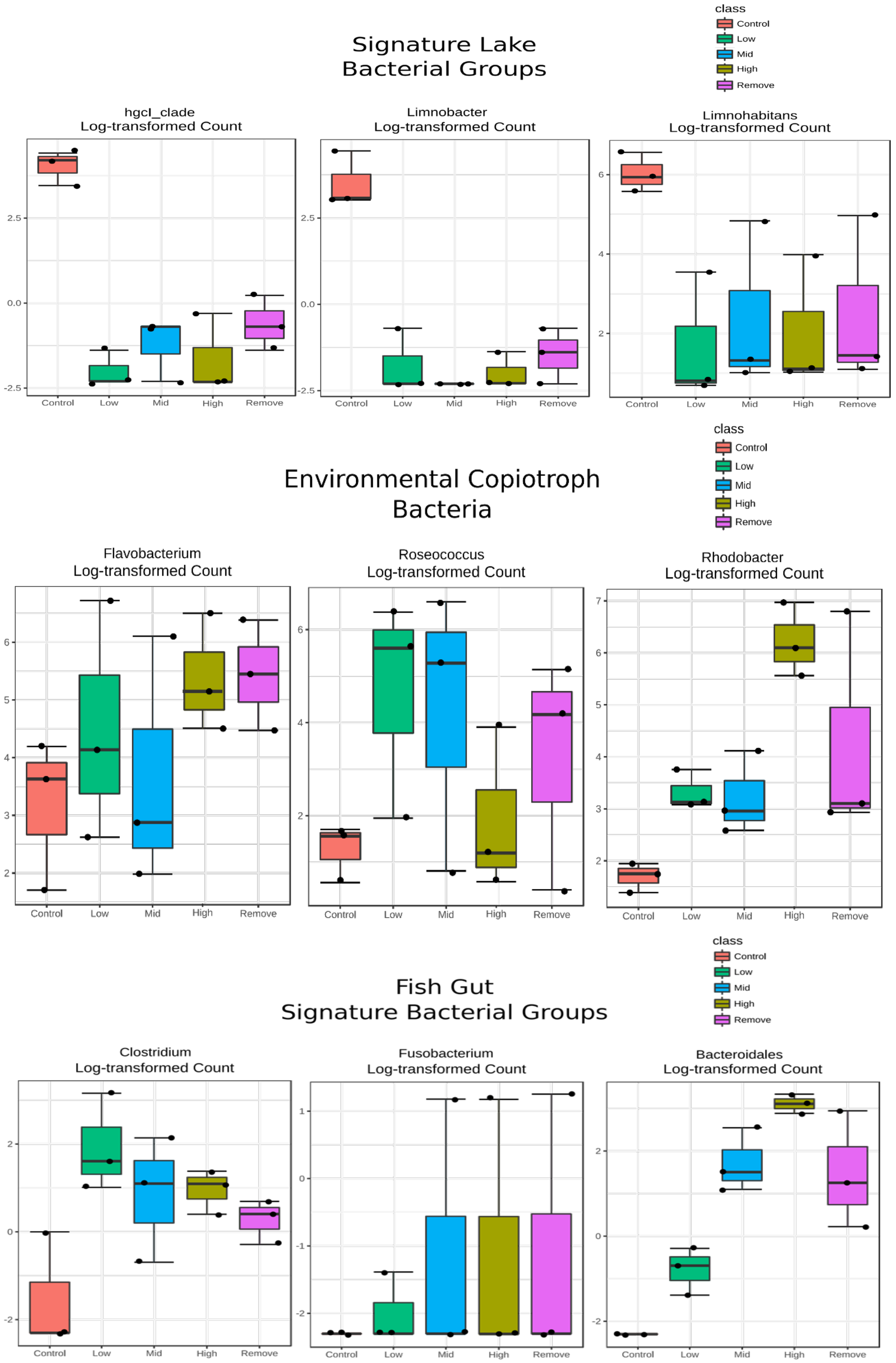

Figure 16 Bacterial assemblages from mesocosm within Prospect Reservoir

Source: Pera *at al*. (2019)

7 Botulism outbreaks in wildlife

The purpose of this review was to identify and evaluate aspects of the epidemiology of botulism in wildlife that may be relevant to the sudden death of carp in Australian waterways, lakes and wetlands in the event of the release of CyHV-3.

7.1 Taxonomy and classification

Clostridium botulinum was first described in 1897 as a result of the investigation of a foodborne illness in Belgium that affected 23 people, 13 of whom became paralysed and 3 of whom died. The outbreak was at that time shown to have resulted from a potent, heat-labile toxin that was produced by a spore-forming, obligate anaerobic bacterium (Barash and Arnon, 2014). *Clostridium botulinum* is rod-shaped and motile, and reacts positively to Gram's stain (Figure 17). It is one of a diverse group of pathogenic spore-forming and generally saprophytic organisms that includes *C. perfringens*, *C. tetani*, *C. haemolyticum*, *C. novyi* and more than 100 other species (Peck, 2009).

Figure 17 *Clostridium botulinum*, a Gram-positive rod (stained here with gentian violet)

Source: Centers for Disease Control and Prevention (CDC) Public Health Image Library (PHIL)[46]

Clostridium botulinum is categorised into four distinct phylogenetic groups, designated by the numerals I to IV (Peck, 2009; Espelund and Klaveness, 2014). All groups of *C. botulinum*, and some strains of *C. butyricum* and *C. barati*, produce botulinum toxin, which paralyses animals by inhibiting acetylcholine release from synaptic vesicles at neuromuscular junctions (Peck, 2009; Espelund and Klaveness, 2014).

There are seven primary strains of botulinum neurotoxin (types A to G), and a significant number of sub-types, including five sub-types of type A toxin (termed A1, A2, A3, A4 and A5), five type B sub-types, and six type E subtypes (Peck, 2009). Each type (A to G) is antigenically distinct (Anza *et al.*, 2014). There are also mosaic strains that are antigenic to (for example) C and D neurotoxins

[46] phil.cdc.gov/details_linked.aspx?pid=2107 (accessed 19/07/2018)

(Anza *et al.*, 2014). Barash and Arnon (2014) described a further strain (designated H) for which there is currently no antitoxin. This strain is not included in most overviews of the taxonomy of *C. botulinum*. The toxin-producing genes for strains type A, B, E and F are encoded on plasmids or chromosomes, while the toxin producing genes for types C, C/D mosaic and D are encoded on bacteriophages (Eklund, 1993; Peck, 2009). *Clostridium butyricum* and *C. barati* have also been shown to produce botulinum neurotoxins of types E and F, respectively (Eklund, 1993; Peck, 2009).

A correlation of neurotoxic *C. botulinum* genomic and toxin strains is given in Table 4.

Table 4 Clostridia species characteristics and types

Group	Characteristics	Types
I	Proteolytic *	A, AB, AF, B, BA, BF, F
II	Non-proteolytic*	B,E,F
III	Non-proteolytic*	C (8 subtypes), D (6 subtypes)
IV	Proteolytic (delayed)*	G
V	*C.butyricum*	E
VI	*C.barati*	F

Source: Eklund (1993)

* *C. botulinum* species, noting that *C. butyricum* and *C. barati* also produce neurotoxins

The seven primary strains of *C. botulinum* have a certain degree of specificity in respect of the animal species affected. Types A, B, E and F are toxic to humans (Satterfield *et al.*, 2010; Espelund and Klaveness, 2014). Outbreaks of botulism in livestock are typically associated with types B, C and D (Long and Tauscher, 2006). Outbreaks in wild birds are generally associated with type C and C/D mosaic strains, with notable large outbreaks of type E occurring in the Great Lakes of North America (Espelund and Klaveness, 2014). These large type E outbreaks also included substantial disease and mortality amongst fish species and are additionally significant in respect of the risk that type E toxin poses to human health (Long and Tauscher, 2006).

7.2 Sporulation and vegetative replication

Although there are marked regional differences in prevalence, the spores of *C. botulinum* are generally considered to be widespread but unevenly distributed in soils and sediments and, in some areas, dust (Rocke and Bollinger, 2007; Espelund and Klaveness, 2014).

The spores of *C. botulinum* are dispersed by wind and water and through passive transfer by terrestrial and aquatic vertebrates and invertebrates (Getchell *et al.*, 2006; Anza *et al.*, 2014; Espelund and Klaveness, 2014). Getchell and Bowser (2006) noted that type E spores can readily be found in the gills and digestive tract of fish. Similarly, Reed and Rocke (1992) reported that more than half of the healthy mallards sampled from wetlands with a history of botulism outbreaks carried type C spores in their caecum. Anza *et al.* (2014) found that waterbirds distributed type C/D spores through their faces and, in a separate study (Anza *et al.*, 2014b), noted that the strains of type C/D isolated from both ends of the Palearctic migratory flyway (between Sweden and Spain) were similar, and concluded from this that the spores had been carried and subsequently shed by migrating birds. Lalitha and Gopakumar (2000) found that 18 percent of fresh or farmed shellfish, and 14 percent of wild fish, from Indian coastal and inland waters were carrying type C or D spores.

Germination of spores occurs when conditions are suitable for the growth of the vegetative bacterium. Rocke and Bollinger (2007) noted that germination is a two-stage process with a slow rate of germination through 18 to 26 hours of incubation, and a sudden fourfold increase in germination following that. Botulinum toxin is released when the vegetative cells undergo autolysis (Rocke and Bollinger, 2007). In culture media, toxin titre is low during the logarithmic phase of growth but increases when cell growth ceases and cell membranes rupture (Rocke and Bollinger, 2007).

All strains of *C. botulinum* require an anaerobic environment, pH in the range 5.7 to 6.2 and the provision of a protein-rich substrate (Getchell and Bowser, 2006; Long and Tauscher, 2006; Anza *et al.*, 2014; Espelund and Klaveness, 2014). Peck (2009) summarised the optimum and minimum growth temperatures for vegetative cells (Table 5). Of note is the tolerance of type C, D and C/D mosaic strains to relatively higher temperatures and the tolerance of type E to lower temperatures. Although the trends appeared to be consistent across the literature, there were some differences in the optimum and minimum temperatures cited by different researchers and reviewers. For example, citing Grecz and Arway (1982), Rocke and Bollinger (2007) maintained that type E spores germinate over a wide temperature range, from 2 to 50C, with optimum germination occurs at approximately 9C. Vegetative growth of type E cells then occurs at temperatures within the range 6 to 41C, and that optimal growth occurs at 32.5C. These authors also noted that only marginal growth occurs between 6 and 14C, although certain strains can produce toxin at a slow rate at temperatures as low as 3.3C.

Table 5 Optimum and minimum growth temperature for vegetative cells

	Group I Proteolytic A, B and F	Group II Non-proteolytic B, E and F	Group III C, C/D, D	Group IV G
Optimum growth temperature	37C	25C	40C	37C
Minimum growth temperature	10 to 12C	2.5 to 3C	15C	

Source: Peck (2009)

The sensitivity of *C. botulinum* to salt and saline conditions was not easily summarised. Many reviewers (for example, Anza *et al.*, 2014; Espelund and Klaveness, 2014) included a blanket statement to effect that increasing salinity will have a protective effect for wetlands in respect of botulism outbreaks. In support of this, Webb *et al.* (2007) found that salting substrate increased the time to germination for type B spores, while Peck (2009) cited a range of foodborne outbreaks of botulism in people, which had been linked to inadequate salting. Offsetting this, however, was a report of distinct marine (saline) and freshwater type C strains of *C. botulinum* (Segner *et al.*, 1971). Similarly, Barras and Kadlec (2000) formulated risk factors for type C botulism in the Great Salt Lakes marshland ecosystem of Utah, while Nol *et al.* (2004) discussed aspects of type C botulism in the Salton Sea—a large inland body of warm and highly-saline water in southern California. Citing unpublished data, Rocke and Bollinger (2007) noted that type E botulism has also occurred in the Salton Sea, and that type E botulism is endemic throughout the Baltic Sea. On balance, it was difficult to draw firm conclusions as to the protective effects of salinity and the relative likelihood of outbreaks of botulism in Australian freshwater ecosystems as opposed to those in estuarine and other saline environments.

7.3 Stability of spores and preformed toxin

Most reviewers noted that the spores of *C. botulinum* are extremely robust and may persist in the environment for decades (for example, Rocke and Samuel, 1999; Getchell and Bower, 2006; Long and Tauscher, 2006; DEPI, 2014; Espelund and Klaveness, 2014). Citing a now-unobtainable United States Centers for Disease Control and Prevention (CDC) Handbook for Epidemiologists, Clinicians, and Laboratory Workers,[47] Villar *et al.* (2006) noted that *C. botulinum* spores were also able to survive up to 2 hours at 100C.

Relatively fewer studies remarked on the stability of the botulinum toxin. Citing very early unpublished Australian works, Bennetts and Hall (1938) noted that rabbit carrion buried in warrens or left in the open for 6 months retained its toxicity to sheep. In a series of experiments, these authors investigated the stability of type C toxin obtained from the replication of vegetative cells inserted into the rectum of rabbit carcasses. The stability of toxin was then assessed through its toxicity to sheep fed a carcass homogenate by stomach tube. These authors found that the toxin obtained a maximum lethality at 3 to 5 days (3 g of homogenate was lethal to sheep) and that 50 g of homogenate administered on day 26 produced no symptoms. Curiously, when the carcasses used in this experiment were sampled 6 months later, 50 g of homogenate was lethal to sheep. Although unable to offer an explanation for this, Bennetts and Hall (1938) did note that cold conditions delayed the onset of putrefaction and the development of toxicity, while rain delayed carcass desiccation and the diminishment of toxicity.

Bennetts and Hall (1938) also had anecdotal evidence that contaminated water had been responsible for botulism in livestock and, to investigate this, undertook their own research. In their first set of experiments, the carcass of a rabbit that had been killed and inoculated per rectum (as above) was placed in two 40-gallon drums of water. The carrion was removed at either 8 or 14 days and the water tested at intervals for toxicity for sheep by voluntary and compulsory ingestion. It was demonstrated that the water became highly-toxic, and retained its toxicity for more than 14 days after removal of carrion, and that by 25 days it had become innocuous to sheep at a dose of 1 L daily. The smallest amount shown to produce symptoms of botulism in sheep was 500 ml drenched 8 days after removal of carrion, and 16 days after the initial contamination. Similar experiments were then undertaken in which the carcass was not removed although, prior to drenching, water was freed from carcass fragments by filtration through several thicknesses of butter muslin. The water was tested for toxicity at 3, 10 and 19 days by drenching sheep (at each point in time) with 250, 500 and 1,000 ml. Toxicity was first demonstrated at 19 days, when all doses were lethal. One month after the commencement of the experiment 350ml of contaminated water remained lethal.

Notermans and Havelaar (1980) also investigated the stability of botulinum toxin in water. These authors examined toxin types A, B and E and focussed separately on surface water, drinking water

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[47] Previously located at: http: //www.cdc.gov/ncidod/dbmd/diseaseinfo/files/botulism_manual.htm#XV

and distilled water. In these experiments, surface water was obtained from a water storage reservoir in The Netherlands (pH 7.9, Total Organic Carbon [TOC] 12.1 mg/L), while drinking water was obtained after slow sand filtration (pH 7.9, TOC 4.2 mg/L) and distilled water was obtained under laboratory conditions. The results of the experiments are reproduced in Table 6 below. In this table, the cell entries depict the percentage of toxicity remaining after each incubation period. The experiments show that botulinum toxin is relatively stable in distilled water, but progressively less so in drinking water and surface water. Notably, surface water retained 33 percent of its toxicity after 3 days but just 0.6 percent after 6 days.

Table 6 Stability of *Clostridium botulinum* toxin type A, B and E in water

Days	Surface water			Drinking water			Distilled water	
Toxin type	A	B	E	A	B	E	A	B
0	100%	100%	100%	100%	100%	100%	100%	100%
3	33%	-	1%	13%	1%	2%	90%	90%
6	0.6%	-	-	3%	-	1%	72%	82%
9	0.3%	-	-	1%	-	-	68%	78%
12	<0.3%	-	-	-	-	-	63%	71%
15	-	-	-	-	-	-	56%	63%

Source: Notermans and Havelaar (1980)

Hubalek and Halouzka (1991) demonstrated that although the botulinum toxin titre dropped 25 to 40-fold, sufficient toxin remained in maggots placed in perforated bottles and buried in wetland sediments over winter (131 days) to cause death of waterbirds. In an earlier publication (Hubalek and Halouzka, 1988) these authors had also found that the time required for 100-fold reduction in the toxicity of a sterile suspensions of botulinum toxin held at 5C was 6 months.

Although human cases of botulism specifically associated with natural drinking water have not been documented (Long and Tauscher, 2006), a German biologist famously threatened in 1973 to contaminate water supplies with *Bacillus anthracis* and botulinum toxin unless he was paid $US8.5 million (Kazdobina, 2001; Villar *et al.*, 2006). Whether for this or unrelated reasons, the body of literature associated with bioterrorism has also reviewed or researched the stability of botulinum toxin. For example, citing unobtainable military research, Burrows and Renner (1999) stated that botulinum toxins are inactivated in sunlight within 1 to 3 hours and in air within 12 hours. These authors also stated that botulinum toxins are inactivated by boiling, or by heating for 30 min at 80C or for several minutes at I00C. With similarly scant substantiation, a table within Khan *et al.* (2001) recorded that botulinum toxin is 'stable in water'. Citing further unobtainable military research, Villar *et al.* (2006) remarked that aerosolised botulinum toxin has been estimated to decay at 1 percent to 4 percent per minute, depending on the weather and dispersal pattern. These authors noted that it would be unlikely for botulinum toxin to contaminate a water supply effectively (from a bioterrorism standpoint) because it is inactivated by sunlight, aeration and chlorine. Warner (1990) noted that botulinum toxin could be inactivated by the vigorous agitation of a dry blend of air at 24C.

7.4 Disease syndromes associated with *Clostridium botulinum*

Botulism, the disease syndrome associated with *C. botulinum*, can follow from: (a) the release of toxin from vegetative cells (termed toxigenesis) within the gastrointestinal tract; (b) the contamination of anaerobic wounds with *C. botulinum* spores (including surgical wounds, such as following the castration of livestock) and subsequent vegetative growth and toxigenesis; or (c) the ingestion of preformed botulinum toxin (Peck, 2009).

The first of these pathways (a) defines the pathogenesis of infant botulism (Satterfield *et al.*, 2010; Barash and Arnon, 2014) and a disease of horses known as 'shaker foal syndrome' (Thomas *et al.*, 1988; Szabo *et al.*, 1994). In some situations, toxigenesis within the gastrointestinal tract may also occur in cattle (Kummel *et al.*, 2012) and has been associated with type C botulism in birds (Rocke and Bollinger, 2007) and fish (Nol *et al.*, 2004). The development of botulism following wounding (b) is a condition seen sporadically in both humans and livestock (Peck, 2009).

The ingestion of preformed botulinum toxin (c) and, less commonly, toxigenesis within the gastrointestinal tract (a), underpin the development of botulism outbreaks involving terrestrial and aquatic wildlife and are largely the focus of this review.

7.5 Environmental and individual-based models for botulism in wildlife

The germination of *C. botulinum* spores, and the initiation of an outbreak of botulism as either an environmental or a local phenomenon, were considered in the early work of Bell *et al.* (1955). These authors formalised the notion of alternative 'sludge bed' (environmental) and 'micro-environment' (individual-based) models for the transition from the endemic persistence of spores within an ecosystem to a typical botulism outbreak.

Environmental model: favourable conditions for the germination of *C. botulinum* spores can follow from an environmental phenomenon, such as eutrophication of a waterbody and the over-proliferation of aerobic microorganisms, that lead to depletion of oxygen (Anza *et al.*, 2014; Espelund and Klaveness, 2014). These conditions may arise in an Australian context when waterways experience, for example, a blackwater event (King *et al.*, 2012) (Section 6.2). Alternatively, significant blooms of phytoplankton (algae or cyanobacteria) may develop across the surface of a waterbody. Death of the bloom, and the accumulation of large amounts of organic debris at the water's edge, can then result in an anaerobic environment (Section 6.2.3). This scenario was described by Galvin *et al.* (1985) who investigated an outbreak of type C botulism in wild birds at a lake in Victoria in November and December 1983 that was believed to have resulted from the decay of a floating mass of phytoplankton and vegetation. A similar scenario was described by Woodall (1982) who investigated separate outbreaks of type C botulism at Seven-Mile Lagoon in south-east Queensland in June 1978 and April 1981 and noted large amounts of decaying vegetation and animal material.

Individual-based model: alternatively, germination of *C. botulinum* spores can be a more local phenomenon — ultimately constrained to the carcass of a single vertebrate or invertebrate animal that was carrying spores (Anza *et al.*, 2014). This scenario underpinned much of the early Australian work on the epidemiology of botulism in sheep in Western Australia, which was linked to an epidemic population of feral rabbits (Bennetts and Hall, 1938). Rabbits carried *C. botulinum* spores within their gastrointestinal tract, and their carcasses provided an anaerobic micro-environment that was then conducive to germination and vegetative growth of the bacterium and the subsequent release of botulium toxin. Sheep and other grazing ruminants will consume carcass materials when certain minerals are deficient from their diets (termed 'pica') and, where such materials contain sufficient quantities of the botulinum toxin, classical botulism may develop. Where animals dying as a result of the botulinum toxin are also carriers of *C. botulinum* spores (or have ingested vegetative cells or spores in conjunction with the preformed toxin) their carcasses will provide substrate for further bacterial multiplication and toxin product and, thus, amplification of the outbreak. This effect was reported by Jubb *et al.* (1993) who investigated an outbreak of deaths in yearling Brahman-cross heifers on a cattle station in northern Western Australia.

Wobeser (1997) extended the individual-level model for the development of botulism outbreaks by adapting it to conventional infectious disease terminology. This approach hinged on the elaboration of a basic reproduction number (R0), the number of new cases that one case generates on average over the course of its infectious period in an otherwise uninfected population. Under this approach, Wobeser (1997) proposed that a case was the carcass of an individual animal, and that R0 could be calculated as M2/M1, where M2 is the number of animals dying of secondary poisoning originating from M1, and M1 = number of animals dying in the location for any reason during the outbreak period. Alternatively, and with some algebraic manipulation, R0 could be calculated as the product of P1, P2 and β. Under this terminology, P1 is the proportion of carcasses that contain spores of toxigenic *C. botulinum* and P2 is proportion of carcasses that become infested with maggots and persist until toxin-laden maggots emerge. The so-called 'intoxication coefficient' (β) then consists of two components: the first (C) represents the frequency of contact between live birds and toxic material, and the second (P3) is the proportion of such contacts that result in intoxication – that is, the proportion of birds that ingest sufficient toxic maggots to cause death. Following this principle, the intoxication coefficient, β, is analogous to the transmission coefficient that underpins the mathematics of infectious disease epidemiology (Anderson, 1982). Using this approach, Wobeser (1997) was able to put forward realistic circumstances, based principally on the prevalence of carcasses in a wetland, whereby: (a) R0 was calculated to be greater than 1, resulting in the development of an outbreak; and (b) R0 was calculated to be less than 1, resulting in the confinement of bacterial multiplication to one or a few carcasses.

Although some authors have strongly favoured one model over another, it seems likely that the particular strain of *C. botulinum*, and its ecology, will dictate whether amplification from a low-grade endemic state to outbreak state occurs through a generalised environmental phenomenon or as a result of a process occurring in individual carcasses — or, ultimately, as a combination of both. Getchell and Bowser (2006), for example, investigated the epidemiology of type E botulism outbreaks in the Great Lakes of North America and the epidemiology they described contained both environmental and individual-level elements. These authors proposed that *C. botulinum* proliferates in the lake sediment, and that benthic invertebrates (principally invasive dreissenid mussels) then filter and concentrate vegetative cells and the botulinum toxin. These invertebrates are predated upon by benthic fish, such as round gobies. As the gobies become intoxicated they surface and behave erratically, and are in turn predated upon by larger fish and by waterbirds.

This work was corroborated through a separate study by Getchell *et al.* (2006), which found both vegetative *C. botulinum* and preformed type E toxin in the tissues of live and moribund fish. The carcasses of fish and birds that succumb to the toxin become individual micro-environments for further vegetative growth and a source of toxin for scavengers and necrophagous invertebrates (Getchell *et al.*, 2006; Espelund and Klaveness, 2014). Decomposing invertebrates and vertebrates also sink to the sediment of waterbodies, thus adding to the reservoir of bacteria and toxin (Eklund *et al.*, 1984; Espelund and Klaveness, 2014).

7.6 Characteristics of botulism outbreaks in wildlife

Outbreaks of botulism in wildlife tend to be focussed on a discrete body of water or wetland, although can nevertheless be devastating to local populations — in particular, those that are threatened and congregated in a particular place for breeding or in the course of migration (Anza *et al.*, 2016). This is notably the case for bird life, although outbreaks have also resulted in significant fish die-offs.

Substantial outbreaks of botulism in wildlife have been associated with *C. botulinum* type C (and C/D mosaic) and type E strains. Type C outbreaks result chiefly in the death of waterbirds, and centre around the so-called 'carcass-maggot' cycle (below). These outbreaks may also, however, be initiated by anoxic aquatic events precipitated by (for example) eutrophication of a waterbody and the proliferation of aerobic microorganisms, or the rapid growth and die-off of algal blooms. Type E outbreaks, on the other hand, result in the death of fish and, generally, waterbirds, and are centred on the cycling of spores, vegetative cells and preformed toxin through an aquatic food web. Type E outbreaks have been included within this assessment as they have resulted in very significant fish and bird kills in North America. To the best of our knowledge, however, type E has not caused human cases of botulism within Australia and although we were unable to obtain a definitive opinion as to whether the strain itself is exotic or endemic, spores are not likely to be commonplace in the sediments of Australian waterways.

The two forms of wildlife outbreaks are discussed individually below. A brief review of botulism outbreaks in Australian wildlife and livestock is given in Section 7.7.

7.6.1 Type C and C/D outbreaks in wild birds

History and distribution: Rocke and Bollinger (2007) noted that large outbreaks of a ‘duck sickness’ (later thought to be type C avian botulism) have occurred in the western United States and Canada since the beginning of the twentieth century — and possibly as early as 1890 in California. The disease was linked to *C. botulinum* in the 1930s, and type C botulism in wild birds was recorded in Russia in 1957 (Kuznetzov 1992; as cited by Rocke and Bollinger, 2007) and in Europe in 1963 (Jensen and Price 1987; as cited by Rocke and Bollinger, 2007), first in Sweden and shortly after in Denmark (1965), Great Britain (1969) and the Netherlands (1970). During this period, the disease was also first recognised in South Africa (1965), New Zealand (1971) and Japan (1973), and later in Argentina (1979), Brazil (1981) (Rocke and Bollinger, 2007). The disease associated with type C botulism has been recognised in Australian livestock since 1928 (Bennetts and Hall, 1938) (Section 7.7).

The global profile of type C botulism as a major cause of wild bird mortality has continued to rise, with outbreaks confirmed on every continent except Antarctica (WHA, 2013), and major events in Russia claiming a million birds in 1982 and 1.5 million or more birds in North America in 1997 (Rocke and Bollinger, 2007). A study focussed on the coastal and inland areas of India found that 18 percent of sediment samples contained type C or D spores, as did 18 percent of fresh or farmed shellfish and 14 percent of wild fish (Lalitha and Gopakumar, 2000). No type E *C. botulinum* was found.

Key species affected: type C strains of *C. botulinum* cause botulism in birds, cattle, sheep and horses. A single reference was identified to substantiate the impact of type C botulinum toxin on turtles (Kendall, 2012). No references were identified to substantiate the disease in frogs, although some early papers about the physiology of botulinum toxin included reference to frog skeletal muscle paralysis (Harris and Miledi, 1971; Antony *et al.*, 1981). All bird species are susceptible and at risk, although the foraging behaviour of particular groups of waterbirds may make them more or less vulnerable in certain outbreak situations (Rocke and Bollinger, 2007; Anza *et al.*, 2016). Filter-feeding and dabbling waterfowl, such as mallards, teal, and shovelers, are among the species at greatest risk for contracting type C botulism, as well as probing shorebirds, such as avocets and stilts (Rocke and Bollinger, 2007). Shorebird species that feed near the surface of wetland soils and sediments appear to be at greater risk than shorebirds that probe deeply into the substrate for food (Adams *et al.*, 2003). Diving ducks may also die in large numbers (Rocke and Bollinger, 2007). Vultures are a possible exception, as there is some evidence that these (turkey vultures, specifically) are relatively less susceptible (Rocke and Bollinger, 2007; Anza *et al.*, 2016), and the lower incidence observed in carrion-feeding Australian raptors, such as whistling kites (Woodall, 1982), may indicate that this is a protective evolutionary adaptation prevalent in avian scavengers. Alternatively, Rocke and Bollinger (2007) suggested that these and some other typically scavenger species, such as crows and ravens, may be more likely to die at a distance from an affected lake or wetland and, for that reason, their carcasses may not be so commonly discovered.

Fish-eating birds are usually associated with type E botulism (below) but in 1996, more than 15,000 pelicans, herons, and other fish-eating birds became sick or died from type C botulism at the Salton Sea, a large inland sea in southern California (Rocke and Bollinger, 2007). The majority of affected birds (> 8,000) were American white pelicans, and the loss represented nearly 15 percent of the western American white pelican population. Since 1996, type C botulism has recurred annually in fish-eating birds at this location, although the numbers of dead birds have been lower (1,000 to 3,000) (Rocke and Bollinger, 2007). In these die-offs at Salton Sea, introduced fish (Mozambique tilapia, *Oreochromis mossambicus*) are thought to harbour the bacteria in their gastrointestinal tracts, where toxin is subsequently formed — perhaps under certain stressful conditions (Nol *et al.*, 2004). Fish are significantly less sensitive to type C toxin than they are to type E (below). Haagsma (1975) found that the sensitivity of carp to type C toxin was many orders of magnitude lower than it was for type E toxin (236 versus 457,000 mouse LD50 per kg). Moribund fish containing vegetative cells and toxin are then consumed by larger fish and by fish-eating birds, and so the disease advances through the food chain.

Outbreak characteristics: although a number of environmental and species-level risk factors have been identified for large-scale outbreaks of type C botulism in wildlife, the reasons why such outbreaks occur in certain situations but not others with the same or similar characteristics, are not well understood (Rocke and Bollinger, 2007; Espelund and Klaveness, 2014).

Citing experimental work in 32 wetlands, Rocke and Bollinger (2007) noted that the risk of type C outbreaks in wetlands was most strongly associated with water pH, although this effect was also influenced by water temperature and redox potential. In general, the risk of botulism outbreaks was increased when water pH was between 7.5 and 9.0, redox potential was negative and water temperature exceeded 20C. The risk declined in wetlands with a pH less than 7.5 or greater than 9.0, when redox potential was positive (greater than +100 mv), and water temperature was lower (10 to 15C). Similarly, Espelund and Klaveness (2014) reviewed a range of primary research and concluded that larger outbreaks were correlated with lower water levels or higher summer surface water temperatures, sediment with a high organic matter content, water pH of between 7.5 and 9.0, an overall negative redox potential and water temperature above 20C.

Wobeser *et al.* (1987) sampled sediments from wetlands throughout the province of Saskatchewan, Canada, and found a strong association between the prior occurrence of type C avian botulism in a wetland and the presence of the bacterium as demonstrated by toxin production from sediments cultured in growth medium. Nearly 60 percent of samples from wetlands with a prior history of botulism outbreaks contained botulinum spores, when compared to 6 percent from lakes with no prior history. These results suggested that wetlands with a history of outbreaks remain contaminated with spores, and the authors hypothesised from this that these wetlands were likely to be at a higher risk of subsequently developing botulism than wetlands without a history of botulism. Countering this, however, Sandler *et al.* (1993) found that the prevalence of type C spores was similar in marshes that experienced either high or low losses of wild birds to botulism during the previous 5 years, and that marshes that had experienced wild bird mortalities arising from botulism had a similar prevalence of type C spores as marshes that had not experienced mortalities.

Any decaying organic matter, insect remains, or other protein particulates can serve as a growth medium for *C. botulinum* (Bell *et al.* 1955). In addition to natural processes of death and decomposition in wetlands, human activity can also increase the available substrate for toxin production (Rocke and Bollinger, 2007). Flooding and draining, pesticides and other chemical inputs into wetlands from agricultural activities may kill aquatic life, thereby providing more substrate for toxin production (Rocke and Bollinger, 2007). Waterbirds may also be secondarily poisoned through ingestion of zooplankton or wetland invertebrates that have consumed toxic material. The carcass-maggot cycle is an example of secondary poisoning through consumption of toxin-laden invertebrates, but other aquatic animals may serve in this role as well (Rocke and Bollinger, 2007). Maggots concentrate the botulinum toxin, and their subsequent predation by a range of wildlife is considered by many authors (including Bell *et al.*, 1955; Wobeser, 1997; Getchell and Bowser, 2006; Rocke and Bollinger, 2007; Anza *et al.*, 2014; Espelund and Klaveness, 2014) to play a key role in the exponential development of type C botulism outbreaks. Wetlands are home to numerous other invertebrates and zooplankton that consume organic debris, particularly in the benthos, and type C toxin has been demonstrated in free-living aquatic invertebrates, crustacea and zooplankton (Rocke and Bollinger, 2007).

In an investigation of triggers for *C. botulinum* outbreaks in Canadian waterbirds, Soos and Wobeser (2006) concluded that the disease in a wetland setting is less likely to be limited by the availability of *C. botulinum* spores or susceptible birds than it is by the availability of suitable protein-rich substrate for bacterial growth and toxin production. These authors described a primary phase of the outbreak in which a number of birds carrying *C. botulinum* spores die within a reasonably constrained period of time, and whose carcasses provide a protein substrate and suitable physical environment for germination and the production of toxin. The more noticeable outbreak then follows from initiation of the carcass-maggot cycle, as discussed above. Anza *et al.* (2014) extend this principle to propose that the cause of the primary die-off can be independent of botulism. These authors were specifically interested in the role of other pathogenic bacteria, particularly given the congregation of high bird numbers at lakes and wetlands, but it follows that a local die-off caused by any toxic process or impingement on the physical environment might serve the same purpose. Murphy *et al.* (2000) had a similar focus and thought it likely that microcystins or anatoxins resulting from algal blooms might be responsible for a sufficient number of waterbird deaths to provide the trigger for botulism outbreaks. Rocke and Bollinger (2007) noted that other sources of mortality, such as hailstorms, algal poisoning, and other disease agents, may also precipitate type C botulism outbreaks.

Of particular relevance to this review is the role that fish carcasses might play in the development of an outbreak of type C or C/D botulism (Figure 31). Although birds are most commonly cited as passive carriers of type C and C/D spores, the same may apply to fish. As noted at the start of this section, 18 percent of samples obtained from fresh or farmed shellfish from coastal and inland areas of India, and 14 percent of wild fish, contained type C or D spores (Lalitha and Gopakumar, 2000). Although a comparative evaluation of Australian waterways was not identified, it seems likely that spores are circulating through a proportion of Australian fish and shellfish. The carcass of a fish carrying *C. botulinum* spores would provide a suitable environment for germination and release of the botulinum toxin and, in doing so, could initiate the carcass-maggot cycle described above.

It is also possible for type C outbreaks to be initiated by anoxic aquatic events precipitated by eutrophication of a waterbody and the proliferation of aerobic microorganisms (Anza *et al.*, 2014; Espelund and Klaveness, 2014) or the rapid growth and die-off of algal blooms (Galvin *et al.*, 1985). Although these events might lead to the death of wild birds, and initiation of the carcass-maggot cycle, they can occur in the first instance as a distinct and separate process. Galvin *et al.* (1985) investigated an outbreak of type C botulism in wild birds at a lake in Victoria in November and December 1983 that was shown to have resulted from the decay of a floating mass of phytoplankton and vegetation. A similar scenario was described by Woodall (1982) who investigated separate outbreaks of type C botulism at Seven-Mile Lagoon in south-east Queensland in June 1978 and April 1981 and noted large amounts of decaying vegetation and animal material. So-called ‘blackwater’ episodes denote the condition of sudden and precipitous anoxia in Australian waterways that can follow from eutrophication (Section 6.2). These usually follow from flooding rains after a prolonged dry period (King *et al.*, 2012), although it is also possible that substantive fish kills would result in local hypoxic events and thus provide both the catalyst and substrate for an outbreak of botulism. Offsetting both the blackwater hypothesis, and the role of fish carcasses as initiators of the carcass-maggot cycle (above), however, are the field observations of Agriculture Victoria who have investigated numerous blackwater events and fish kills in Victorian waterways and wetlands and have not to-date identified any cases of botulism in associated waterbirds.[48] The peer-reviewed literature is also effectively silent on the role of large fish kills as initiators of botulism outbreaks in natural settings. Indeed, the single record that was identified during this review was a 2005 media statement[49] in which a range of intrinsic factors within a lagoon in northern New South Wales were thought to have led to a fish kill that subsequently resulted in waterbirds dying from suspected botulism.

A further possibility for the initiation of a botulism outbreak is that preformed type C toxin persists over winter in toxin-bearing maggots, or other invertebrates that collect in the sediment of waterways, and precipitates outbreaks in the spring (Wobeser *et al.*, 1983; Hubalek and Halouzka, 1991; Rocke and Bollinger, 2007). In support of this, Hubalek and Halouzka (1991) demonstrated that although the titre had dropped 25 to 40-fold, sufficient toxin remained in maggots placed in perforated bottles and buried in wetland sediments over winter (131 days) to cause death of waterbirds. This scenario was evaluated for completeness, although may be more applicable to colder northern hemisphere winters.

Finally, although most botulism cases in birds result from the ingestion of preformed toxin, empirical evidence suggests that gut toxigenesis may also occur in some situations (Rocke and Bollinger, 2007). In support of this, several cases of type C botulism have been confirmed in moribund waterfowl that were simultaneously diagnosed with severe cases of lead poisoning and vitamin A deficiency while other birds in the vicinity remained healthy (unpublished data cited by Rocke and Bollinger, 2007). The sick birds were severely emaciated and were not ingesting food. Because the wetlands they inhabited were heavily seeded with botulinum spores, it was postulated that these debilitated birds developed botulism secondarily through gut toxigenesis. The theory has merit, although it is not difficult to imagine that these birds might also have ingested preformed toxin in the water they were drinking — whether free or associated with aquatic invertebrates.

7.6.2 Type E outbreaks in fish and wild birds

History and distribution: Botulism in humans, associated with the consumption of fish or marine mammals, has been reported since the early 19th century (Rocke and Bollinger, 2007). An isolate was labelled as type E in 1935, and the disease continues to be a small but important concern for public health. The organism has a wide geographic distribution, although is particularly associated with northern temperate zones (Rocke and Bollinger, 2007). The ability of type E spores to germinate at cold temperatures, and the cold tolerance of vegetative cells, may provide *C. botulinum* type E with an ecological advantage over other anaerobic bacteria in cold water environments (Rocke and Bollinger, 2007).

Type E botulism in wild birds has a more recent history, with the first reported die-off of wild birds occurring in late 1963 on the shores of Lake Michigan's Lower Peninsula (Rocke and Bollinger, 2007). Since then, the disease has become established through the Great Lakes ecosystem (Brand

[48] Personal communication with Prof. Grant Rawlin, Research Leader, Veterinary Pathobiology, Biosciences Research, Agriculture Victoria Research, Victorian Department of Economic Development, Jobs, Transport and Resources (August, 2018).

[49] See: http: //www.abc.net.au/site-archive/rural/content/2005/s1529708.htm

et al., 1983; Brand *et al.*, 1988). *Clostridium botulinum* type E has been found in the intestinal contents of fish taken from the Great Lakes (Getchell *et al.*, 2006; Yule *et al.*, 2006b), as well as in dead fish collected from Lake Michigan (Getchell and Bowser, 2006), but less reliably in fillets of fish muscle (Yule *et al.*, 2006b). The presence of *C. botulinum* type E in the sediments of the Great Lakes (Getchell and Bowser, 2006) and the Gulf of St. Lawrence (Laycock and Loring, 1972) has been demonstrated. The only other location where significant wildlife mortalities from type E botulism has been noted is the Canche Estuary in France (Gourreau *et al.* 1998; as cited by Rocke and Bollinger, 2007). In February 1996, 5,000 to 10,000 birds died in the estuary and diagnostic testing confirmed type E botulism. In November of that year the disease recurred, killing 4,000 to 6,000 individuals of the same species. Contaminated fish waste in a nearby dump where the birds were known to feed was the suspected source of toxin. Type E botulism has, however, been associated with outbreaks in farmed fish (Cann and Taylor, 1982; Eklund *et al.*, 1984).

To the best of our knowledge, type E *C. botulinum* has not caused human cases of botulism within Australia. A review of foodborne botulism in Australia found only two cases in the period 2002 to 2017, and these were attributed to type A and type B (Ramachandran *et al.*, 2017). Although spill-over from an essentially aquatic environment to human health is not common (Rocke and Bollinger, 2007), it would seem likely that a small number of human cases would have been observed had type E been endemic and widely distributed within the sediments of Australian waterways during this period. That notwithstanding, the importance of type E outbreaks to birdlife and aquatic animals in North America – and the special role that fish play in the ecology of the disease – warranted its consideration within this review.

Key species affected: although fewer bird species have been recorded as affected by outbreaks of type E botulism than by outbreaks of type C, this seems likely to reflect the fact that type E outbreaks involving wild birds have to-date been limited to a specific region within North America and to France. There is no evidence to suggest that type E toxin is less pathogenic to birds than type C toxin. The feeding and foraging habits of particular species is, however, likely to dictate their level of exposure to particular sources of type E toxin — whether this is associated with mussels, fish or aquatic invertebrates – and, thus, their vulnerability and mortalities (Getchell and Bowser, 2006). These authors noted a repeated pattern of annual occurrence on Lake Erie in the Great Lakes ecosystem. In late July or early August, there were small-scale die-offs of gulls and, less commonly, of other species such as cormorants and terns, which shared colony sites. Later, migrating shorebirds, such as sanderlings, plovers, and sandpipers, were affected. Large-scale die-offs began in late September, peaked in late October and November, and involved primarily predatory fish-eating species.

Fish are considered significantly more sensitive to type E botulinum toxin than they are to type C toxin (Rocke and Bollinger, 2007). That notwithstanding, individual species of fish are also differentially sensitive to type E toxin and react differently in terms of their behaviour and changes to skin pigmentation (Getchell *et al.*, 2006). Carp, in particular, are very sensitive to type E toxin (Haagsma, 1975). Carp orally administered type E toxin died from doses as low as 0.24 mouse lethal doses per gram of fish, although survived oral dosing with type C of up to 427 mouse lethal doses per gram of fish (Haagsma, 1975). Some fish species endemic in the Great Lakes ecosystem (for example, the round goby) showed pigment change upon challenge with type E toxin while others (for example, the rainbow trout) showed marked behavioural changes (Getchell *et al.*, 2006). Similarly, Yule *et al.* (2006) found that that yellow perch artificially-challenged with type E toxin survived significantly longer than rainbow trout, round goby or walleye. Both groups of authors postulated that such changes in behaviour, which included erratic swimming or breaching (where the fish swims head-first upwards in water), may attract bird predators to the affected fish, and that this might help to explain why fish-eating birds are particularly vulnerable in outbreaks of type E botulism.

Outbreak characteristics: the preferred habitat of *C. botulinum* type E is temperate, fresh and brackish water sediments, although cases and outbreaks of type E botulism have occurred infrequently at the Salton Sea in southern California — a hot marine environment (Rocke and Bollinger, 2007).

In a study based on Lake Michigan, Lafrancois *et al.* (2011) found that type E botulism outbreaks that included significant avian mortality occurred most frequently in years with low mean annual water levels and higher mean surface water temperatures. Although trends in fish populations at this location did not strongly correlate with contemporary outbreaks, Lafrancois *et al.* (2011) noted that outbreaks in the 1960s coincided with a high abundance of alewife (an anadromous species of herring), and that recent outbreaks coincided with rapidly increasing abundance of the round goby. As noted previously, this benthic species was identified by Getchell and Bowser (2006) as likely to play a key role in the epidemiology of type E botulism outbreaks in the Great Lakes ecosystem. Lafrancois *et al.* (2011) also found that botulism outbreaks occurred cyclically, and that the frequency of outbreaks did not increase over the period of record.

Type E spores are ingested routinely by healthy fish and cause no harm as germination or growth of *C. botulinum* type E does not generally take place in living fish (Eklund *et al.* 1984). Ingested spores pass through the digestive tract. Under experimental conditions, most fish fed approximately 1,000 type E spores were no longer carrying spores after 2 days (Sugiyama *et. al*., 1970; as cited by Rocke and Bollinger, 2007).

The source of toxin in outbreaks of type E botulism in wild birds is not known with certainty. Vegetative cells replicate in decomposing anaerobic carcass materials and produce type E toxin (Rocke and Bollinger, 2007). Spores, vegetative cells and preformed toxin can also accumulate within sediment (Eklund *et al.*, 1984). These authors noted that toxins obtained from pure cultures of type E *C. botulinum* isolated from the sediments of ponds produced clinical botulism in salmon. Toxin was not, however, detectable in samples of water or salmon feed. The key source of toxin (4 to 20 000 MLD/g) was found to be dead fish that had accumulated on the pond bottoms. These were fish that had died with spores within their gastrointestinal tracts.

Getchell and Bowser (2006) investigated the role that mussel beds may play as a substrate for vegetative multiplication and a means by which preformed toxin could be filtered and concentrated. The mussels themselves were unaffected, although the benthic fish (such as gobies) and invertebrates that feed on them, or amongst their detritus, were exposed to the bacterium and toxins (Getchell and Bowser, 2006). While a toxigenic process was not associated with type E botulism in fish (Getchell *et al*, 2006; Rocke and Bollinger, 2007), small benthic fish may remain alive sufficiently long as to be predated upon by larger fish and fish-eating birds, allowing the disease to be amplified through the food web.

The role of carrion-feeding invertebrates in type E botulism outbreaks has not been established with certainty. Eklund *et al.* (1984) found that low titres toxin (2 MLD/g) could be detected in tubifex worms collected under dead fish in the sediment of salmon ponds. Blowfly larvae may also be a source of type E toxin in some situations, but do not appear to play the key role observed in type C outbreaks (Rocke and Bollinger, 2007). Involvement of non-piscivorous shore birds in some type E botulism die-offs suggests that aquatic or terrestrial invertebrates, or sources of toxin other than fish, may be important (Rocke and Bollinger, 2007).

Chun *et al.* (2013) found that approximately 75 percent of green *Cladophora* (algal) extracted from shorelines of the Great Lakes between June and October 2011 contained type E botulinum toxin. These authors also noted that the mats contained vegetative *C. botulinum* cells and concluded that accumulations of phytoplankton in this setting were likely to provide a niche habitat for *C. botulinum* and might be a risk factor for the development of type E outbreaks. Lafrancois *et al.* (2011) found that type E outbreaks within Lake Michigan occurred most frequently in years with a lower than average annual water level and higher surface temperature. These authors suggested that climate factors may impact directly on the bacterium and its spores, or may act to stimulate benthic invertebrates, which then concentrate the organism and its toxin and facilitate its passage upward to higher orders of the food chain.

Of key importance to this review is the potential for fish kills resulting from causes other than botulism to generate sufficient carcasses to initiate an outbreak of botulism. This was noted by Eklund *et al.* (1984), for example, who had observed that salmon carcasses harbouring type E spores, vegetative cells and toxin gathered within the sediment of ponds and were then cannibalised by living fish.

7.7 Botulism outbreaks in Australia

The early seminal work of Bennetts and Hall (1938) was focussed on type C cases of botulism and outbreaks in Australian sheep. In this scenario, feral rabbits were present in epidemic numbers in Western Australia and their carcasses were predated upon by sheep deficient in certain minerals (a phenomenon termed 'pica'). Bennetts and Hall (1938) undertook a number of critical field trials to establish the infectivity of rabbit carcass materials and contaminated drinking water. Some parts of this work are discussed in Section 7.3. Later, Harrigan (1980) described an outbreak of type C botulism in broiler chickens. Kelly *et al.* (1984) discussed a type C or D outbreak of botulism in Australian horses, while Thomas *et al.* (1988) and Szabo *et al.* (1994) described type B outbreaks in horses. Smart *et al.* (1988), Jubb *et al.* (1993) and Main and Gregory (1996) discussed a range of type C and D outbreaks in cattle and diagnostic tools that could be used for this species.

Outbreaks of botulism in Australian wild birds were reviewed in 2013 by Wildlife Health Australia (Grillo *et al.*, 2013; WHA, 2013). In these reviews it was noted that botulism had been observed in wild birds since the 1930s. Most of the earlier reports were of local outbreaks, with relatively low numbers of affected birds (less than 100), although approximately 1,000 were killed in two outbreaks of type C botulism investigated by Woodall (1982) at Seven-Mile Lagoon in south-east Queensland, and approximately 1,500 birds were killed in an outbreak of type C botulism investigated by Galvin *et al.* (1985) at Lake Lalbert in north-western Victoria.

Wildlife Health Australia (WHA, 2013) paid particular attention to the period 2006 to 2012, during which 68 events of suspected or confirmed avian botulism were observed in Australian wild birds and reported to the eWHIS database.[50] In 10 of these events, multiple species of birds from several bird orders were affected, although Anseriformes (ducks, geese and swans) were involved at the majority of events. Most of the reported cases of avian botulism took place during a 6 month window from November to April, while fewer cases were reported in the cooler months of the year. An unusually high incidence of outbreak events was recorded for the periods (quarters) January and March 2011 and October to December 2012.

- **January and March 2011**: in New South Wales, reports were received from areas near Tweed Heads, Newcastle, Albury and Wagga Wagga. Reports were also received from close to Brisbane, Alice Springs, Melbourne, Shepparton and Bendigo. Anseriformes were involved in all events, with multiple species affected including magpie geese, Australasian grey teal, Pacific black ducks, muscovy ducks, masked lapwings and black swans.
- **October to December 2012**: 10 wild bird mortality events were attributed to suspected or confirmed cases of botulism. Botulism was confirmed by ELISA in one pelican and five ducks found dead in a lake near Port Headland, Western Australia. In the other nine events, a presumptive diagnosis of botulism was based on clinical signs, environmental conditions and a lack of lesions upon necropsy. Five of the events reported were from Victoria, and involved mainly Australian white ibis and (variably) wild ducks and pelicans. Two events each were also recorded in New South Wales and Queensland with only wild ducks affected.

[50] http: //www.wildlifehealthaustralia.com.au/DiseaseIncidents.aspx

8 Impact of fish kills on food webs and ecosystems

Paton and McGinness (2018) used a formal process of scientific review to identify and evaluate papers from the published literature concerned with the ecosystem effects of fish kills. This CSIRO report was carried through to the peer-reviewed literature in McGinness *et al.* (2019a and supplementary material in 2019b) with an emphasis on the risks associated with the proposed release of CyHV-3.

The most visible outcome of Paton and McGinness (2018) and later works was an acknowledgement of the paucity of research in this area. The authors were able to identify just 18 studies that fulfilled their inclusion criteria. Of these, 14 focussed on seabirds, three on freshwater plankton and one on marine macroinvertebrates. **No studies describing the ecosystem-wide effects of fish kills on freshwater communities were identified**. Likewise, no studies in any context addressed the consequences of fish kills on amphibians, crustaceans, reptiles or mammals (McGinness *et al.*, 2019a). Details of the review process were provided by Paton and McGinness (2018) and demonstrated that the search and analysis of the literature was comprehensive and exhaustive.

The balance of the papers described the authors' deductive reasoning about the likely ecosystem effects of fish kills and the special case of a carp die-off following release of CyHV-3. Much of what follows in Sections 8.1 to 8.3 below was adapted from Paton and McGinness (2018), with cross-references to McGinness *et al.* (2019a) and the supplementary material provided in McGinness *et al.* (2019b).

Section 8.4 describes a quantitative exercise that was undertaken to elicit the opinion of experts in waterbird ecology about the likely impacts that a reduction in juvenile carp might have on waterbird population strength and recruitment.

8.1 Spatial scale and timing

One of the key considerations when evaluating the possible ecosystem-wide effects of a carp die-off is its spatial extent – including the connectedness of the affected waterway(s) and whether vulnerable species are inherently mobile or otherwise able to avoid exposure to the most severely affected areas (Paton and McGinness, 2018). The extent of clustering of vulnerable species will also be important, in the sense that a relatively small carp die-off within a given disconnected wetland could be significant if that wetland is one of a few remnant locations where particular species or ecosystems are known to exist. Although the nature of the stressor was quite different, the impact of the 2010-11 blackwater event within the Murray River (Section 6.2) was particularly devastating for the Murray crayfish as: (a) the blackwater event spanned such a large section of the Murray River channel (approximately 1,800 km); and (b) the Murray River is one of the few remaining strongholds for this species in the wild (McCarthy *et al.*, 2014).

The ecosystem effects of a carp die-off might also be influenced by its timing – in particular, if the die-off coincided with spawning or breeding of vulnerable fish, bird or amphibian species (Paton and McGinness, 2018). Many species are less mobile during breeding events and are thus less able to avoid the outcomes of a fish kill. Many species also have increased, altered or very specific dietary needs during the breeding season – in particular, during the period when young are being raised. Many native piscivorous waterbird species, for example, will rely on a ready supply of small fish suitable for growing chicks and will be reluctant to venture far from nests. McGinness *et al.* (2019a) also explain that the nutrient characteristics of food required by growing chicks (specifically, the lipid content) differs from that required by adult birds. Adult birds could in some settings switch to alternative prey (for example, frogs, crustaceans or small-bodied native fish) although the lipid content of these alternatives is unlikely to match that of juvenile carp. Adult birds could also, at other times of the year move from the wetland to forage for alternative sources of food. Waterbirds, native fish and amphibians may not recruit strongly each year, and if a fish kill coincided with an otherwise successful breeding season then the long-term outcomes for the population could be more substantial.

8.2 Impact on food webs

The ecosystem effects of a carp die-off are likely to be affected by a range of factors specific to the vulnerable species within each ecosystem.

Diet is a key consideration. There has been speculation around the importance of carp in the diets of native fauna in Australia, with some authors suggesting that carp are not of major importance in the diet of waterbirds (Koehn, 2004; McColl *et al.*, 2016). Paton and McGinness (2018) maintained, however, that this supposition has been based largely on the fieldwork of Barker and Vestjens (1989) whose surveys preceded the expansion of the carp population throughout (in particular) the Murray-Darling Basin. Given the extent of this expansion, and the high proportion of the biomass in most freshwater rivers that is now composed of carp, it seemed plausible to Paton and McGinness (2018) that carp could represent a larger proportion of the diet of many piscivorous, carnivorous and generalist consumers, detritivores and scavengers than otherwise considered. For example, waterbirds known to consume fish regularly include spoonbills, ibis, egrets, pelicans, darters, cormorants and kingfishers. Citing Taylor and Schultz (2008), McGinness *et al.* (2019a) noted that, in one study, 94 percent of the dietary biomass of eastern great egrets (*Ardea modesta*) in the Murray-Darling Basin consisted of carp.

Some predator species may be limited by their gape size to predation of only juvenile carp, and because juvenile carp are more susceptible to CyHV-3 (Perelberg *et al.*, 2003; Ito *et al.*, 2007) this may translate to a more significant population-level impact. A comparative example of this phenomenon was provided by Paton and McGinness (2018) in the little tern, *Sterna albifrons sinensis*, which can only consume fish of a size class less than 10 cm in length (Taylor and Roe, 2004). For this reason, little terns only feed only on juvenile pilchard (*Sardinops sagax*). A 1995 mortality of adult pilchards (fork length 12 to 18 cm) that occurred along the southern coastline of Australia consequently had minimal impact on little tern breeding (Taylor and Roe, 2004). However, the 1998 mortality that affected principally juvenile pilchards (fork length less than 10 cm) within the same area was correlated with a significant reduction in little tern breeding success (Taylor and Roe, 2004).

Other species may prefer to predate upon the larger, adult size class of a species to minimise foraging effort, but can choose to feed on the smaller juveniles at the cost of expending more energy foraging if the need arises (Paton and McGinness, 2018; citing Dann *et al.*, 2000; Chiaradia *et al.*, 2003; Taylor and Roe, 2004; Österblom *et al.*, 2006). In this respect and more broadly, the ability of a predator species to cope with the effects of a fish kill may be influenced by foraging flexibility and adaptability (Paton and McGinness, 2018; citing Bunce and Norman, 2000; McLeay *et al.*, 2009; Chiaradia *et al.*, 2010; Cohen *et al.*, 2014; Kowalczyk *et al.*, 2014). The initial or short-term impact of a fish kill on predators was strongly negative within all articles reviewed by Paton and McGinness (2018) yet, given time, most of the populations studied recovered by switching to alternative prey items, by changing foraging locations or, in some cases, by changing foraging strategies (Paton and McGinness, 2018; citing Williams and Bunkley-Williams, 1990; Dann *et al.*, 2000; Chiaradia *et al.*, 2003; Chiaradia *et al.*, 2010). Generally, a piscivorous predator species can substitute one prey species for another, when available, but in doing so may also increase its foraging time or effort, particularly if their new prey item is smaller or of lower quality (Paton and McGinness, 2018; citing Österblom *et al.*, 2006; Gjosaeter *et al.*, 2009, McLeay *et al.*, 2009; Cohen *et al.*, 2014). This increased energy output for foraging may result in lowered breeding success and offspring survival in the first few seasons after the fish kill (Paton and McGinness, 2018; citing Chiaradia *et al.*, 2010).

One of the concerns in the context of the CyHV-3 is that a sudden and precipitous die-off of carp might result in an increase in predation pressure on native species (Paton and McGinness, 2018). This is likely to be particularly relevant when juvenile carp within a wetland are killed in large numbers by the virus and piscivorous breeding birds then turn to the juveniles of native fish, or amphibian species, as alternative food sources. Australian freshwater piscivorous birds and other fauna are not known to be selective in terms of the fish species they target and are likely to consume whichever species is most abundant and accessible (Paton and McGinness, 2018).

Food quality can also be an important factor to consider, particularly when young animals and breeding are involved. Many bird species have chicks that rely on a high-calorie diet. This means that while adult birds may be able to compensate for the loss of a fish species with another lower quality food source, the chicks may not (Paton and McGinness, 2018; citing Dann *et al.*, 2000; Bunce *et al.*, 2005; Kowalczyk *et al.*, 2014). A switch from lipid-rich to lipid-poor prey may have been responsible for changes in the breeding success and population structure of some piscivorous seabirds (Paton and McGinness, 2018; citing Wanless *et al.*, 2005). Moreover, studies have shown that feeding chicks on a lipid-poor diet negatively impacts fledgling body condition and cognitive ability (Paton and McGinness, 2018; citing Kitaysky *et al.*, 2006). Hence, if a lipid-rich species of fish is killed in high numbers, the chicks of piscivorous birds that feed on that species may be affected regardless of the foraging flexibility and dietary adaptability of the adult. This principle extends not only to seabirds, but to freshwater birds, mammals, reptiles and amphibians that have specialised dietary requirements when breeding – often requiring increased calories and nutrients (Paton and McGinness, 2018; citing Bronson, 1985; White, 2008). If these requirements are not met, then breeding success is likely to decline and may ultimately have longer term impacts on the size of the population (Paton and McGinness, 2018; citing White, 2008).

8.3 Long-term adjustments within natural ecosystems

The ecosystem-wide impacts of carp were reviewed in Section 4.3. In brief, the foraging behaviour of carp results in the suspension of sediments and nutrients and the destruction of macrophytes, increasing the turbidity of the water and thereby limiting light to aquatic plants, which further decreases water quality. Without carp constantly suspending sediment as they forage, turbidity should reduce. Increased light availability would facilitate the re-establishment of some aquatic macrophytes. Macrophyte establishment could in turn be expected to further encourage sedimentation, increase oxygenation and increase habitat suitability for native fauna. Improved water quality would also reduce pressure on water-quality-sensitive fauna, potentially allowing for population increases

Carp predate upon micro- and macro-invertebrates, as well as on the eggs and larvae of native fishes and amphibians. The eggs of bottom-nesting fish, such as the freshwater catfish and Macquarie perch, are particularly vulnerable. Adult carp compete with native benthic foraging fish, and both adult and juvenile carp compete for zooplankton with planktivorous fish and with the planktivorous larvae of most native fish species. If the density of zooplankton is reduced, then grazing on phytoplankton (including algae and cyanobacteria) will also be reduced and the incidence and severity of algal blooms may increase. A long-term reduction in the biomass density of carp has the potential to lessen each of these impacts, although the response is likely to be constrained in many settings by other ongoing stressors – including competition for water flows, agricultural production in catchments and continued deforestation of floodplains.

One of the most striking features of freshwater aquatic ecosystems through much of south-eastern Australia is the dominance of carp. This has the effect of creating an unnaturally simplified food web. The removal or diminution of this single dominant species is likely result in a lowered pressure on the species currently preyed upon or otherwise suppressed by carp. Conversely, the piscivorous fish and bird species that currently feed upon carp will be forced to seek alternative prey and this may increase the pressure on smaller or juvenile native fish. Collectively, the rearrangement of predator-prey relationships may lead to a more complex and diverse food web than is currently the case with carp as the dominant species and, as a result, one that is ultimately more resilient (Chiaradia *et al.*, 2010).

The above notwithstanding, it is difficult to be prescriptive as to the eventual structure and dependencies within re-arranged food webs in individual geographic locations, or types of freshwater waterways, or the species that are most likely to be advantaged or disadvantaged. Speculatively, some native waterbird, fish or amphibian species may encounter greater hardships than they currently face. Others may be able to re-establish thriving and geographically-distributed populations. Conversely, given the current fragility of many freshwater aquatic ecosystems, and their diminished ability to withstand major shocks, it is possible that some local populations of waterbirds, fish or amphibians may be heavily impacted by the immediate effects of a substantial carp die-off – in particular, where this results in catastrophic short-term changes in water quality, a marked decrease in the availability of a staple food source or a significant increase in predation. If these effects are widespread, or extend to the limits of a species' mobility, and occur at a time when vulnerable species are spawning or breeding, then some local populations may not be sustainable.

Ultimately, the characteristics of re-established ecosystems across geographic locations and types of freshwater waterways are likely to be quite different. It is also likely that human management will influence food webs and inter-species dependencies. This will include the management of environmental flows and may include the translocation of individual species once particular local environments have restabilised. As an example, the endangered Murray hardyhead is currently being reintroduced to areas of the Chowilla Floodplain as part of the redevelopment of that ecosystem as regional salination is addressed.[51]

8.4 Elicitation and analysis of expert opinion

8.4.1 Objectives of the study

The purpose of this study was to seek the views of experts to help clarify the importance of juvenile carp to the diet of piscivorous waterbirds (including the strictly piscivorous waterbirds, as well as those whose diet may include some fish), and the likely impact that removal of this source of food might have on waterbird population strength or sustainability.

8.4.2 Approach to the enrolment of experts and analysis of opinion

The study was based on the quantitative analysis of a questionnaire that was completed by a group of experts. For the purpose of the study, experts were defined as waterbird or fish ecologists with sufficient observations or knowledge of wetlands to draw inference about the likely impact of a change in the recruitment of carp on waterbird breeding success and population dynamics.

In total, 34 experts with the requisite experience and skills were identified through networking and by directly approaching jurisdictions for nominations of people who were considered suitable (Appendix B.1). All nominated individuals were approached, with an initial email sent to explain their selection and provide some background to the study. The survey was then distributed with an accompanying letter providing background material and instructions (Appendix B.2). Three voluntary webinars were held to help clarify the study's goals and the use of the spreadsheet-based questionnaire. Those experts that did not enrol in the study were followed up by email and telephone.

8.4.3 Response to the survey

Of the 34 identified experts, 16 declined to participate. Where provided, reasons for declining included the time that would be required to complete the exercise (1 to 2 hours was suggested in the approach letter) and the difficulty inherent to estimating an ecosystem response quantitatively.

In particular, experts felt that there was too much uncertainty in:

[51] See: http: //www.environment.gov.au/water/cewo/media-release/endangered-fish-returns-western-nsw

- Contribution of juvenile carp to the diet of piscivorous waterbirds
- Response of breeding waterbirds to a marked decline or removal of juvenile carp
- Extent to which native fish species (small-bodied and juvenile large-bodied native fish) would be predated upon given a marked decline or removal of juvenile carp.

Of the remaining 18 experts, four completed a questionnaire within the study period and a further four undertook to complete a late questionnaire.[52] Of these four, three attempted the questionnaire but found that they could not provide estimates, hence the final sample size was five responses. The balance (10 experts) did not respond to the invitation to take part in the survey.

8.4.4 Outcomes for waterbird food webs

The response rate (number of returned surveys) was too low to enable robust quantitative estimates to be drawn as to expert belief about the likely effects of reducing juvenile carp abundance. For those experts who gave reasons for not being able to provide responses (see above), the clear conclusion was that there is a significant knowledge gap with regard to: (a) the importance of juvenile carp in the breeding biology of waterbirds; (b) how the waterbirds would respond to less juvenile carp; and (c) the extent to which native fish populations (for example, bony bream) may recover and compensate for the reduction in carp. Finally, the species of waterbirds involved are highly mobile, and well adapted to utilising wetlands across eastern Australia, and the experts found it challenging to factor this into answers to the questions posed.

The results obtained, however, were illustrative of the level of understanding of this topic amongst waterbird ecologists.

For waterbirds that are predominantly-piscivorous (Figure 18), a reduction the survival (and, therefore, availability) of juvenile carp was believed likely to equate to a reduction in breeding success. Whilst this trend remained for all levels of reduction in juvenile carp, the extent of the reduction in breeding success wasn’t quite commensurate with the reduction in juvenile carp survival. This indicated that the experts did not believe that predominantly-piscivorous waterbirds were completely reliant on juvenile carp as a source of food for chicks.

For waterbirds that are partly-piscivorous (Figure 19), the elicited responses were more variable and the impact (if any) was substantially smaller. Some experts believed that a reduction in juvenile carp could be beneficial to variably piscivorous waterbirds, although the overall general trend was negative.

[52] The late questionnaires had not been received at the time of compiling this Draft Report

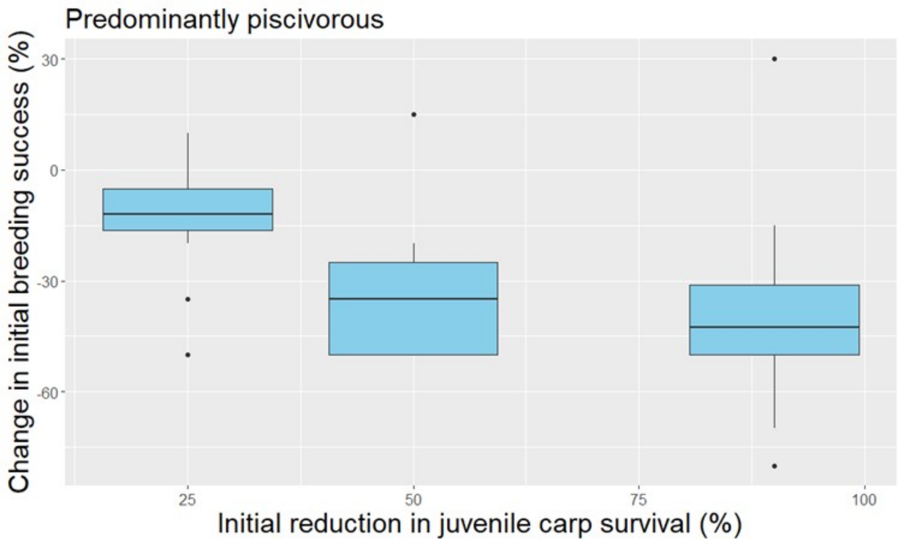

Figure 18 Estimated change in breeding success for predominantly-piscivorous waterbirds

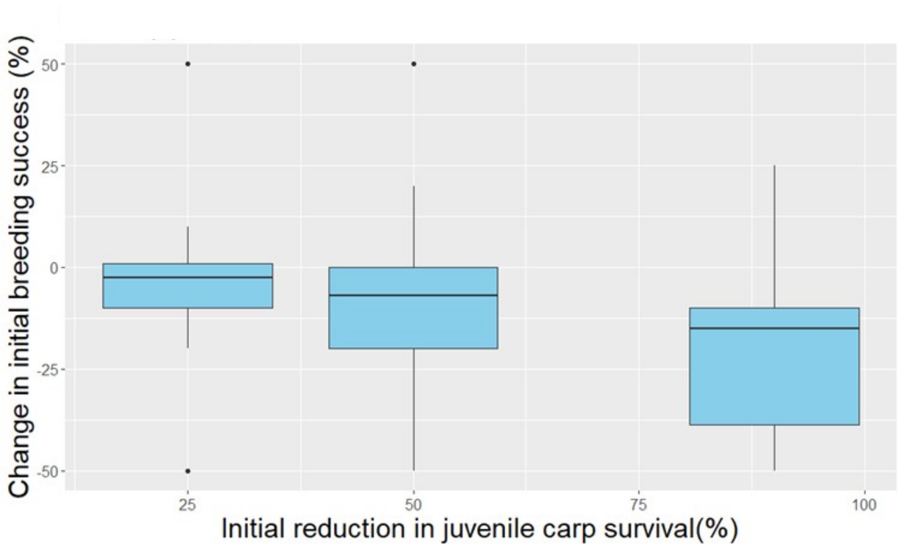

Figure 19 Estimated change in breeding success for partly-piscivorous waterbirds

The elicited responses for the long-term impact of a reduction in juvenile carp survival on predominantly-piscivorous waterbirds tended to be negative, and largely variable (Figure 20).

By comparison, experts believed that a long-term reduction in the survivability of juvenile carp would result in a slight increase in the long-term population strength of partly-piscivorous waterbirds (Figure 21).

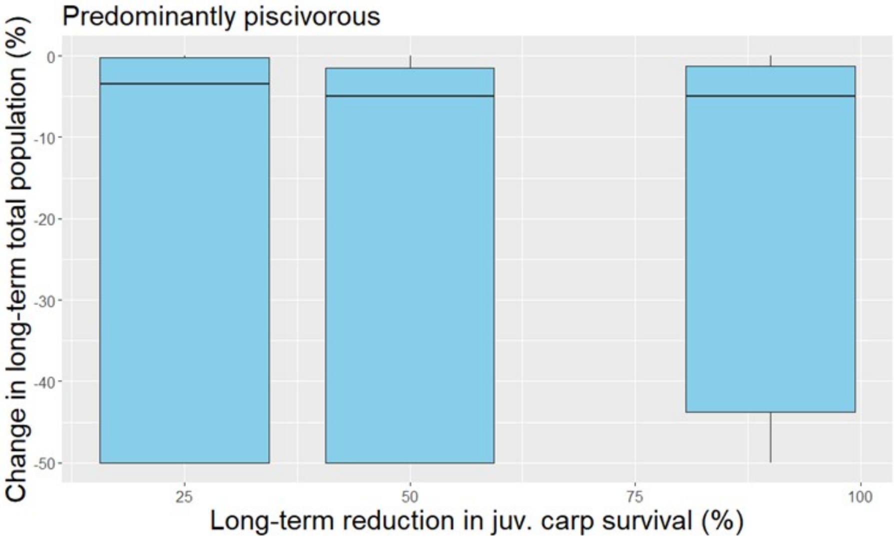

Figure 20 Estimated change long-term population of predominantly-piscivorous waterbirds

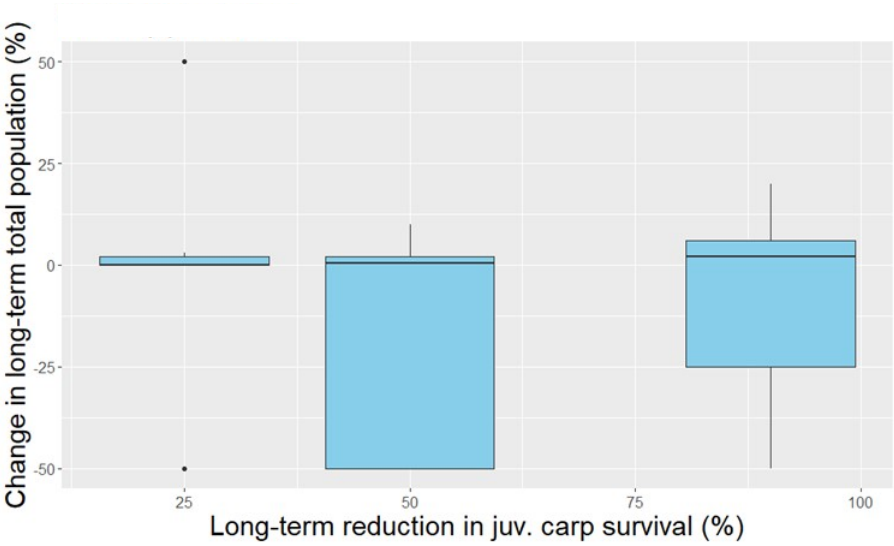

Figure 21 Estimated change in the long-term population of partly-piscivorous waterbirds

8.4.5 Conclusions from the elicitation of expert opinion

None of the elicited responses indicated a belief that even a high level of loss of juvenile carp would result in a catastrophic impact on waterbird population strength or sustainability.

There was a belief that predominantly-piscivorous waterbirds would be affected negatively, implying that prey-switching to other fish (native or introduced) would not be completely successful in the short term. The results for partly-piscivorous waterbirds were more variable. Short-term impacts tended to be negative while long-term impacts tended to be slightly positive. The implication of a positive trend is that the removal of carp would result in a long-term improvement in habitat quality – and that this would then lead to an increase in biodiversity and ecosystem health, and a consequent abundance of other food sources.

The responses elicited from the study must be considered in light of the high proportion of experts who felt that the exercise was simply too difficult on account of the inherent uncertainty in how both waterbirds and native fish population would respond to a reduction in juvenile carp survival. Some of this uncertainty can be reduced through further basic research. The importance of juvenile carp to the diet of nesting waterbirds, for example, could be evaluated through reasonably straightforward field studies. The impact that decline in, or removal of, juvenile carp might have on species of native fish, frogs and crustaceans would be more difficult to investigate.

9 Evaluation of the resurgence of other invasive animal and plant species

9.1 Resurgence of invasive animal species

9.1.1 Introduction

Australian freshwater fish communities are generally species-poor, although there are a number of non-native invasive species that occur in the river systems (García-Díaz *et al.*, 2018). Many of the non-native fish have long residence times as they were released as part of 'acclimatisation' practices in the mid-late nineteenth century, whereas later introductions escaped, or were released from, aquariums (García-Díaz *et al.*, 2018). Non-native species that have had a sufficient period of time to naturalise across their new distributions may exist in an ecosystem at a new-equilibrium. It follows then that their removal could result in disruption to ecological interactions that have re-stabilised following initial invasions.

One indirect ecological consequence of the removal of carp may be the competitive release of other non-native fish species that will outcompete native fishes for a vacant niche presently occupied by carp. This scenario is an important consideration in the biocontrol of a species as prolific as carp, as the success of removal of one invasive species may not lead to any ecological restoration of native species if they are faced with competition from an alien species previously suppressed by carp.

Tench (*Tinca tinca*) is an example of an invasive freshwater species whose distribution and ecological niche overlap with carp. Tench were introduced to Australia in 1876. The introduction of carp appears to have lowered the population of tench, to the point that they are now rare in the Murray-Darling Basin and New South Wales and are not present in the Australian Capital Territory or Queensland (Lintermans and Murray-Darling Basin Commission, 2007). Elsewhere, DeVaney *et al.* (2009) looked at carp and tench (and two other species) using species-distribution modelling with broadscale climatic and topographic predictors. As tench were not filling their range, it was suggested they could be limited in establishment success by biotic interactions.

Without experimental evidence to examine competition between species (for example, experiments based on mesocosms), observational data in the form of location or abundance of different fish species, at different times, allows for correlations between species to be quantified, leading to the formation of hypotheses of interaction. Joint-species-distribution modelling (for example, Pollock *et al.*, 2014; Hui, 2015) allows for communities of species across multiple sites to be modelled in combination. For example, Inoue *et al.* (2017) examined communities of freshwater fishes with mussels, rather than each species individually as is typical in species distribution modelling. Using joint-species-distribution modelling in this way quantifies a broad-scale interaction network by jointly modelling observations of fish occurrence alongside environmental information. The strength of joint-species-distribution modelling is that it allows for latent processes that may describe different interactions not evident from the abiotic predictors employed alone. Understanding different habitat use patterns between species provides important information for predicting how different species may respond to management actions (Laub and Budy, 2015). In the case of the removal of carp, the focus was on quantifying latent processes that may describe species interactions, with interaction strength putatively indicating the opportunity for recolonisation.

The aims of this evaluation were to:

1. Describe patterns of co-occurring non-native freshwater species in south-eastern Australia
2. Determine which, if any, species have distributions that may be explained by competitive exclusion, in particular with carp.

It was proposed that the stronger the interaction strength with carp, the more likely that the removal of carp will present opportunities for the associated fish to recolonise the habitats made vacant in the event of successful biological control.

9.1.2 Approach to the evaluation

9.1.2.1 Species list

The evaluation focused on non-native fish species that are found in the Murray-Darling Basin, as this encapsulates the key distribution of carp in Australia. Although the distributions of a range of non-native species found within the Murray-Darling Basin may extend outside this river system, we were mainly interested in categorising sites that were accessible to carp across the extent of its invasion history. A list of alien fish species of the Murray-Darling Basin was obtained from Lintermans and Murray-Darling Basin Commission (2007). Eleven species were included in the study for further analysis. Table 7 shows the 11 species, introduction dates and their pathways of introduction.

Table 7 Species included in the evaluation of co-occurrence

Pathway	Family	Species	Common name	Introduction	ALA records	Unique sites in this study
Aquaculture	Cyprinidae	*Cyprinus carpio*	Carp	1800s/1960s	38057	1219
Acclimatisation	Cyprinidae	*Carassius auratus*	Goldfish	1860s	8284	861
Acclimatisation	Cyprinidae	*Rutilus rutilus*	Roach	1860s	779	69
Acclimatisation	Cyprinidae	*Tinca tinca*	Tench	1876	1581	125
Acclimatisation	Percidae	*Perca fluviatilis*	Redfin perch	1861	9386	674
Acclimatisation	Salmonidae	*Oncorhynchus mykiss*	Rainbow trout	1894	5085	262
Acclimatisation	Salmonidae	*Salmo salar*	Atlantic salmon	1864-1870	52	1
Acclimatisation	Salmonidae	*Salmo trutta*	Brown trout	1864	19216	662
Acclimatisation	Salmonidae	*Salvelinus fontinalis*	Brook char	1870s	195	0
Biocontrol	Poeciliidae	*Gambusia holbrooki*	Eastern mosquitofish	1925	16066	1671

Pathway	Family	Species	Common name	Introduction	ALA records	Unique sites in this study
Ornamental	Cobitidae	*Misgurnus anguillicaudatus*	Oriental weatherloach	1861	958	136

ALA = Atlas of Living Australia (see: http://www.ala.org.au - accessed 13/05/2019)

The term 'unique sites' refers to unique grid cells following the rescaling process (see main text)

9.1.2.2 Distribution

Distribution data for each of the species was obtained from the Atlas of Living Australia,[53] using the 'ALA4R' package (Newman *et al.*, 2019) in the statistical computing package 'R'. To account for potential differences in the observation and recording processes, and inherent spatial bias in sampling, steps were undertaken to standardise the data across species. As the Atlas of Living Australia data is a repository for many data sources it was important to remove some of the error associated with the different methods and places of data collection. First, presences were rescaled to a 5 km^2 grid cell to aggregate observations that might have been recorded with different coordinates or other sources of spatiotemporal error. While this changed the scale of the analysis (including the assumption that interactions between species are apparent and relevant at the 5 km^2 level), it allowed for grid cells to reflect a 'site' and, thus, the presence or absence of multiple species in the waterways contained within. This was considered reasonable in view of the mobility of the fish species included. The final extent included 2,879 5 km^2 cells. This included the distribution points across the Murray-Darling Basin, with a western boundary of 138.1°E and a northern boundary of 22.1°S. A convex hull was drawn around the points in this area to define the study extent.

Following setting the study extent, the temporal resolution of the data was evaluated. Given differences in introduction rates, observations reflect processes linked to abundance, such as lag-phases and spread rates of non-native species. It was therefore important to identify a time period that reflected similar stages of invasion for all species being considered, so that each species was as close to equilibrium as possible. It was equally important not to select too long a time period, so that observations reflected the chance of each species observed at a given site being actually present at the same time. To do this, the trajectories of the number new grid cells occupied per year for all species were explored. This gave an indication of the rate of change or spread in a distribution. A yearly stepwise process was used to identify the cumulative number of new grid cells for observations per species. After visualising the observation rates, observations made between 2000 and 2015 were selected (Figure 22 and Figure 23). After setting the grid cell size, the extent, and time frame, two species (Atlantic salmon and brook char) had too few unique sites remaining for further analysis, leaving a list of nine species for the balance of the evaluation.

[53] See: http://www.ala.org.au (accessed 20 May 2019)

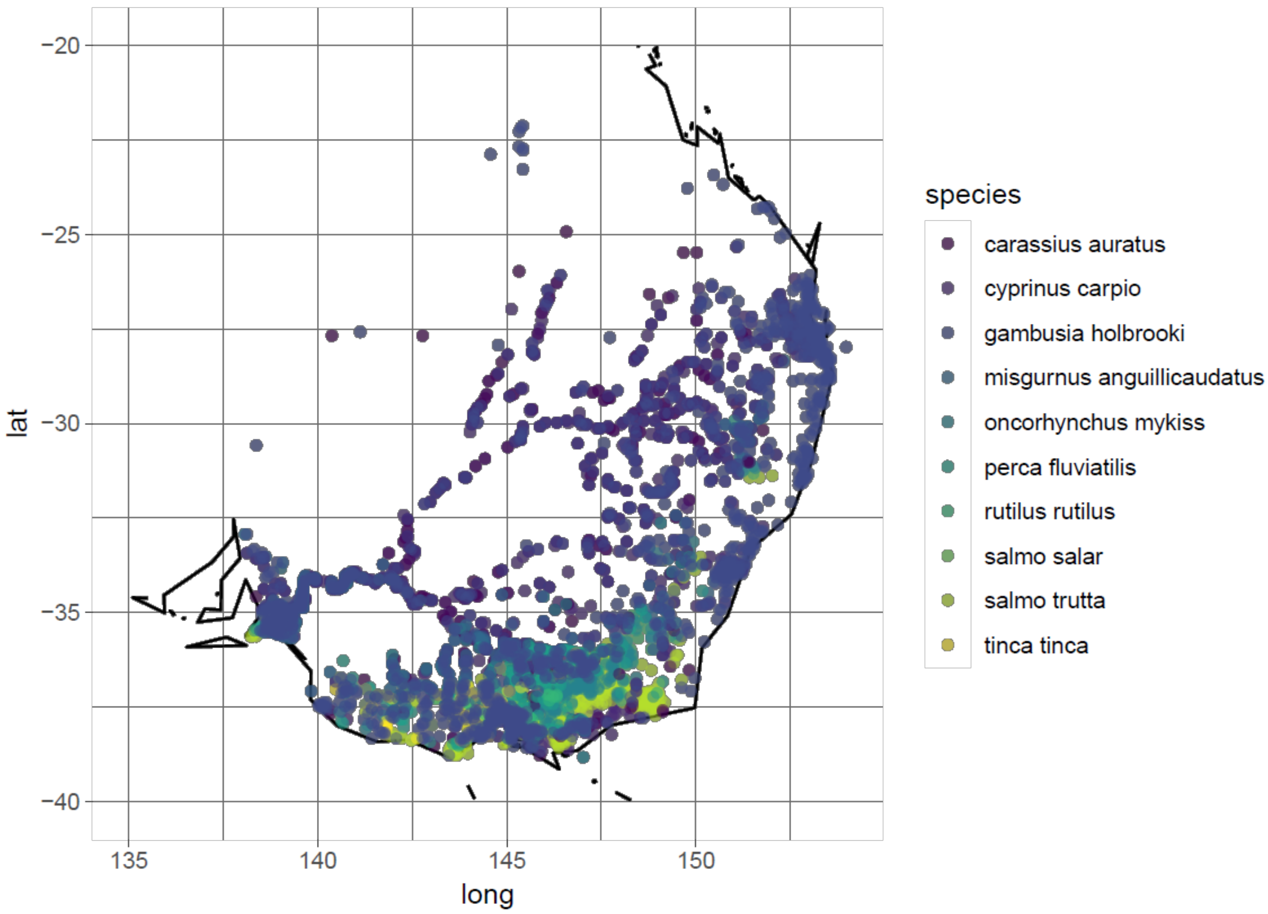

Figure 22 Distribution data for the nine invasive fish species in this study

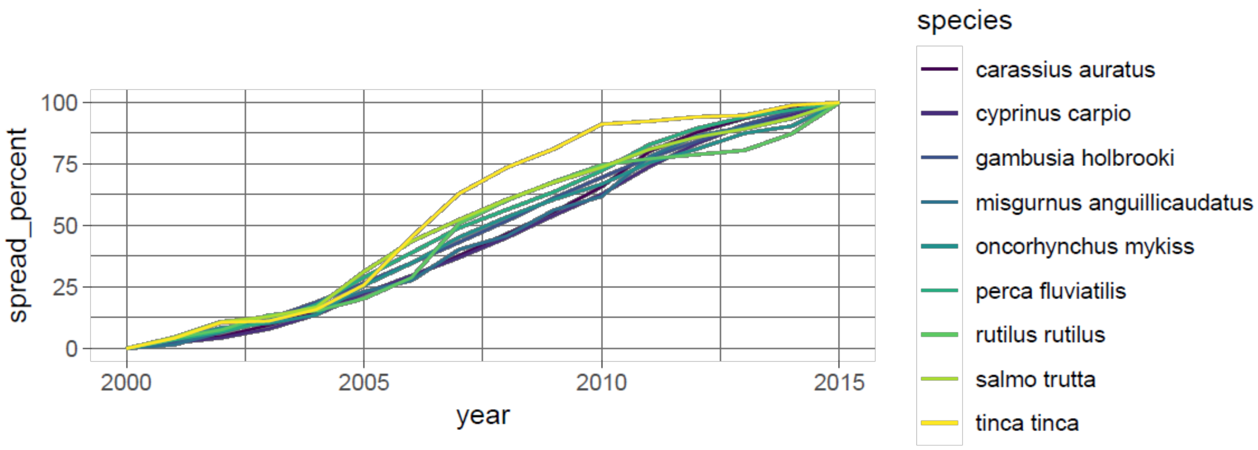

Figure 23 Cumulative new records

Cumulative new records (new grid cells per year, expressed as a percentage of total of cells present in 2015) of the fish species from 2000-2015

9.1.2.3 Environmental data

Predictors were based on those used for species distribution models of freshwater fish elsewhere in the literature. Elith *et al.* (2008), for example, contains an example that was used as a guide.

Table 8 shows the predictors used. All environmental data were obtained from the Environmental Stream Attributes (v1.1.1, August 2012) linked to the National Catchment and Stream Environment Database version 1.1.5 (Stein *et al.*, 2014), including foundation layers for the Australian Hydrological Geospatial Fabric.[54] Data at each point (centroids of rescaled grid cells) were extracted using the NNJoin (Tveite, 2017) plug-in for QGIS (QGIS Development Team, 2016).

Table 8 Environmental predictors used in this study

Predictor Description	Predictor Name
Catchment average Saturate Hydraulic Conductivity	CAT_A_KSAT
Stream and environs average driest quarter mean temperature	STRDRYQTEM
Stream and environs average coldest quarter mean temperature	STRCOLDQTE
Maximum slope in downstream flow path	DOWNMAXSLP
Catchment average annual mean temperature	CATANNTEMP
Stream and environs average wettest quarter rainfall	STRWETQRAI
Stream and valley percentage natural forest cover	STRFORES_1
Flow Regime Disturbance Index	FRDI

9.1.2.4 Joint-species-distribution modelling

The R package BORAL (Hui, 2015) was used to examine species co-occurrence through joint-species-distribution modelling. This is one of a number of joint-species-distribution modelling packages that allow for modelling community data and co-occurrence within a generalised linear modelling framework (Wilkinson *et al.*, 2019). BORAL allows for models of counts of species at multiple sites to be fitted with environmental covariates and trait data to determine how much observed distributions can be explained by either the covariates, or residual covariates (Hui, 2015).

When using environmental covariates, BORAL fits a correlated response model and separates the correlations between species due to environmental (abiotic) response and residual correlations (due to other sources of covariation). The latent variables can be interpreted as accounting for residual covariation not explained by the environmental covariates, that is either a missing environmental predictor, or some species interaction (Hui, 2015; Ford and Roberts, 2019). This correlated response model can thus be used to investigate the similarities of species-environment relationships for species abundance at sites, or across a presence-absence matrix, to detect patterns of co-occurrence after accounting for amounts of environmental similarity (Ford and Roberts, 2019). The amount of species co-occurrence explained by covariates can be examined in the trace of the estimated covariance matrix (Hui, 2015; Warton *et al.*, 2015), which is induced by the latent variables, to give pairwise correlation scores across the species included. While the direction of the correlation is not explicitly the direction of the interaction (Ford and Roberts,

[54] See: http://www.bom.gov.au/water/geofabric/index.shtml

2019), it allows for assessment of the influence of the environmental response or residual correlations on species pairs (Inoue *et al.*, 2017)

BORAL also allows for the incorporation of trait data to the random effects normal distributions of correlated response models (Hui, 2015). Trait data for the non-native fish species were obtained from FishBase (Froese *et al.*, 2010) using the R package RFishBase (Boettiger *et al.*, 2012). Traits were only selected if they were complete across all species included in analysis. This had one exception, the Oriental weatherloach did not have a trait record for trophic level, so we included this as 3.3, based on information from García-Díaz *et al.* (2018). The traits that were included in subsequent analysis are shown in Table 9.

Table 9 Traits for the 11 species of invasive freshwater fish included in the evaluation

Species	TL	SL	FL	HL	Troph	DemersPelag	AnaCat	BodyShapeI
Carassius auratus	575.00	467.00	536.00	129.00	2.00	1	2	2
Cyprinus carpio	579.50	491.00	532.50	106.50	2.63	1	2	2
Gambusia holbrooki	530.00	458.00	530.00	116.00	3.10	1	2	2
Misgurnus anguillicaudatus	559.00	489.50	559.00	71.50	3.30	2	1	2
Oncorhynchus mykiss	607.00	542.50	594.00	125.50	3.50	1	1	2
Perca fluviatilis	553.00	468.50	523.00	148.00	3.68	2	1	2
Rutilus rutilus	568.00	461.50	509.00	98.50	2.87	1	2	2
Salmo salar	604.67	550.00	589.67	154.33	3.80	1	1	2
Salmo trutta	609.00	535.00	587.00	125.00	3.28	3	1	2
Salvelinus fontinalis	606.00	550.00	599.00	118.00	3.02	2	1	2
Tinca tinca	596.50	508.00	589.50	110.50	3.78	2	2	2

TL =Total Length (mm); SL = Standard Length (mm); FL = Fork Length (snout → central rays of caudal fin, mm); HL = Head length (mm); Troph (Continuous variable describing trophic level / diet); DemersPelag (1= benthopelagic , 2= demersal, 3= pelagic-neritic); AnaCat (1 = anadromus, 2 = potamodromous); BodyShapeI (1 = elongated, 2= fusiform / normal)

A presence-absence matrix was constructed for nine species across 2,879 sites (5 km^2 grid cells). A binomial model employing purely latent variables was first investigated for the fish species data alone. From this, a two-variable biplot of the unconstrained ordination was constructed. A correlated response model was then constructed. This incorporated the full set of environmental predictors, the trait data and two latent variables. This binomial model was run using 300,000 iterations, a thinning factor of 100 and an initial burn-in of 100,000. Convergence of the models was assessed using Dunn-Smyth residual plots against the linear predictors, sites (rows) and species (columns) respectively, as well as a normal quantile plot of Dunn-Smyth residuals. A biplot of the residual ordination based on the posterior mean estimates (Hui, 2015) was then constructed. From the correlated response model, pairwise species correlations were plotted as a matrix for both the environmental response and residual correlations using the Corrplot package (Wei and Simko, 2017) and as a network graph using the Corrr package (Jackson *et al.*, 2019).

9.1.3 Results of the evaluation

9.1.3.1 Distribution data

Following the downloading of all species occurrences, and subsequent clean-up Figure 22 shows the extent and coverage of rescaled distribution data for the nine invasive fish species in this study, for the period 2000 to 2015. It can be seen from Figure 22 that most of the invasive fish species records have been recorded in the south-eastern corner of the study extent, with observations for carp, goldfish and eastern mosquitofish extending further inland than the other species. Figure 23 shows the cumulative new records (new grid cells per year, expressed as a percentage of total of cells present in 2015) of the fish species from 2000 to 2015. This figure reveals similar patterns of observation (cumulative records) for the species across the period of interest, despite being large differences in abundance.

9.1.3.2 Joint-species-distribution modelling

There is no discernible patterning (identifiable clusters) in both the unconstrained biplot (variation due to species occurrences alone) and the residual biplot (how much variation is due to residuals in full model) of the joint-species-distribution modellings (Fig. 2). Eastern mosquitofish and brown trout are further away from the cluster of sites than the other species in the unconstrained biplot. In the residual biplot, eastern mosquitofish is again at some distance from the cluster of sites, as is the roach.

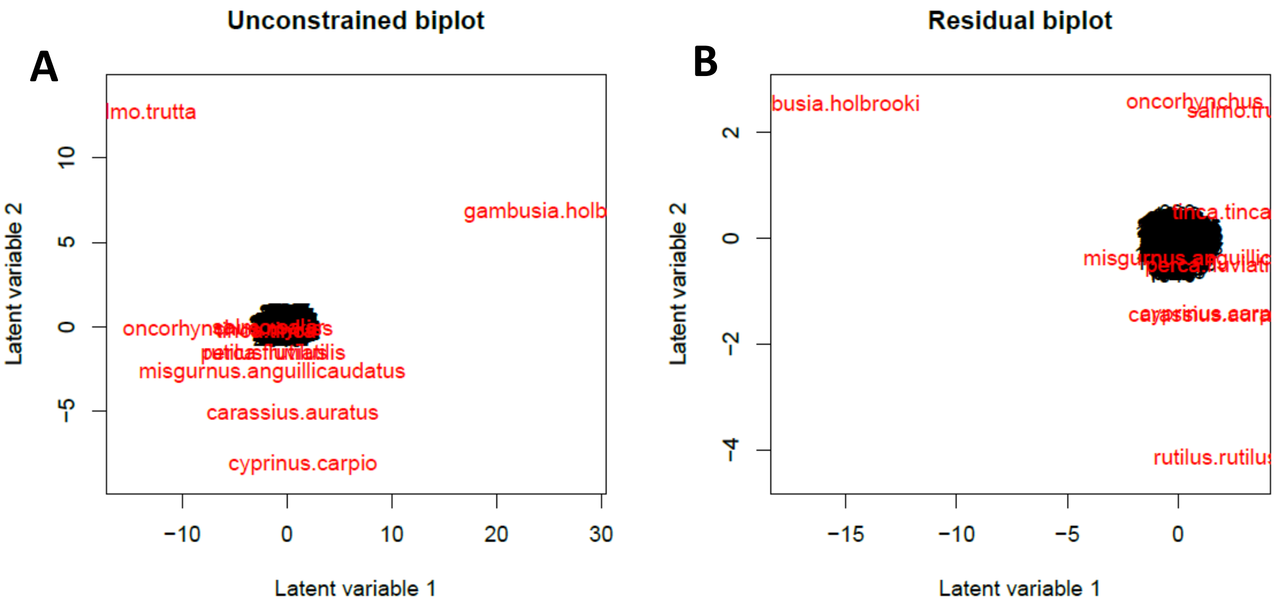

Figure 24 Distribution data for the nine invasive fish species in this study

a) Model based unconstrained ordination (from the latent variable model)

b) residual ordination based on the correlated response model posterior mean estimates. Each of the sites is labelled by its number (too many to decipher patterns)

The environmental correlations between the species are shown in Figure 25. For carp there is a very strong positive correlation with goldfish. There are also moderate positive correlations with eastern mosquitofish and redfin perch. There is also a strong negative correlation with the brown trout, which also has strong negative correlations with goldfish and the Oriental weatherloach, but a strong positive correlation with rainbow trout. There is a moderate positive correlation between tench and redfin perch.

When looking at the residual correlations, carp has strong positive correlations with oriental weatherloach, redfin perch, roach and tench (Figure 26). In addition to carp, tench has strong positive residual correlations with the Oriental weathloach, redfin perch and roach (Figure 26). Goldfish have strong negative residual correlations with the eastern mosquitofish, Oriental weatherloach, redfin perch and roach; moderate negative residual correlations with carp and tench; and a strong positive residual correlation with rainbow trout (Figure 26).

None of the included traits influenced either the environmental or residual correlation scores. When examining the credible intervals of the trait coefficients, they all included zero. This result suggested that the traits were not explaining co-occurrence patterns in respect to the environmental information (Hui, 2015).

In these plots, (a) shows pairwise correlations between the species due to the environmental response. Colours indicate strength and direction of correlation; while (b) is a network plot showing the same relationships in (a), but with clustering of species based on similarities. The colours and direction of interaction are the same for both plots.

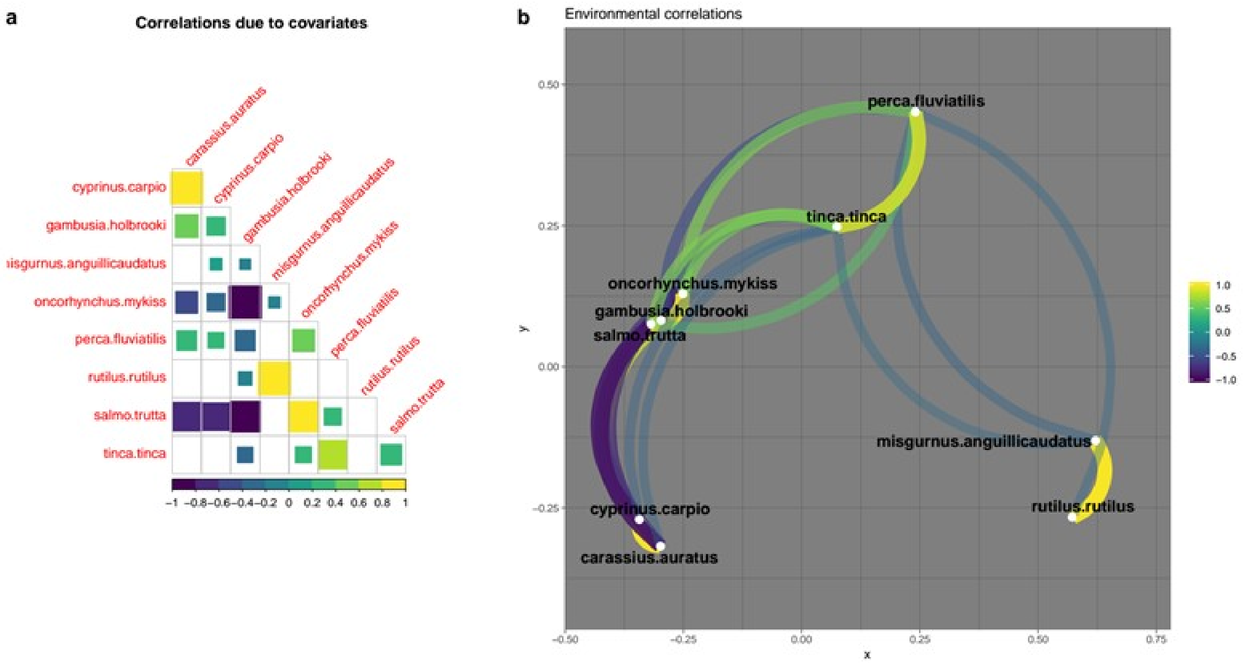

Figure 25 Environmental correlations between the species

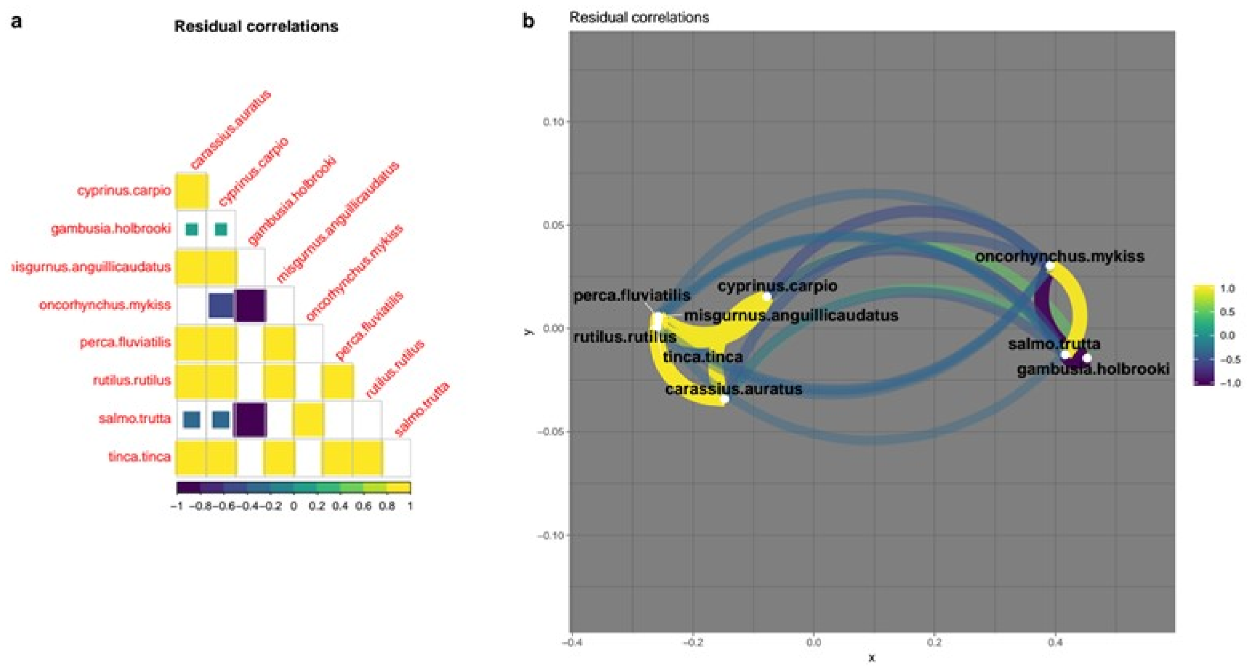

Figure 26 Pairwise correlations between the species due to the residual correlations

9.1.4 Discussion and conclusions

The objective of the evaluation was to identify environmental and residual correlations between species that may provide an indication as to the interactions to consider when examining the possible impacts of CyHV-3. Importantly, direct interactions between these fish species are likely to be at a finer scale than the 5 km^2 grid cell size that was employed here, so interpretation of how correlations may indicate interactions between species needs to be made with caution (Zurell *et al.*, 2018). Other studies have been successful in using similar methods and scale of analysis to determine regional patterns. For example, Inoue *et al.* (2017) built joint-distribution models at 5 km^2 to examine community composition of freshwater fishes in association with mussel species and, thus, help to define restoration and conservation goals. When interpreting the correlations between species, the residual correlation may also reflect the absence of a more suitable predictor. Identifying a variable set that is general enough to be relevant to all fishes, but also proximal enough to the processes that define distributions, is a challenge for any form of community-level modelling. Given the range of predictors that were employed have been used elsewhere for understanding fish community patterns, it was assumed that they were somewhat correlated with causal predictors and therefore applicable to the questions at hand.

Establishment of CyHV-3 within Australian freshwater waterways is expected to result in partial eradication of carp at a local, catchment and national level. The ecological effects of this will then also scale accordingly, including the ability of other species to recruit within the newly-released habitat and resources. While this of course includes native fishes and benthic invertebrates, given their demonstrated ability to be successful invaders, other non-native fishes are perhaps likely to recolonise quicker. Importantly, how quickly the habitats that have been modified by carp will recover, if ever, will play a large role in determining which species are able to reoccupy those environments under a scenario of carp population reduction.

The results of the evaluation presented here suggest that at a broad spatial scale, there are putative interactions between carp and six other non-native fish species – in particular, with goldfish, but also with tench, redfin perch, Oriental weatherloach, roach and eastern mosquitofish.

In general, there is little known about competitive interactions between carp and other non-native fish species of south-eastern Australia. Key pieces of information that are likely to determine the ability of other non-native fish species to recolonise a potential niche left vacant by removal of carp include diet item overlap (in terms of individual species diets, such as chironomid larvae) and levels of affinity for the muddy and turbid waters that are likely to remain for the short-medium term. Secondary effects on fish populations resulting from shifts in pressures on zooplankton and phytoplankton are a complex issue and will also drive community changes given removal of carp. These mechanisms are discussed in the context of each of the six putative non-native species that may have some competitive interactions with carp.

Goldfish: the relatively close relatedness of this species to carp (from both a phylogenetic and ecological standpoint) suggests that goldfish may benefit from the removal of carp. Countering this, however, is the observation that widespread and strong populations of goldfish currently coexist with carp. Additionally, goldfish are known to hybridise with carp (Hume *et al.*, 1983), and the degree to which these hybrids are susceptible to CyHV-3 may present a range of outcomes for the biological control of carp. Goldfish are thought to be a more benign invader than carp (Lintermans and Murray-Darling Basin Commission, 2007), although may be responsible for some structural damage to wetlands (Wilson, 2005).

Tench: this species is found in slow-flowing water, often with a muddy bottom (Lintermans and Murray-Darling Basin Commission, 2007). Tench are similar in diet and feeding strategy to carp, and both species can exploit a wide range of resources. This means that they share similar trophic positions (Britton *et al.*, 2018) and are likely to undergo some form of competition. In terms of functional responses, a recent study found there were no significant differences between carp, tench and goldfish, on chironomid prey (Guo *et al.*, 2017). Tench have experienced a range contraction and population demise resulting from the presence of carp (Moore *et al.*, 2007, citing some reports of this process), to the point that they are now rare in the Murray-Darling Basin and New South Wales and are not present in the Australian Capital Territory or Queensland (Lintermans and Murray-Darling Basin Commission, 2007). The impacts of tench are largely considered non-significant (Wilson, 2005), although at high densities they may have an adverse effect water quality and hence on habitat (Moore *et al.*, 2007). This suggests that this species is able persist under the habitat modification by carp, but that direct competition for food and habitat is the mechanism restricting their populations at this point.

Redfin perch: redfin perch are pelagic carnivores, and feed on yabbies and other crustaceans, zooplankton and small fish (Lintermans and Murray-Darling Basin Commission, 2007). Studies in Europe have suggested that redfin perch compete with carp for around half of the available food items. This is particularly the case for adult fish, as both feed on chironomid larvae (Adámek *et al.*, 2004). While there is little evidence in the literature, carp may also predate upon redfin perch eggs, which are generally not palatable to other fish due to a gelatinous layer surrounding them (Moore *et al.*, 2007). Redfin perch are recognised as a significant threat to the Murray-Darling Basin (Wilson, 2005), although the competitive release of this species may be limited by how successful they are as predators in the turbid waters that are likely to remain for the short-medium term following a reduction in carp populations.

Roach: little is known about this species in terms of its ecology in Australia or its impacts on native fish (Lintermans and Murray-Darling Basin Commission, 2007). Roach are largely restricted to the cooler waters of southern Victoria. They are predominantly a benthic feeder, although can also take insects from the water's surface (Lintermans and Murray-Darling Basin Commission, 2007). For roach, the impacts of the removal of carp are likely to focus on how other predatory species such as redfin perch recover – given that these may subsequently predate upon roach (Moore *et al.*, 2007) – or their ability to compete with other non-natives such as tench and redfin perch, and with native species, for food sources such as chironomids.

Oriental weatherloach: this species is a substantially smaller fish than carp and a benthic feeder in low flowing and still waters, with sand, mud, and detritus substrates for burrowing (Lintermans and Murray-Darling Basin Commission, 2007). It has a similar impact on water quality to carp (Keller and Lake, 2007) although its overall ecology in Australian waters is poorly understood. Importantly, Oriental weatherloach can tolerate low DO and may alter macro-invertebrate communities (Keller and Lake, 2007). Interactions with carp are unknown.

Eastern mosquitofish: there is some evidence that there is competition between juvenile eastern mosquitofish and juvenile carp, to the detriment of carp (Macdonald *et al.*, 2012). Eastern mosquitofish is considered a key threat to the Murray-Darling Basin (Wilson, 2005), although given the currently high biomass and extensive spread of eastern mosquitofish, the presence of carp does not appear to be limiting this species. There is also some possibility that an increase in the predatory redfin perch would result in a decline in the eastern mosquitofish as this is known to be a prey species (Wilson, 2005).

From the above, one or a number of the six identified species might benefit from the removal of carp if they are able to recruit strongly and quickly within turbid and degraded waters. Two other species, Mozambique tilapia and guppies (*Poecilia reticulata*) have been identified as threatening to the Murray-Darling Basin (Wilson, 2005) but have not as yet invaded. Both species have established populations in Queensland and elsewhere in (mostly northern) Australia where there is little to no overlap with carp. Whether the presence of carp is a factor in this, or lack of opportunity for colonisation, is not possible to examine with the current datasets.

In concluding: the evaluation revealed important distribution and co-occurrence patterns between non-native fish species in south-eastern Australia. While it is difficult to infer direct relationships from these data, the results provided context around possible interaction scenarios – or at least did not rule them out. The correlations calculated between species presented avenues for hypotheses of the nature of these interactions. For species that were negatively correlated with carp, the likelihood is that the net benefit to those species will be relatively less than the positively correlated ones. For the species that have positive correlations to carp (including goldfish, tench, redfin perch, roach, Oriental weatherloach and eastern mosquitofish) further testing of interactions through additional datasets or experimental means would help elucidate the potential effects under scenarios of the biological control of carp.

9.2 Resurgence of invasive plant species

In theory, the removal of carp, or a substantive reduction in biomass, may allow exotic plant species that are currently established, although not exhibiting invasive tendencies due to suppression by carp, to exhibit invasive behaviour. Exotic plants may currently be suppressed directly by the feeding behaviour of carp, which includes the uprooting of aquatic macrophytes and reduced macrophyte diversity and abundance; or indirectly through increased turbidity and a reduction in the penetration of light (Weber and Brown, 2009). The removal of carp, or a substantive reduction in biomass, is thus likely to favour (in decreasing order) submergent, emergent and floating plant forms. This trend notwithstanding, the breadth of exotic aquatic plant species that might benefit from the removal or suppression of carp is substantial (Mitchell 1978). There is also likely to be considerable geographic variation in the importance of individual exotic aquatic plant species across the distribution of carp in Australia, and local areas where particular species have become established. Some species that are currently quite cryptic, and consequently quite poorly understood, may also establish a stronger presence in particular waterways or catchments.

Offsetting this is the observation that invasive species in any setting would need to out-compete any native aquatic plants, such as the submergent *Hydrilla verticillata*, that are endemic to that area.

An alternative (applied) approach to assessing the likelihood that exotic aquatic plant species will benefit from the removal or suppression of carp, and develop invasive capabilities, is to compare waterways with and without carp. This approach presupposes that a similar range of exotic species is present in both waterways, and that opportunities for developing invasive potential are similar. Hydrological and other conditions within the comparison waterways should also be reasonably well matched. This approach was taken in the observational experimental work of Marshall *et al.* (2019), for example, who compared aquatic macrophytes in reasonably well-matched river catchments in southern Queensland that either included carp (the Paroo, Warrego, Nebine catchments) or were free of carp (the Bulloo catchment). Surprisingly, these authors noted a greater proportion of sites with macrophytes in the catchments with carp present. They did not observe, however, any exotic aquatic plant species developing invasive capabilities in the absence of carp.

Ultimately, it is plausible that a range of exotic aquatic plant species (in particular, submergent species) will benefit from the removal or suppression of carp and, in some settings, will develop invasive capabilities. This may be a short-term adjustment within the ecosystem, at that location, or may be a more permanent shift in the balance of species and, ultimately, a shift that requires rectification. In either case, the resilience of Australian freshwater ecosystems to invasive aquatic plant species is likely to be improved in the absence of carp and robust native species such as *Hydrilla verticillata* are likely to have more opportunity to compete and flourish.

Part III Ecological risk assessment

Ecological risk assessment for the release of cyprinid herpesvirus 3 (CyHV-3) for carp biocontrol in Australia

Important note:

Part III of the ecological risk assessment is only lightly referenced.

Readers are directed to the review of literature in Part II for corroboration of the underlying science.

10 Outbreak scenarios for CyHV-3 in Australian waterways

The key factors that are likely to underpin the development of the first CyHV-3 outbreaks in Australian freshwater waterways,[55] and their aggressiveness, are opportunities for close contact between infected and susceptible fish and a permissive water temperature. These considerations were discussed in Part II: Section 5.3.4. Close contact is particularly pertinent to an aggregation event, as shown in Figure 27 below, but also occurs during spawning (Figure 6).

A third factor that may be important to the transmission of CyHV-3 in some settings is the high pathogenicity of CyHV-3 for juvenile (post-larval) fish. This is discussed in Part II: Section 5.3.2.

These three factors, and the role of river flows, may come in to play in different ways within:

- Wetland or floodplain environments
- Irrigation reservoirs and other permanent or semi-permanent waterbodies
- Riverine environments.

The three settings are illustrated schematically in Figure 28 (overleaf) and discussed in turn in the text that follows.

Figure 27 An aggregation of carp in the Murray River

Source: Dr Peter Jackson, Journal Ecological Management and Restoration Project Summary (5 January, 2014)[56]

[55] In this context, the first outbreaks are those that will follow from the release of CyHV-3 into a naïve population within a given waterbody or catchment

[56] See: https: //site.emrprojectsummaries.org/

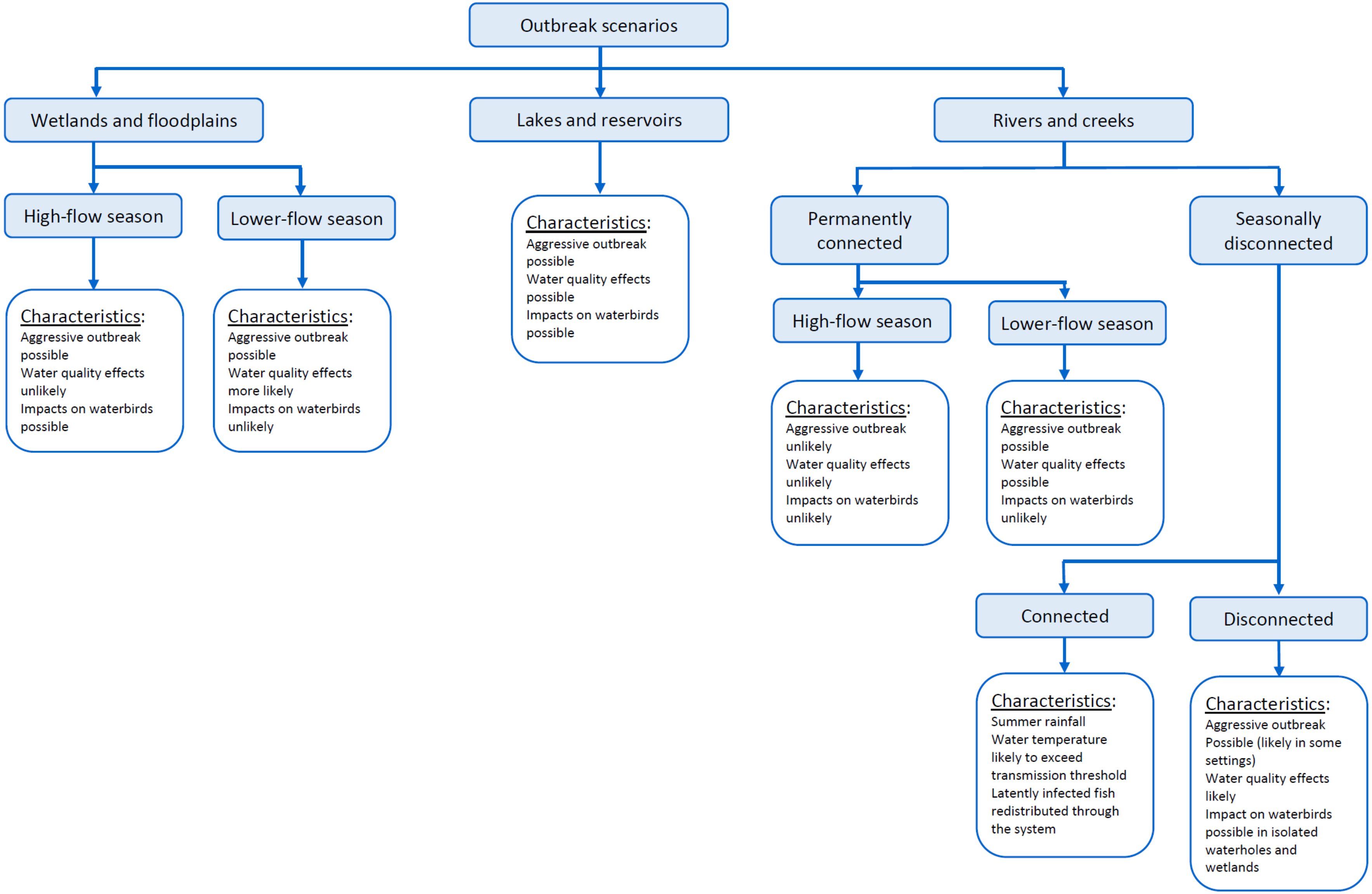

Figure 28 Outbreak scenarios for CyHV-3 in Australian waterways

10.1 Outbreaks of CyHV-3 in ephemeral wetland and floodplain environments

The reproductive cycle of carp in Australian waterways was discussed in Part II: Section 4.2.2, where it was noted that carp tend to aggregate in early spring, prior to spawning. Spawning itself is triggered by a rise in water temperature to approximately 16 to 17C, which also represents what is broadly understood to be lower end of the permissive range for the transmission of CyHV-3 (approximately 18C, Part II: Section 5.3.4). In Australia, spawning results in substantial recruitment into the major river systems when carp have access to inundated ephemeral wetlands, floodplains or marshes (Part II: Section 4.2). This occurs in what can loosely be considered ‘high-flow’ years when river flow is sufficient to enable inundation of these areas – whether naturally, or by way of regulators. Conversely, spawning in ‘lower-flow’ years will generally be limited (by water level) to the river channel and to permanently-inundated anabranches and wetlands. Some recruitment under lower-flow conditions is likely, although under the source-sink model described in Part II: Section 4.2.2 the adult population of carp within the broader river system may slowly decline between high-flow seasons without sufficient recruitment.

While high-flow seasons are reasonably definitive, as these alone are likely to result in substantive inundation of floodplains, there may be a considerable range in the extent of wetland (c.f. floodplain) inundation under various lower-flow seasons and irrigation scenarios. This is chiefly because regulation through locks, weirs, regulators and other structures may enable parts of some wetland ecosystems to remain inundated under relatively lower channel flows. Flows may also be purposively channelled to parts of a wetland ecosystem at certain times during lower-flow seasons under an environmental watering plan. This degree of water management is particularly relevant to identified high-value wetland ecosystems, including many of Australia’s Ramsar-listed wetlands (for example, Chowilla Floodplain as discussed in the case study in Section 12.2).

The above notwithstanding, a clear-cut delineation between ‘high-flow’ and ‘lower-flow’ is useful from a conceptual and analysis standpoint as it enables a division between settings where: (a) channel flows are relatively rapid, the floodplain is inundated and the wetland-floodplain ecosystem is fully charged; and (b) some individual wetlands may remain inundated, but channel flows are slower and lower, the floodplains are not inundated and only part of the larger wetland ecosystem is supporting active wetland or marshland communities.

High-flow and lower-flow spawning scenarios for carp in Australian ephemeral wetland and floodplain environments are likely to result in two quite different scenarios for the first outbreaks of CyHV-3.

In a **high-flow season**, transmission of CyHV-3 is likely to occur during the aggregation of carp prior to spawning (Figure 27). The extent of this will depend on the timing of the rise in water temperature, which may be just below the accepted minimum for effective transmission at the point of pre-spawning aggregation (approximately 18C, Part II: Section 5.3.4). If the water temperature is permissive for transmission, then the active close contact of a large number of fish is likely to enable the exposure of a wide cross-section of a given population of carp (Durr *et al*., 2019a). Importantly, successful transmission during an aggregation event may also mean that further transmission is less dependent on the density of carp (Durr *et al*., 2019a). Spawning follows from the aggregation and, in a high-flow season, may occur at the fringes of permanently-inundated waterbodies or on the inundated floodplain – and the predilection for one or other may depend on the timing of the rising water level and water temperature, and the extent to which the broader floodplain is inundated and accessible to carp at the time of spawning (Part II: Section 4.2.2). If spawning occurred at the fringes of permanently-inundated waterbodies, then the carp biomass density in those places will be lower than it was during the pre-spawning aggregation – and during the quiescent winter months – but may be sufficiently high to enable significant further close-contact transmission of CyHV-3 between a large number of infected and susceptible fish. When access to the inundated floodplain is available, however, the biomass density of carp will be substantially diminished. Brown *et al.* (2005), for example, estimated that the average biomass of carp within the Barmah forest floodplain in January 2001, at the end of a prolonged flood peak, was approximately 22 kg/ha. By comparison, when carp were confined to Moira Lake the biomass increased to approximately 190 kg/ha. In the floodplain setting, most clinically affected fish – and fish carcasses – could be avoided by healthy carp, and there would be less opportunity for transmission by way of contaminated water. Close contact between individual actively-spawning fish remains likely, however, and if a moderate or high proportion of these were exposed during the aggregation event, or prior to the movement of carp out onto the floodplain, then the outbreak may continue to be propagated, albeit at a lower intensity. The outward visibility of the outbreak at this stage of the season is also likely to be lower, given the low biomass density of carp on the floodplain and the likely sparse distribution of carcasses, and there is also likely to be less opportunity for impacts on water quality (Part II: Section 6). As summer progresses, the water temperature on the floodplains will continue to rise and, in many parts of Australia (including much of the Murray-Darling Basin), may reach and exceed the maximum permissive temperature for transmission of CyHV-3 (approximately 28C). Although larvae are not susceptible to CyHV-3, juveniles drifting back toward the channels and anabranches with the receding floodwater are highly-susceptible. The water temperature within channels and anabranches, while elevated in summer, is less likely to exceed the threshold for transmission in autumn and if the virus has persisted in these locations then a significant rate of infection and mortality amongst the juveniles is possible. Importantly, this may mean that recruitment from a high-flow season is substantially less than it would otherwise have been. Because of the source-sink dynamic, this outcome is likely to be more significant in terms of its impact on the broader carp population than a locally aggressive outbreak amongst adult carp (Part II: Section 4.2.2).

In summary, an aggressive outbreak of CyHV-3 in carp is possible within an ephemeral wetland during a high-flow season if sufficient transmission occurs during the pre-spawning aggregation of carp, or prior to their movement out onto the inundated floodplains. The outward appearance of the outbreak, however, and its impacts on water quality, are likely to be minimised by the relatively low biomass density of carp on the floodplain during a significant inundation event. If the virus persists through summer in deeper river channels, then there may also be substantial mortality of drifting juvenile carp and this may have an impact on the long-term sustainability of the broader carp population.

In a **lower-flow season**, some transmission of CyHV-3 is likely to occur during the aggregation of carp prior to spawning, and the extent of this is again likely to depend on water temperature – which may be just below the accepted minimum for effective transmission (approximately 18C, Part II: Section 5.3.4). In a lower-flow season, spawning is likely to occur at the fringes of permanently-inundated off-channel waterbodies and the ensuing larvae and juveniles will then be confined to these waters (Part II: Section 4.2.2). Because the carp in this setting will be more physically-restricted by the extent of inundation than under the high-flow scenario, opportunities for close-contact transmission of CyHV-3 will be more numerous. It is also likely that the temperature of the water within deeper permanently-inundated waterbodies will remain sufficiently low for the ongoing transmission of CyHV-3 through most of the summer, if not for its entirety. Juvenile carp, while less numerous, are likely to mix with adults in this setting, thus maximising opportunities for exposure to the virus and helping to ensure that it continues to replicate and be excreted within the contained population. Collectively, this scenario means that an aggressive outbreak of CyHV-3 within an ephemeral wetland setting is more likely during a lower-flow season. The outbreak is also likely to be more visible, and the higher biomass density of carp will mean that there is a higher likelihood of substantive impacts on water quality. A single aggressive outbreak of CyHV-3 will generally be of relatively short duration (approximately 3 to 6 weeks, Part II: Section 5.3.5), but it is also possible that a series of outbreaks might follow from each other under the lower-flow scenario. Because recruitment to the broader river systems is in general less significant when the floodplains are not inundated, however, the overall impact of the outbreak(s) on the sustainability of the carp population may be lower than the impact of a less aggressive outbreak during a high-flow (and recruitment-critical) season.

10.2 Outbreaks in lakes, irrigation reservoirs and other permanent waterbodies

The two scenarios above describe the implications of high-flow and lower-flow seasons to ephemeral wetland and floodplain environments, noting that these settings provide for much of the recruitment of carp into the broader river systems within the Murray-Darling Basin and elsewhere. These scenarios notwithstanding, it is important to acknowledge that carp also exist at relatively high biomass densities within some stable and relatively contained bodies of water – that is, permanent lakes or wetlands (including some urban or peri-urban lakes and wetlands, and some irrigation reservoirs). These waterbodies may have marshland at their peripheries, and this and the littoral provide important habitat for a wide range of waterbirds (including colonial-nesting waterbirds) during lower-flow seasons when ephemeral wetlands and floodplains are unlikely to be inundated. To variable degrees, these lakes and other permanent waterbodies may also include populations of both large-bodied and small-bodied native fish (for example, Kow Swap as discussed in the mini case study in Section 13.1). These populations may be augmented through the strategic release of farmed fingerlings, if fish from the river system do not effectively navigate regulators and other barriers in sufficient numbers to support recreational fishing. Other native aquatic wildlife (including frogs and freshwater turtles) are also likely to be present. Carp spawn amongst macrophytes and other aquatic plants in the shallow waters and marshland at the periphery of permanent lakes and reservoirs. Aggregation prior to spawning may not be as striking, given that the carp are in any case reasonably confined. Spawning will occur when the temperature of the water within the shallows reaches approximately 16 to 17C, and eggs then adhere to aquatic vegetation (Part II: Section 4.2.2). Larvae and juveniles will then remain within the waterbody.

In this setting, a high carp biomass density has the potential to result in an aggressive and highly-visible outbreak, with the potential for marked impacts on water quality (Durr *et al*., 2019a). As for the lower-flow ephemeral wetland scenario described above, a succession of outbreaks is also possible and, if the virus is able to persist through to early summer, there may be a subsequent high mortality amongst juveniles. Although this scenario could result in the rapid loss of a significant proportion of a local carp population, recruitment from connected ephemeral floodplains (by way of a parent waterway) is in many settings likely without intervention, and the population may, thus, rebound. There is also the possibility that the virus would become established and endemic within a relatively contained body of water and the population of carp that reside in it. This may result in seasonal outbreaks that continue to suppress the population of carp, albeit without the outwardly dramatic signs of a significant fish kill (Durr *et al*., 2019a).

The extent of flow through the river system is likely to be of less importance to the character of outbreaks of CyHV-3 within lakes, reservoirs and other permanent waterbodies than it is to outbreaks within ephemeral wetland or floodplain environments. The reproductive cycle for carp will be reasonably stable, and the majority of native fish may be constrained from migrating for spawning by regulators and other barriers. The point of difference in many permanent waterbodies may be the breadth of the population of nesting waterbirds (including colonial-nesting waterbirds), as these may tend to move to inundated ephemeral wetlands and floodplains during high-flow seasons to take advantage of an optimal environment for raising chicks.

10.3 Outbreaks of CyHV-3 in riverine environments

Riverine environments are diverse in respect of water depth, velocity, temperature and quality; the existence and extent of weirs and other regulators; the biomass density of carp; the presence and sustainability of native fish populations, and populations of other native aquatic biota; and the extent to which they provide for nesting waterbirds (in particular, colonial-nesting waterbirds). Some riverine environments are also highly-regulated, while others depend on local rainfall or rainfall within the broader catchment. This diversity has marked implications both for the initiation and likely aggressiveness of an outbreak of CyHV-3 in carp, and for the possible effects of an outbreak on native and migratory biota and ecological communities.

With this as background, outbreaks of CyHV-3 within riverine settings can be separated into: (a) those that occur in perennially-connected rivers (for example, the mid-Murray River channel as discussed in the case study in Section 12.3); and (b) those that occur in rivers that contract seasonally to disconnected waterholes which are then reconnected briefly with substantial (generally summer) rains (for example, the Moonie River as discussed in the case study in Section 12.4).

The general principles governing outbreak development and its outcomes that were discussed for wetlands and permanent waterbodies (above) apply broadly to **perennially connected riverine environments**. Foremost, the aggressiveness of an outbreak of CyHV-3 within a given reach – and its potential to harm species or communities – is likely to reflect the extent of aggregation of carp prior to spawning and the biomass density of carp within the reach during the ensuing spring and summer (Durr *et al*., 2019a). In some settings, the extent of flow within the broader river system may also be important, while in others the flow within a given reach may be regulated to an approximately stable state. Where flow is not regulated, then lower-flows within the broader river system will in general be more likely to result in the concentration of carp and the development of aggressive outbreaks. Other species may also be less able to avoid the possible outcomes of the outbreak under lower-flow conditions – and these outcomes may include harmful processes such as lowered DO or the development of a widespread cyanobacterial bloom. In some settings, reaches of an unregulated river (including billabongs and anabranches) may become disconnected under lower-flow conditions, and this may then lead to an increase in the exposure of individual species and communities. Conversely, higher flows may be protective to unregulated or partly-regulated riverine environments in the sense that the rapid ongoing exchange of water will help to dilute or flush through the detritus from an outbreak of CyHV-3 in carp, and thus minimise opportunities for it to affect water quality. Finally, highly-regulated riverine reaches may also include impoundments in which the character of outbreaks of CyHV-3 is likely to be governed by similar parameters to those described for permanent lakes or irrigation reservoirs (above). Likewise, the impacts of outbreaks within such impoundments on species and ecological communities may be similar to the impacts that would be observed in permanent lakes or irrigation reservoirs.

Outbreaks of CyHV-3 that occur in **seasonally-disconnected riverine settings** are likely to be quite different. These settings occur in the northern and western Murray-Darling Basin, where water temperature is likely to be suited to the transmission of CyHV-3 during spring and autumn and summer rains briefly reconnect the waterholes and allow rivers to flow (Section 12.4.5). The contraction of waterholes between flow events results in the concentration of aquatic biota (including carp) and a deterioration in water quality (Section 12.4.2). In spring, prior to summer rains, when the waterholes are maximally contracted, carp biomass density may be high (as a result of concentration) and with it the potential for an aggressive outbreak of CyHV-3 and the accumulation of carcasses in numbers sufficient to impact further on water quality (in particular, DO and the risk of cyanobacterial blooms) (Section 12.4.5). These outbreaks may be terminated either by their direct impact on the population of susceptible carp, or by an indirect impact on the ecosystem within each waterhole and its ability to sustain a population of aquatic biota. If this does not occur, then, as the water warms in late spring toward the upper threshold for the transmission of CyHV-3, a proportion of infected carp may transition to from being actively infected to latently infected. These and susceptible carp will then be redistributed through the river system with summer flow events. When the flow ceases, the river will return to a baseline of disconnected waterholes (Section 12.4.4). As the water within these disconnected waterholes cools in late summer, latent infections may then transition once again to active infections (recrudescence) and new outbreaks commence (Section 12.4.5). If summer flows were significant, and substantial recruitment of carp occurred, then autumn outbreaks may be aided by the presence of highly-susceptible juvenile carp. Autumn outbreaks may again be terminated by the outcomes of the outbreak process, or a proportion of infected carp may transition to a latent state with winter and the ongoing cooling of the water within waterholes. These latently-infected carp will then transition back to active infection when the water warms in spring. In this way, the cycle will continue (Section 12.4.5).

11 Analysis of exposure pathways

Exposure pathways represent the biological, ecological and physical scenarios that might follow from the release of CyHV-3 and result in the exposure of species, ecological communities, properties and places to the harmful effects of the virus, or to the harmful outcomes of its impacts on carp. In this way, the exposure pathways link the release of CyHV-3 to one or more of the risk assessment endpoints.

Nine exposure pathways were included in the assessment.

- Exposure of native aquatic species to low dissolved oxygen (DO)
- Exposure of native species, livestock and humans to widespread cyanobacterial blooms
- Exposure of native species, livestock and humans to the microorganisms associated with decomposing fish carcasses
- Exposure of native piscivorous species to the removal of a dominant and stable food source
- Exposure of native species to increased predation as a result of prey-switching
- Exposure of native species, livestock and humans to an outbreak of botulism
- Exposure of native species to the direct pathogenic effects of CyHV-3
- Exposure of native species to the direct pathogenic effects of a mutated strain of CyHV-3 with altered species specificity
- Exposure of humans to the direct pathogenic effects of CyHV-3 in drinking water or consumed fish.

The pathways with a non-negligible likelihood (termed throughout, a ‘real chance or possibility’) for one or more endpoints were taken forward to the individual case studies (Section 12).

The assessment of exposure pathways focussed on events that may unfold under a maximally aggressive outbreak. In most settings, this will be the period immediately after release of the virus. In disconnected riverine environments, however, outbreaks are more likely to be maximally aggressive during the dry season following from reconnection of the river system – that is, after affected fish have had an opportunity to be redistributed through the population.

11.1 Low dissolved oxygen

This pathway considers the exposure of aquatic native biota and ecological communities to the harmful effects of low DO as a result of the aerobic decomposition of carp carcasses.

Key references for the science underpinning this pathway can be found in Part II: Section 6.

11.1.1 Likelihood of exposure

As a general rule, native fish and other higher aquatic organisms require a DO concentration of at least 2 mg/L to survive – although may begin to be stressed at levels below 4 to 5 mg/L (Part II: Section 6.2.2). Few species can tolerate conditions of less than 3 mg/L for prolonged periods.

Larvae and young-of-year juveniles may survive at DO as low as 20 percent saturation (for example, 1.8 mg/L at 20C), but growth is likely to be restricted. With these broad parameters in mind, the Murray-Darling Basin Plan[57] targets of ≥50 percent saturation and a DO concentration of at least 4.5 mg/L (at approximately 20C) are widely regarded as appropriate critical values for river channels and anabranch creeks.

The experimental work of Walsh *et al.* (2018) confirmed that the decomposition of fish carcasses can result in a low DO and, in some situations, anoxia (Part II: Section 6.3.2). The mean oxygen demand of decomposing carp carcasses in warm and cold water was found to be approximately 1 and 0.5 mg/kg/min, respectively. The biomass density used in the experiments of Walsh *et al.* (2018) was very high, although the action of wind or currents may mean that carcasses accumulate in large numbers in certain places within a waterbody. Additional factors that influence the extent and duration of oxygen drawdown include temperature, water quality (including salinity), thermal stratification within the water column and the rate of reaeration through the air-water interface (primarily as a function of wind). The aggressiveness of the outbreak will also be relevant as it will determine the density of the carcass accumulation at any given point in time. Other factors such as the connectedness of the affected population, and its spatial arrangement, will also be important in this respect. Outbreaks are most likely to occur in spring, following the aggregation of carp immediately prior to spawning.

Two key scenarios were described in Section 10 for outbreaks of CyHV-3 in ephemeral wetland or marshland settings. In these scenarios, the key determinant of the outward visibility of the outbreak is the flow scenario.

- An aggressive outbreak of CyHV-3 in carp is possible within an ephemeral wetland during a **high-flow** season if sufficient transmission occurs during the pre-spawning aggregation of carp, or prior to their movement out onto the inundated floodplains. The outward appearance of the outbreak, however, and the potential for carcass decomposition to impact on DO, are likely to be minimised by the relatively low biomass density of carp on the floodplain during a significant inundation event. Some individual wetlands will be shallow, and relatively disconnected, and without a substantial source of uncontaminated water some local areas of low or zero DO may develop. It is unlikely, however, that an outbreak of CyHV-3 that occurred during a high-flow season would result in hypoxia or anoxia within a significant proportion of an ecosystem.
- In a **lower-flow** season an aggressive and outwardly visible outbreak is more likely and may result in the accumulation of a significant number of carcasses in a limited body of water. The decomposition of these carcasses may then lead to low DO or anoxia. The spatial extent of the impact will depend on the number and distribution of carcasses at any point in time, and the size and connectivity of the waterbody. Overall, it is possible that an outbreak of CyHV-3 that occurred during a lower-flow season would result in hypoxia or anoxia within a significant proportion of an ecosystem.

While the same general principles can be applied to outbreaks of CyHV-3 in permanent lakes or irrigation reservoirs, or in riverine reaches or impoundments, the role of high-flow and lower-flow

[57] See: https: //www.mdba.gov.au/basin-plan/plan-murray-darling-basin

seasons will depend to a large extent on the connectedness of the waterway and the extent of its regulation. Three further points are relevant:

- DO falls naturally overnight in many freshwater settings, as the respiratory needs of phytoplankton and water plants will continue in the absence of photosynthesis. Overnight low DO commonly results in fish rising toward the surface in the early morning and, in extremis, engaging in ASR (as shown in Part II: Figure 10). If the overnight low DO falls substantially below the tolerance of particular fish and other aquatic animals, then this may be sufficient to cause physiological stress and, ultimately, death.
- Under either of the scenarios above, the period of hypoxia or anoxia that might follow from an outbreak of CyHV-3 is unlikely to be sustained – as can be the case with some substantial blackwater events – as most carcasses will have decomposed to a large extent within approximately 2 weeks of death (Part II: Section 5.3.5).
- The high DOC observed during a true blackwater event includes phenols and other compounds that are toxic to fish and other aquatic animals (Part II: Section 6.2.3). This results in a synergism with low DO and, when DOC is higher than about 100 mg/L, may override the primary effect of low DO. The high DOC that accompanies a fish kill, however, is not derived from toxic materials and does not act in synergism with low DO (Part II: Section 6.3.2).

11.1.2 Likely consequences of exposure

A summary of the potential impacts of hypoxic or anoxic conditions on risk assessment endpoints (Part I: Section 2.2) is given in Table 10. In chief, these impacts focus on direct harm to local populations of aquatic native biota, including large-bodied and small-bodied fish, crustaceans, zooplankton and macroinvertebrates. The effects of carcass decomposition on DO are likely to be relatively short-term. This means that while the size of a population or its area of occupancy might be affected, populations are less likely to be fragmented. Low DO is also unlikely to have an effect on habitat, nor result in the establishment of a disease or invasive species. None of the water-breathing species affected by low DO are considered migratory in the regulatory sense used throughout this risk assessment. Similar logic was applied across ecological communities and wetlands. Low DO will not affect livestock or humans.

The likely consequences of hypoxic or anoxic conditions for individual species or functional groups are discussed in parts (a) to (d) below, while the consequences for wetland or marsh ecosystems are discussed in part (e).

For this and other exposure pathways, the likely consequence provide a preliminary or baseline assessment of the possible impacts of CyHV-3, in the sense that they do not relate to particular places or ecosystems. The site-specific case studies undertaken in Section 12 build on these baseline assessments and give an evaluation of the possible impacts of CyHV-3 on the communities and species that characterise each location.

Table 10 Relevance of hypoxic or anoxic conditions to risk assessment endpoints

Endpoints (significant impact criteria)	Relevance
Migratory and non-migratory native species	
Lead to a long-term decrease in the size of a population of a non-migratory native species	✓
Reduce the area of occupancy of a non-migratory native species	✓
Fragment an existing population into two or more populations of a non-migratory native species	✗
Adversely affect habitat critical to the survival of a non-migratory native species	✗
Disrupt the breeding cycle of a population of a non-migratory native species	✓
Modify, destroy, remove, isolate or decrease the availability or quality of habitat to the extent that a non-migratory native species is likely to decline	✗
Result in invasive species that becomes established in a non-migratory native species' habitat	✗
Introduce disease that may cause a non-migratory native species to decline	✗
Interfere with the recovery of a non-migratory native species	✓
Migratory species	
Substantially modify (including by fragmenting, altering fire regimes, altering nutrient cycles or altering hydrological cycles), destroy or isolate an area of important habitat for a migratory species	✗
Result in an invasive species that is harmful to the migratory species becoming established in an area of important habitat for the migratory species	✗
Seriously disrupt the lifecycle (breeding, feeding, migration or resting behaviour) of an ecologically significant proportion of the population of a migratory species	✗
Ecological community	
Reduce the extent of an ecological community	✓
Fragment or increase fragmentation of an ecological community, for example by clearing vegetation for roads or transmission lines	✗
Adversely affect habitat critical to the survival of an ecological community	✗
Modify or destroy abiotic (non-living) factors (such as water, nutrients, or soil) necessary for an ecological community's survival, including reduction of groundwater levels, or substantial alteration of surface water drainage patterns	✗
Cause a substantial change in the species composition of an occurrence of an ecological community, including causing a decline or loss of functionally important species, for example through regular burning or flora or fauna harvesting	✓
Cause a substantial reduction in the quality or integrity of an occurrence of an ecological community, including, but not limited to • Assisting invasive species, that are harmful to the ecological community, to become established, or • Causing regular mobilisation of fertilisers, herbicides or other chemicals or pollutants into the ecological community which kill or inhibit the growth of species in the ecological community, or interfere with the recovery of an ecological community	✗
Wetland ecosystem	
Areas of the wetland being destroyed or substantially modified	✓
A substantial and measurable change in the hydrological regime of the wetland, for example, a substantial change to the volume, timing, duration and frequency of ground and surface water flows to and within the wetland	✗
The habitat or lifecycle of native species, including invertebrate fauna and fish species dependent upon the wetland being seriously affected	✓
A substantial and measurable change in the water quality of the wetland – for example, a substantial change in the level of salinity, pollutants, or nutrients in the wetland, or water temperature which may	✓

Endpoints (significant impact criteria)	Relevance
adversely impact on biodiversity, ecological integrity, social amenity or human health	
An invasive species that is harmful to the ecological character of the wetland being established (or an existing invasive species being spread) in the wetland	×
Livestock	
Local exposure of livestock to the clinical effects of a pathogenic organism or its toxin	×
Establishment of a pathogenic microorganism in livestock in areas not previously affected	×
Public health	
Exposure of individuals to the clinical effects of a pathogenic organism or its toxin	×
Exposure of groups of individuals or communities to the clinical effects of a pathogenic organism or its toxin	×

(a) Large-bodied native fish

The tolerance of some juvenile large-bodied native fish to low DO was evaluated in one of the few formal experimental trials focussed on Australian native fish (Small *et al.*, 2014, as discussed in Part II: Section 3.1.1). These authors found that juvenile Murray cod experienced 50 percent mortality (LC50) when the DO concentration in freshwater was held at 1.58 mg/L for 48 hours at 25 to 26C. Under the same conditions, the LC50 for juvenile silver perch was 1.04, for golden perch was 0.85 and for freshwater catfish was 0.25 mg/L. This analysis was extended by Gilmore *et al.* (2018), who measured the tolerance of juvenile silver and golden perch to low DO before and after 10 months of exposure to either normoxic (6 to 8 mg/L) or hypoxic (3 to 4 mg/L) conditions. These authors found that golden perch had a higher tolerance to hypoxia than silver perch, and that golden perch also adapted more effectively to persistently hypoxic conditions. These experiments suggested that golden perch could acclimate to water with a DO of approximately 3 mg/L.

The implications of low DO concentration for eight key species of large-bodied Australian native fish are discussed below.

<u>Murray cod</u> utilise a diverse range of habitats from clear and rocky upland streams to slower-flowing, turbid lowland rivers and billabongs (Part II: Section 3.1.1). The species is a main-channel specialist, with only limited use of minor tributaries and inundated floodplain habitats. Murray cod migrate upstream prior to spawning over 4 to 5 weeks in late spring or early summer, when day length is increasing and water temperature reaches 16 and 21C. The adhesive eggs are laid on solid substrates, and larvae then drift downstream before settling out in suitable protected channel habitat. Juveniles become territorial at a young age and remain aggressively so as adults. Adult Murray cod are solitary and return to their territory following spawning.

Murray cod are likely to be the least tolerant of the large-bodied native fish to low DO. Although this may to some extent reflect their size, the experiments undertaken with juvenile fish of a range of species (above) showed that the species itself is not physiologically adapted to low DO. Offsetting this, however, is their preference for deeper water and channel habitat where the likelihood of DO falling below physiologically tolerable limits as a result of an outbreak of CyHV-3 would in general be less than it would in shallow, ephemerally-inundated water. The exception to this is slow-moving or stationary water in (possibly disconnected) channels or impoundments. In this setting, the water column is likely to be stratified and the layer of acceptable oxygenation quite limited. Spawning carp may also be constrained and concentrated within channels or off-channel waters, and an outbreak of CyHV-3 thus relatively more aggressive. Under these conditions, DO could fall sufficiently to cause stress to Murray cod and, if sustained for a long period, death.

Overall, it is plausible that, in some settings, low DO associated with an outbreak of CyHV-3 would result in a long-term decrease in the size of the population of Murray cod or interfere with its recovery. This reflects the assumption that a population is defined in this assessment as an occurrence of the species in a particular area. In this context, populations of Murray cod in lentic or stationary lowland waterbodies, or those that have migrated to these areas to spawn, would be most at risk – in particular, in unregulated waterways or wetlands during a lower-flow season. It is also plausible that low DO would disrupt the breeding cycle of Murray cod in these settings and, thus, recruitment, although Murray cod are long-lived, and their sustainability does not rely heavily on individual recruitment years. In the longer term, there is not a real chance or possibility that low DO associated with outbreaks of CyHV-3 would reduce the area of occupancy of Murray cod.

Trout cod occupy comparatively deep and rapidly moving water. The single extant naturally-occurring population is restricted to a small (approximately 120 km) stretch of the Murray River from Yarrawonga Weir to the Barmah-Millewa Forest and, occasionally, downstream to Gunbower (Part II: Section 3.1.1). Trout cod pair to spawn in late September to late October, when water temperatures are between 14 and 22C – often about 3 weeks prior to Murray cod. Trout cod display high site fidelity, with a small home range, and do not routinely undertake migratory movements for spawning other than to move from the main channel to off-channel branches or to floodplains in the event of a significant flood. The tolerance of trout cod to low DO does not appear to have been evaluated, although is likely to be comparable to Murray cod as the two species are phenotypically similar and closely related.

The reach of the Murray River below Yarrawonga Weir, and the Barmah-Millewa Forest, are case studies and are examined in detail in Sections 12.3 and 12.1, respectively. In brief, the reach of the Murray River below Yarrawonga Weir is a lentic and highly-regulated channel environment. An outbreak of CyHV-3 in this reach is unlikely to differ markedly between high-flow and lower-flow seasons as water will in either event be diverted through the main channel to service irrigation needs. An outbreak in this reach is also unlikely to result in a markedly lowered DO. By contrast, Barmah-Millewa Forest includes an ephemeral floodplain that is a focus for the recruitment of carp during high-flow seasons. In lower-flow seasons, carp will spawn in, and remain constrained to, the permanently-inundated off-channel waters. Accumulation of carp carcasses in the Barmah-Millewa Forest is likely to be more marked during a lower-flow season, when the carp themselves are concentrated and contained and opportunities for close contact are maximised. Under these conditions, it is possible that DO would fall sufficiently to stress trout cod and, if sustained, result in the death of some fish.

Overall, it is plausible that low DO associated with an outbreak of CyHV-3 would result in a long-term decrease in the size of the population of trout cod or interfere with its recovery. The populations of trout cod within the Barmah-Millewa Forest are most at risk – in particular, during a lower-flow season. It is also plausible that low DO would disrupt the breeding cycle of trout cod in this setting and, thus, recruitment, although trout cod are long-lived, and their sustainability does not rely heavily on individual recruitment years. In the longer term, the fact that the species does not readily migrate means that it is plausible that low DO associated with outbreaks of CyHV-3 would reduce the area of occupancy of trout cod.

Silver Perch are strongly migratory, travelling long distances upstream to spawn (Part II: Section 3.1.1). Larvae and eggs then drift downstream. Silver perch can spawn in lower-flow and high-flow settings, and at relatively cool water temperatures, although a flood event does maximise spawning and recruitment. Spawning occurs in warm water (generally above 23C) and in faster-flowing areas of the river – generally over gravel or rock-rubble substrates. The only sizeable and self-sustaining population of silver perch is in the middle reaches of the Murray River (including the Edwards/Wakool anabranches), from the base of Yarrawonga Weir to the Torrumbarry Weir, and then to the Euston Weir and downstream. This population may also extend down to the South Australian border and up to the lower Darling River. Silver perch is slightly more tolerant to low DO than the larger Murray cod and trout cod, although are not a species that has adapted to this condition. The species occupies a larger reach of the Murray-Darling Basin than trout cod (above), including the lower turbid reach of the Darling River, and this may both extend its exposure to low DO associated with an outbreak of CyHV-3 and perhaps provide a buffer in the form of a more spatially distributed population.

Overall, it is plausible that low DO associated with an outbreak of CyHV-3 would result in a long-term decrease in the size of the population of silver perch or interfere with its recovery. The populations of silver perch within the Barmah-Millewa Forest or the lower Darling River are most at risk – in particular, during a lower-flow season. It is also plausible that low DO would disrupt the breeding cycle of silver perch in these settings and, thus, recruitment. In the longer term, there is not a real chance or possibility that low DO associated with outbreaks of CyHV-3 would reduce the area of occupancy of silver perch.

Golden perch are predominantly found in lowland, warmer, turbid, slower-flowing rivers and have a higher tolerance for low DO than most other large-bodied native fish (aside from the freshwater catfish, *Tandanus tandanus*) (Part II: Section 3.1.1). As noted above, there is some evidence that golden perch can adapt to a DO as low as 3 mg/L, in response to prolonged exposure. Golden perch are nomadic, with schools of fish occupying home ranges of about 100 metres for weeks or months before relocating to another site where a new home range is established. Upstream movements by both immature and adult fish are stimulated by small rises in streamflow and may extend as far as a 1,000 km if fish are able to navigate weirs and other barriers. Spawning occurs in temperatures ranging from 17 to 25. The large eggs semi-buoyant and drift downstream. Juvenile golden perch can be found in both channel and inundated floodplain habitats.[58]

The wide distribution, mobility and resilience of golden perch (to low DO) mean that the species is likely to be less exposed to low DO resulting from an outbreak of CyHV-3. That notwithstanding, it is plausible that, in some settings, an outbreak would lead to a long-term decrease in the size of the population or interfere with its recovery. The likelihood of this would not vary greatly between a lower-flow or high-flow season, although small populations in disconnected waterbodies may be

[58] See: https: //vfa.vic.gov.au/

relatively more exposed. It is also plausible that low DO associated with an outbreak of CyHV-3 would disrupt the breeding cycle of golden perch in some settings. There is not a real chance or possibility that its area of occupancy would be reduced.

Macquarie perch is a schooling species closely related to golden perch, although adapted to be an upland riverine specialist that prefers clear deep water and rocky holes (Part II: Section 3.1.1). Spawning occurs just above riffles (shallow running water), where rivers have a base of rubble (small boulders, pebbles and gravel). Although this species can tolerate temperatures less than 9C it requires a temperature of at least 16.5C for spawning. The eggs, which are adhesive, stick to the gravel, and newly-hatched yolk sac larvae shelter amongst pebbles. Some fish use the same river each year for spawning. Migrations are undertaken by fish resident in lakes, but not otherwise. Published evidence of the tolerance of Macquarie perch to low DO could not be located, although it seems likely that as a result of adaptation to lotic upland rivers and streams the Macquarie perch would not share the relatively high tolerance of the closely related and phenotypically similar golden perch. That notwithstanding, it is also less likely that the cooler and readily flowing upland rivers and streams will experience an aggressive and localised outbreak of CyHV-3 or a fall in DO as a result of the decomposition of large numbers of carp carcasses – this is particularly the case for reaches that includes riffles or rapids, which will tend to aerate the water passing through them more effectively that flow alone. The possible exception to this is an extremely dry season, when the flows within some upland rivers and streams may be significantly diminished and both carp and native fish may be concentrated in remaining waterholes. Here a more aggressive outbreak is possible and, following from that, it is also possible that the water quality would deteriorate to the point of causing the stress or death of Macquarie perch.

Overall, it is plausible that, in some settings, low DO associated with an outbreak of CyHV-3 would lead to a long-term decrease in the size of the population of Macquarie perch or interfere with its recovery. The likelihood of this may be increased in some settings in the event of an extremely dry season, with substantially decreased river and stream flows. It is also plausible that low DO associated with an outbreak of CyHV-3 would disrupt the breeding cycle of Macquarie perch in some settings, although its propensity to undertake migrations means that there is not a real chance or possibility that its area of occupancy would be reduced.

Freshwater catfish is a benthic species that prefers slower-flowing streams and lake habitats (Part II: Section 3.1.1). Because it is adapted to micro-environments with a low DO, its tolerance is substantially higher than other large-bodied fish. Juveniles, for example, require approximately 15 percent of the DO concentration required by juvenile Murray cod. Freshwater catfish are relatively sedentary, and do not migrate to spawn, which occurs in the spring and summer when water temperatures are between 20 and 24C. The eggs are comparatively large (approximately 3 mm) and non-adhesive and settle into the interstices of the coarse substrate of the nest in which they are laid. Freshwater catfish within a lacustrine environment may be exposed to the outcomes of an aggressive outbreak of CyHV-3, as carp in these settings may reach a high biomass density. If the lake is not large, and is partially disconnected, then the accumulation of carcasses might result in localised areas of very low DO or, for a short period, complete anoxia. In this situation, the survival of freshwater catfish may depend on opportunity to move away from poor quality water. If this is not possible then, while they may the last of the native species to be stressed or die, they will not be completely immune to effects of persistently low DO.

Overall, and despite the physiological adaptions of freshwater catfish, it is plausible that, in some settings, low DO associated with an outbreak of CyHV-3 would lead to a long-term decrease in the size of the population or interfere with its recovery. The likelihood of this event would not vary greatly between a lower-flow or high-flow setting, although small populations in disconnected waterbodies may be relatively more exposed. It is also plausible that low DO associated with an outbreak of CyHV-3 would disrupt the breeding cycle of freshwater catfish in some settings, although the resilience of the species means that there is not a real chance or possibility that its area of occupancy would be reduced.

Bony herring are a widespread, abundant and hardy fish, tolerating high temperatures (up to 38C), high turbidity and high salinity (up to at least 39 ppt) (Part II: Section 3.1.1). Published experiments examining the tolerance of bony herring to low DO were not located, and there appeared to be some disagreement in this respect between the general guidance provided by the Murray-Darling Basin Authority and the Australian Museum. The former declared bony herring to be tolerant of low DO, whereas the latter described bony herring as susceptible to low DO and likely to be the first species to become stressed or die in the event of the drying of ephemeral waterbodies. It may be that the ubiquity and relatively high abundance of bony herring mean that deaths within a local population are visible earlier and more obviously than some other species. Bony herring are a relatively short-lived fish (2 to 3 years is common) but are highly-fecund. This combination means that a failure to recruit in a given season may result in a noticeable drop in the population at that location – with rapid a rebound when conditions for breeding are more favourable. This observation may be clouded, however, by the ability of bony herring to migrate in order to colonise new habitat or to move away from unfavourable habitat. Spawning is generally understood to take place in the still waters of shallow, sandy bays in October to February (depending on water temperature). The Character Description for the Barmah-Millewa Ramsar site, however, describes bony herring as a wetland specialist that spawns and recruits in floodplain wetlands and lakes, anabranches and billabongs during in-channel flows (Hale and Butcher, 2011). The disparity may reflect the widespread distribution of bony herring and their versatility within a range of habitats.

Overall, although there appears to be some disagreement as to the behaviour and ecology of bony herring, their ubiquity, abundance and mobility within freshwater water systems mean that it is not likely that low DO associated with an outbreak of CyHV-3 would lead to a long-term decrease in the size of a population or interfere with its recovery. This assessment would not vary greatly between a lower-flow or high-flow setting, although small populations in disconnected waterbodies may be relatively more exposed. It is plausible that low DO associated with an outbreak of CyHV-3 in carp would disrupt the breeding cycle of bony herring in some settings, although the mobility of the species means that the likelihood that its area of occupancy would be reduced is negligible.

Australian grayling are unique amongst fish in this group in that they are diadromous, spending part of its lifecycle in freshwater and at least part of the larval or juvenile stages in coastal seas (Part II: Section 3.1.1). Adults (including pre-spawning and spawning adults) inhabit cool, clear, freshwater streams with gravel substrate and areas alternating between pools and riffle zones. Spawning occurs in freshwater from late summer to early winter, cued by an increase in river flows from seasonal rains and a fall in water temperatures to 12 to 13C. Eggs are scattered over the substrate and newly hatched larvae drift downstream and out to sea, where they remain for approximately six months. Juveniles then return to the freshwater environment around November of their first year, where they remain for the remainder of their lives. Most Australian grayling die after their second year, soon after spawning, however a small proportion reach five years of age. The short life span and high fecundity mean of the species mean that local populations may undergo substantial year-to-year variations in size. The Australian grayling is believed to be absent from the inland Murray-Darling system, occurring only in streams and rivers on the eastern and southern flanks of the Great Dividing Range, from Sydney southwards to the Otway Ranges of Victoria. There is also a Tasmanian population. The tolerance of the Australian grayling to low DO does not appear to be understood, although its absence from the Murray-Darling system means that it is less likely to be exposed to low DO as a result of the decomposition of large numbers of carp carcasses. An exception to this may be the Gippsland Lakes system, in which carp are present at a moderate biomass density (50 to 150 kg/ha, Stuart *et al.*, 2019) and the Australian grayling are also present in reasonable numbers.

Overall, however, there is a negligible likelihood that low DO associated with an outbreak of CyHV-3 would lead to a long-term decrease in the size of the population of Australian grayling or interfere with its recovery. It is very unlikely that a breeding cycle would be disrupted, and the likelihood that the area that Australian grayling currently occupy would be reduced is negligible.

(b) Small-bodied native fish

Small-bodied native fish are a mixed group including wetland or floodplain specialists, and foraging generalists (Part II: Section 3.1.2). The small-bodied native fish feed principally on aquatic and terrestrial invertebrates (including both insects and small crustaceans). They breed in spring, leaving eggs adhered to submerged vegetation or substrate. Low-flow settings are generally more favourable for recruitment, although a small pulse may help to inundate spawning grounds. Two to three cycles of spawning may occur each year.

- The <u>wetland or floodplain specialists</u> that are listed under the EPBC Act and whose distribution overlaps with that of carp include the critically endangered flathead galaxias, the endangered Murray hardyhead and the vulnerable eastern dwarf galaxias. These and other wetland specialists
- The listed <u>foraging generalists</u> whose distribution overlaps with that of carp include the endangered barred galaxias, and the vulnerable variegated pygmy perch and Yarra pygmy perch.

There is also a wide range of small-bodied native freshwater species that are not listed under the EPBC Act, although occur within waters inhabited by carp. These include Australian smelt, some of the freshwater galaxias, some of the rainbowfish, river blackfish, southern purple-spotted gudgeon and some other freshwater gudgeons, some of the carp gudgeons, the southern pygmy perch, the olive perchlet (or Agassiz's glass fish), and the unspecked hardyhead and some other hardyheads.

The small-bodied native fish (including both the wetland or floodplain specialists and the foraging generalists) are relatively more tolerant to low DO than are most of the larger fish (with the exception of the benthic freshwater catfish). Although individual fish within the small-bodied category will be relatively more or less tolerant, most will begin to use aquatic surface respiration (ASR) at around 2.5 mg/L (McNeil and Closs, 2007) and may survive for some time while the oxygen concentration remains above about 1 mg/L (McMaster and Bond, 2008). This is not surprising given that many of these species are either wetland specialists, or able to use wetlands and inundated floodplains opportunistically, and recognising that the DO in warm shallow wetland water is in general quite low. Relative tolerance to low DO may mean that the small-bodied native fish as a group are likely to be less affected to the fall in DO that may accompany an outbreak of CyHV-3. The highest risk scenario for most small-bodied species would be an aggressive outbreak in a contracted waterway, in a lower-flow setting. In this situation, small-bodied native fish may be less likely than many of the larger fish to leave off-channel waterways with poor water quality and seek out more viable habitat. This may result in the stress or death of adults or juveniles, or the failure of annual recruitment. Because the small-bodied fish are also in general quite short-lived, local populations may be relatively exposed in the event of recruitment failures.

Overall, it is not likely that low DO associated with an outbreak of CyHV-3 would lead to a long-term decrease in the size of a local population of small-bodied native fish or interfere with its recovery. This event may become more plausible in an extremely dry season, with substantially decreased river and stream flows. It is plausible that low DO would disrupt the breeding cycle of individual species of small-bodied native fish in some settings. If the species is threatened, and the remaining populations of that species are spatially distributed, then the combined effects of mortality and failed recruitment might lead to a reduction in the species' area of occupancy.

(c) Freshwater crustaceans

This category includes the endangered Glenelg spiny freshwater crayfish as well as the Murray crayfish (Part II: Section 3.5) whose population strength is thought to have declined markedly following the widespread and long-lasting blackwater event within the mid-lower Murray River in 2010-11 (Section 6.2.2). Both species prefer permanent rivers and large streams, where the water flow is moderately rapid, and are sensitive to water quality and the addition of sediment, thermal pollution, pollutant discharges and bushfire impacts. Emergence occurs at approximately 2 mg/L with juveniles having a LC50 of 2.2 mg/L. Emergence leads to a high fatality rate through dehydration and predation (including opportunistic harvesting by humans). A range of other native freshwater crustaceans was also considered, including the yabby and freshwater shrimp. The yabby is perhaps the most tolerant of the crustaceans to low DO and other forms of poor water quality and survives well in farm dams for this reason. This notwithstanding, both yabbies and freshwater shrimp were observed at the water's edge during the widespread and long-lasting blackwater event of 2010-11.

Although significant blackwater events have been shown to affect the population strength of Murray crayfish and other freshwater crustaceans, it is unlikely that the decomposition of carp carcasses would result in a similarly widespread and enduring drop in DO within the lotic river Murray River channel or the major anabranches and tributaries favoured by these species. In this setting, Murray crayfish, yabbies and other detrivorous crustaceans are likely to be advantaged by the increase in available food. The risk may be higher in a lower-flow setting, when the water level is lower and local populations of Murray crayfish and other crustaceans may be sequestered in poorly-connected parts of the larger river system. In this scenario, carp biomass is also likely to be more concentrated, potentiating a more aggressive outbreak of CyHV-3 and a more marked accumulation of carp carcasses.

Overall, it is not likely that low DO associated with an outbreak of CyHV-3 would lead to a long-term decrease in the size of a local population of crustaceans or interfere with its recovery. This likelihood may be increased to a possibility in the event of an extremely dry season, with substantially decreased river and stream flows. Because eggs hatch during late spring and juveniles remain attached to the mother's pleopods until they have completed a series of moults, it is plausible that low DO associated with an outbreak of CyHV-3 in carp would disrupt a breeding cycle of (in particular) Murray crayfish and that this would result in a reduced area of occupancy.

(d) Freshwater zooplankton and macroinvertebrates

Hypoxic conditions (less than about 2 mg/L) significantly reduce the richness and abundance of zooplankton emerging from wetland sediments. This effect may be partially reversed if DO returns to normal values within about 3 weeks. If hypoxic conditions are sustained, then there will be a reduction in the availability of food resources to planktivorous biota, and this effect may cascade through the aquatic food web. Macroinvertebrates are also affected by hypoxic conditions. Although the threshold DO concentration for adult mortalities in many aquatic insect species may be slightly lower than it is for most higher-order species (about 0.85 to 1.6 mg/L) a DO concentration in the range of 2 mg/L can also reduce emergence and, thus, population sustainability. Aquatic insect species do not appear to detect and move from an area with hypoxic water until DO falls to a concentration around 0.75 mg/L, meaning that local populations can be significantly affected while DO remains above this. This again has implications through the food chain.

Overall, it is plausible that, in some settings, low DO associated with an outbreak of CyHV-3 would lead to a long-term decrease in the size of a local population of zooplankton or macroinvertebrates or interfere with its recovery. It is also plausible that low DO would disrupt a breeding cycle of zooplankton and macroinvertebrates and less likely that this would result in a reduced area of occupancy.

(e) Wetland and marshland ecological communities and ecosystems

These include the assemblage of native flora, fauna and micro-organisms associated with and dependent upon the floodplain and river wetland ecosystem (Part II: Section 3.6). Communities extend to both aquatic and terrestrial ecosystems of interconnected environmental subunits, and may include river channels, lakes and estuaries, tributaries and streams, floodplain wetlands and marshes, woodlands, and groundwater. In ecological terms, these complex communities may be considered landscape-scale ecological meta-ecosystems whose hydrological connectivity is recognised as being essential to their long-term health. The impact of low DO on such communities is likely to depend on the level to which it falls, the extensiveness of the change within the wetland or marsh community and its duration. A widespread and sustained fall in DO that affected much of a wetland community might have an effect that mirrored the drop in DO associated with a blackwater event. This can impact on the biomass, species composition and food webs within the wetland. The latter might extend to nesting waterbirds that rely on aquatic biota as a staple source of food for young. A drop in DO associated with the decomposition of carp carcasses is more probable, however, during a lower-flow carp spawning season when the extent of inundation within most wetland or marshland ecosystems will be lower and the likelihood of widespread exposure will be less. During a high-flow season the ecological community will be significantly wider-reaching and richer, although the biomass density of carp will be lower and the opportunity for carp carcasses to result in a lowered DO will be less.

Overall, it is plausible that an outbreak of CyHV-3 would lead to lowered DO that results in a reduction in the spatial extent of a wetland or marshland ecological community, or a substantial change to its species composition – the latter including: (a) harm to the habitat or lifecycle of native species, including invertebrate fauna and fish species dependent upon the wetland being seriously affected; and (b) a decline or loss of functionally important species as a result of a substantial and measurable change in the water quality. It is not likely, however, that lowered DO associated with an outbreak would lead to areas of the wetland being destroyed or substantially modified, although the plausibility of this scenario may be increased in the context of an aggressive outbreak of CyHV-3 that occurs in a part of a wetland or marsh that remains inundated (naturally or through environmental watering) during a lower-flow carp spawning season.

11.1.3 Conclusions: risks, uncertainty and treatments

The key factor influencing the likely consequences of exposure to a low DO in most settings is the flow scenario. This is because the flow scenario is in most settings linked to the accumulation of carp carcasses, to the proximity of native aquatic biota and, in some cases, to the reproductive biology of individual species. An outbreak in a wetland or a disconnected waterhole during a lower-flow setting is likely to have a greater impact on DO as carp (and carp carcasses) will be concentrated within available spawning sites. Native aquatic biota will be sharing the same constrained waterbodies and are more likely to be exposed to low DO. In some settings, wetlands or waterholes may be disconnected, thus minimising opportunities for native biota to move from an affected area. Overall, it is plausible that an outbreak of CyHV-3 that occurred within a wetland or waterhole during a lower-flow season would result in hypoxia or anoxia within a significant proportion of an ecosystem, while this scenario is less likely in a high-flow season. While the same general principles can be applied to outbreaks of CyHV-3 in permanent lakes or irrigation reservoirs, or in regulated riverine reaches or impoundments, the role of high-flow and lower-flow seasons will depend to a large extent on the connectedness of the waterway and the extent of its regulation.

If hypoxia or anoxia was to develop within a significant proportion of an ecosystem, then any Murray cod, trout cod and silver perch that are present are likely to be most at risk and, in some situations, exposure may interfere with population recovery. The area of occupancy of trout cod, some small-bodied native fish and some crustaceans (in particular, the Murray crayfish) might also be threatened, while the breeding cycle for most aquatic species might be disrupted. Low DO could reduce the extent of wetland ecological communities or cause a substantial change to their species composition. It is less likely, however, that this effect would result in areas of a wetland ecosystem being destroyed or substantially modified in a permanent sense. Evaluation of the pathway in a range of practical settings is provided in the case studies (Section 12).

The important **areas of uncertainty** encountered in this evaluation concerned the aggressiveness of an outbreak of CyHV-3 under different scenarios, and the impact that accumulating carcasses are likely to have on DO within a wide range of riverine, lacustrine and palustrine settings. In view of these uncertainties, conservative assumptions[59] were made in respect of the outbreak scenarios considered most likely in different settings. Conservative assumptions[60] were also made as to the vulnerability of key species, functional groups and ecological communities to low DO, although in this respect a partial parallel was available in the experience of ecologists and water managers with the low DO that can be associated with blackwater events.

The **treatment of risks** associated with the low DO that may accompany an outbreak of CyHV-3 will focus on the timely removal of carp carcasses. The extent to which this will be necessary, and practicable, will vary amongst settings although the overarching goal will be to prevent the DO within a given waterbody falling below about 3 to 4 mg/L or to enable it to return to that level. If the physical removal of carcasses is not practicable, then consideration could in some settings be given to flushing carcass components from a waterbody, or diluting their effect, using environmental watering. This strategy may have complex side-effects at the level of an ecosystem, functional group or individual species, and these should be reviewed by a panel with both ecological expertise and local knowledge prior to the release of environmental water. Whichever approach is taken, priority should be given to scenarios where local populations of Murray cod, trout cod, silver perch, threatened small-bodied native fish or crustaceans (in particular, the Murray crayfish) or significant wetland ecosystems are likely to be at risk. Vigilance should be particularly high where an aggressive outbreak of CyHV-3 has occurred in a wetland or off-channel environment during a lower-flow carp spawning season. In this situation, the biomass of carp carcasses will be higher although the spatial extent of the outbreak will be less, and individual waterbodies are likely to be more readily accessible than would generally be the case in a high-flow season when the floodplain is fully inundated.

11.2 Widespread cyanobacterial blooms

This pathway considers the exposure of native aquatic and terrestrial animals, livestock and humans to the effects of cyanobacterial blooms that may result from the death of large numbers of carp and the consequent eutrophication of waterways. Cyanobacterial blooms are often termed ‘harmful algal blooms’, or HABs.

It must be stressed that there is an ongoing baseline incidence of cyanobacterial blooms within the Murray-Darling Basin and elsewhere in Australia’s freshwater waterways. Signage warning of hazards associated with the use of particularly high-risk reaches, billabongs or wetlands are commonplace (Figure 29). Given this, the scenario that underpins the pathway considered in this part of the assessment was loosely described as the occurrence of a ‘widespread cyanobacterial bloom affecting a broader range of species and a more substantial part of an ecosystem than is generally the case’.

Key references for the science underpinning this pathway can be found in Part II: Section 6.

[59] Here and throughout Section 11, a conservative assumption about an outbreak scenario inferred that an outbreak will propagate as rapidly as feasible for that scenario and to its fullest likely extent in that setting

[60] Here and throughout Section 11, a conservative assumption about the vulnerability of species, functional groups or communities inferred that exposure to a given stressor would have a maximal biologically-plausible impact

Figure 29 Signage warning of cyanobacteria in the Murrumbidgee River

Source: P. Caley (January 2019)

11.2.1 Likelihood of exposure

A pictorial representation of the pathways leading to, and sustaining, a widespread cyanobacterial bloom is given in Figure 30. The particular relevance of a fish kill is that it will provide a potent source of DOC and phosphorous. In contrast to a blackwater event, where DOC is generally attributed to leachate from the accumulated leaves of river red gums and other forest trees, and is toxic to fish and other aquatic animals at high concentrations, the key relevance of DOC arising from the breakdown of fish carcasses is that it will provide a substrate for aerobic bacteria and for cyanobacteria (Part II: Section 6.3). Thus, if cyanobacteria are present in a freshwater waterway in which a fish kill has occurred, and the temperature of the water is suitable – or the water column is stratified, with the surface layer of a suitable temperature – then a bloom will in most situations be likely. This was observed by Walsh *et al.* (2018) whose experimental work was discussed within Part II: Section 6.3. For the process to extend to multiple cyanobacterial blooms, or to a single very large cyanobacterial bloom affecting a wider range of species and a more substantial part of an ecosystem than is generally the case, the source of DOC and phosphorous must be widespread and the water conditions suitable throughout a larger area.

Two key scenarios were described in Section 10 for outbreaks of CyHV-3 in ephemeral wetland or marshland settings. In these scenarios, the key determinant of the aggressiveness of the outbreak is the flow scenario.

- If a spring or early summer outbreak of CyHV-3 was to occur in a relatively high-flow season, when floodplains are widely inundated and carp are not constrained to river channels and permanent or semi-permanent off-channel waters, then the biomass density of carp at any particular point is likely to be relatively low. Under this scenario, an aggressive outbreak of CyHV-3 would require substantial transmission to have occurred during the pre-spawning aggregation. If a widespread outbreak was to occur, then although the water temperature and other conditions on the inundated floodplains are likely to be conducive to cyanobacterial blooms, the much lower biomass density of carp carcasses would be unlikely to trigger either: (a) the concurrence of multiple cyanobacterial blooms; or (b) the occurrence of a single very large cyanobacterial bloom affecting a wider range of native and other species and a more significant part of an ecosystem than is generally the case.

- If a spring or early summer outbreak of CyHV-3 was to occur in a relatively lower-flow season, when the floodplains are not widely inundated and carp are constrained to river channels and permanent or semi-permanent off-channel waters, then the biomass density of carp at points of aggregation is likely to be relatively high (with a consequently higher likelihood of concurrent significant disease events), water temperature is likely to be sufficiently high to support cyanobacterial growth and (in flowing waterways) water velocity will be lower than in a high-flow season – increasing the likelihood of stratification. Under these conditions, widespread outbreaks of cyanobacteria would be possible.

While the same general principles can be applied to outbreaks of CyHV-3 in permanent lakes or irrigation reservoirs, or in riverine reaches or impoundments, the role of high-flow and lower-flow seasons will depend to a large extent on the connectedness of the waterway and the extent of its regulation.

Once initiated, a cyanobacterial bloom would persist for as long as the conditions remained suitable and substrates remained available. An ongoing source of DOC and phosphorus will be important, as will adequate sunlight to maintain water temperature and to support a high rate of photosynthesis. An abrupt change in conditions (for example, a cool change with cloudy weather) may precipitate an imbalance whereby the respiratory needs of the cyanobacteria for oxygen outweigh the oxygenation of the waterbody through photosynthesis. When this or another imbalance occurs, the cyanobacteria will begin to die-off. Death of cyanobacteria results in aerobic decomposition and a concomitant elevated BOD and, with continued declining photosynthesis (as a result of a reducing volume of living cyanobacteria), to an ongoing fall in DO – ultimately to the point of anoxia. When this is reached, decomposition will become anaerobic and the prevalence of organisms such as *C. botulinum* (Section 11.6) may increase.

The fall in DO can be catastrophic to fish and other aquatic biota, and it may be difficult to delineate the secondary deaths associated with this from the deaths directly caused by cyanotoxin (noting that not all cyanobacteria are cyanotoxic). Once a widespread cyanobacterial bloom has been initiated, however, its progression through this process is effectively certain, with the only variables being the time before the death of cyanobacterial cells commences and the precipitousness of the resulting decline in DO. If the key factor triggering the death of cyanobacteria was an exhaustion of DOC or phosphorous, then it is also possible for the decomposing carcasses of fish and other aquatic animals killed by cyanotoxins or low DO to replenish these and to thus initiate a further bloom (Figure 30).

Once the dead cyanobacteria and carcasses of fish and other aquatic life have been consumed by anaerobic digestion, or otherwise incorporated into sediments, the water quality will begin to normalise.

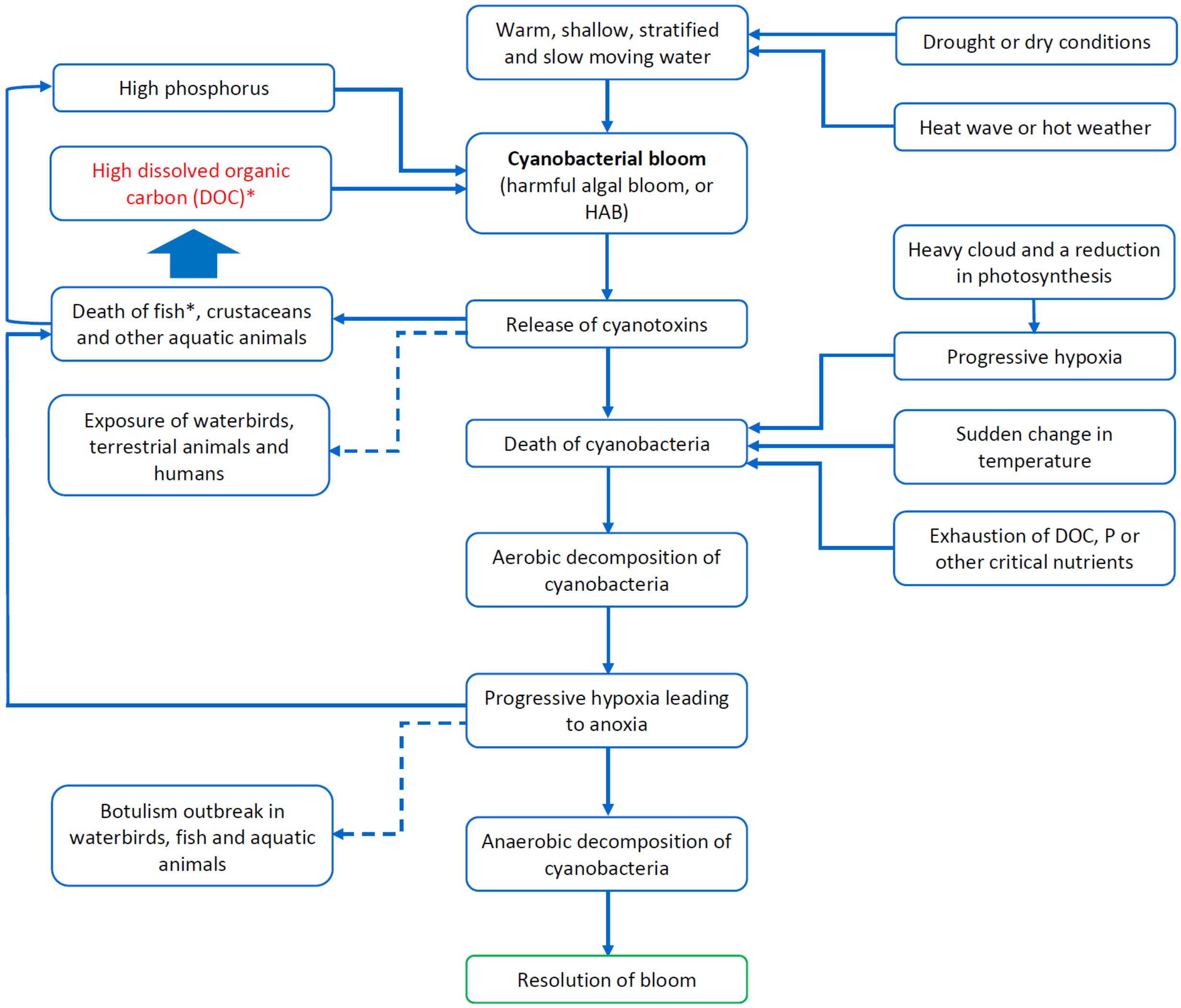

Figure 30 Ecology of a widespread cyanobacterial bloom

* Parts of the cycle that may be augmented through fish kills

11.2.2 Likely consequences of exposure

A widespread cyanobacterial bloom can harm an ecological community in four key ways:

- The pathogenic effects of cyanotoxin (if the strain involved is cyanotoxic)
- The precipitous drop in DO that will follow from collapse of the bloom
- The decline in water quality that will follow from the death and decomposition of cyanobacteria and aquatic biota
- The ecosystem-wide impacts of these processes on food webs.

A summary of the potential impacts of cyanobacterial blooms on risk assessment endpoints (Part I: Section 2.2) is given in Table 11. In chief, these impacts focus on direct harm to local populations of aquatic native biota, including large-bodied and small-bodied fish, crustaceans, zooplankton, macroinvertebrates, waterbirds (including native and migratory waterbirds), frogs and freshwater turtles. There may also be an impact on humans and livestock. A widespread cyanobacterial bloom that followed from extensive carcass decomposition is likely to be a relatively short-term event. This means that while the size of a population or its area of occupancy might be affected, populations are less likely to be fragmented. A widespread bloom is also unlikely to have an effect on habitat, nor result in the establishment of a disease or invasive species. The definition of an impact on habitat when applied in the context of migratory species is slightly broader, would be likely to extend to the effects of a widespread cyanobacterial bloom. A widespread bloom might also impact on the lifecycle of a migratory species, although would not be likely result in the establishment of a harmful invasive species. Similar logic was applied across ecological communities and wetlands.

The likely consequences of cyanobacterial blooms for individual species or functional groups are discussed in parts (a) to (d) below, while the consequences for ecological communities – including wetlands or marshes, forests and woodlands, and grasslands – are discussed in parts (e) and (f). The consequences for public health and livestock are discussed in parts (g) and (h).

For this and other exposure pathways, the likely consequence provide a preliminary or baseline assessment of the possible impacts of CyHV-3, in the sense that they do not relate to particular places or ecosystems. The site-specific case studies undertaken in Section 12 build on these baseline assessments and give an evaluation of the possible impacts of CyHV-3 on the communities and species that characterise each location.

Table 11 Relevance of widespread cyanobacterial blooms to risk assessment endpoints

Endpoints (significant impact criteria)	Relevance
Migratory and non-migratory native species	
Lead to a long-term decrease in the size of a population of a non-migratory native species	✓
Reduce the area of occupancy of a non-migratory native species	✓
Fragment an existing population into two or more populations of a non-migratory native species	✕
Adversely affect habitat critical to the survival of a non-migratory native species	✕
Disrupt the breeding cycle of a population of a non-migratory native species	✓
Modify, destroy, remove, isolate or decrease the availability or quality of habitat to the extent that a non-migratory native species is likely to decline	✕
Result in invasive species that becomes established in a non-migratory native species' habitat	✕
Introduce disease that may cause a non-migratory native species to decline	✕
Interfere with the recovery of a non-migratory native species	✓
Migratory species	
Substantially modify (including by fragmenting, altering fire regimes, altering nutrient cycles or altering hydrological cycles), destroy or isolate an area of important habitat for a migratory species	✓
Result in an invasive species that is harmful to the migratory species becoming established in an area of important habitat for the migratory species	✕
Seriously disrupt the lifecycle (breeding, feeding, migration or resting behaviour) of an ecologically significant proportion of the population of a migratory species	✓
Ecological community	
Reduce the extent of an ecological community	✓
Fragment or increase fragmentation of an ecological community, for example by clearing vegetation for	✕

Endpoints (significant impact criteria)	Relevance
roads or transmission lines	
Adversely affect habitat critical to the survival of an ecological community	✕
Modify or destroy abiotic (non-living) factors (such as water, nutrients, or soil) necessary for an ecological community's survival, including reduction of groundwater levels, or substantial alteration of surface water drainage patterns	✓
Cause a substantial change in the species composition of an occurrence of an ecological community, including causing a decline or loss of functionally important species, for example through regular burning or flora or fauna harvesting	✓
Cause a substantial reduction in the quality or integrity of an occurrence of an ecological community, including, but not limited to • Assisting invasive species, that are harmful to the ecological community, to become established, or • Causing regular mobilisation of fertilisers, herbicides or other chemicals or pollutants into the ecological community which kill or inhibit the growth of species in the ecological community, or interfere with the recovery of an ecological community	✕
Wetland ecosystem	
Areas of the wetland being destroyed or substantially modified	✓
A substantial and measurable change in the hydrological regime of the wetland, for example, a substantial change to the volume, timing, duration and frequency of ground and surface water flows to and within the wetland	✕
The habitat or lifecycle of native species, including invertebrate fauna and fish species dependent upon the wetland being seriously affected	✓
A substantial and measurable change in the water quality of the wetland – for example, a substantial change in the level of salinity, pollutants, or nutrients in the wetland, or water temperature which may adversely impact on biodiversity, ecological integrity, social amenity or human health	✓
An invasive species that is harmful to the ecological character of the wetland being established (or an existing invasive species being spread) in the wetland	✕
Public health	
Exposure of individuals to the clinical effects of a pathogenic organism or its toxin	✓
Exposure of groups of individuals or communities to the clinical effects of a pathogenic organism or its toxin	✓
Livestock	
Local exposure of livestock to the clinical effects of a pathogenic organism or its toxin	✓
Establishment of a pathogenic microorganism in livestock in areas not previously affected	✕

(a) Large-bodied and small-bodied fish, crustaceans, zooplankton and macroinvertebrates

The exposure pathway centred on the fall in DO concentration that may follow from the decomposition of carcasses in the course of an outbreak of CyHV-3 (Section 11.1) focussed on large- and small-bodied native fish, the crustaceans (in particular, the Murray crayfish), zooplankton and macroinvertebrates. Differences amongst these species and functional groups in respect of the risks associated with low DO largely reflected aspects of their spawning habitat and behaviour, and the spatial connectivity of their remaining sustainable populations. Differences amongst the species and functional groups in their susceptibility to low DO were relatively less important, as even those that are relatively tolerant will begin to experience physiological stress once DO falls below about 2 mg/L.

A similar situation is likely for the risks associated with cyanobacterial blooms, in that all of these aquatic species will be susceptible to cyanotoxins (if present) and to the precipitous fall in DO that is likely to accompany the collapse of a cyanobacterial bloom. The same will be true of the impact of decomposing cyanobacteria and aquatic biota on water quality, and the broader food-web effects of these events. Given that, differences in risk amongst the species and functional groups of native fish and other aquatic biota are again likely to reflect aspects of breeding habitat and behaviour, and the spatial connectivity of their remaining sustainable populations.

Accepting this, the risks for large- and small-bodied native fish, the crustaceans (in particular, the Murray crayfish), zooplankton and macroinvertebrates associated with cyanobacterial blooms are likely to mirror those associated with low DO. Further detail about their underpinning can be found within the discussion of low DO in Section 11.1.

The balance of this pathway focusses on the risks associated with cyanobacterial blooms that may be faced by native and migratory waterbirds, amphibians and freshwater turtles, as these groups were not included within the discussion of low DO. The broader risks to wetland and marshland ecological communities are also discussed.

(b) Native and migratory waterbirds

The implications of cyanobacterial blooms for each of nine identified groups of native or migratory waterbirds (Part II: Section 3.2) are discussed below.

Seabirds, including pelicans, cormorants and darters, are piscivorous and in general migratory, although none of those whose distribution within Australia overlaps with carp are listed under international agreements for the protection of migratory species to which Australia is a party. These species are colonial nesters and tend to settle within an area as a group and to move from it as a group. The importance of this is that stresses applied to seabird colonies are likely to be felt by many or most members of the colony – and this may mean that a large number of birds are exposed to those stresses, and that in turn can have marked impacts on the broader population of the species.

Cyanobacterial blooms may be toxic (if the species concerned is cyanotoxic) and their collapse is likely to result in a precipitous fall in DO and the resultant death of aquatic biota. This is turn will impact negatively on water quality and may disturb aquatic food webs. Seabirds spend much of their time on the water, and this will tend to maximise opportunities for exposure to cyanotoxins. Likewise, nests are often constructed from mats of vegetation, and either float within wetlands or rest close to the water's edge. In either setting, chicks will in general be placed in close proximity to affected water. Early in the breeding season, nesting seabirds may be able to avoid an affected waterbody – or move from it to one that is not affected – with no, or minimal, disruption to the breeding cycle. Once eggs have been laid, however, or chicks have hatched, it will be difficult for the colony to move without sacrificing the young from that breeding event. In this situation, parent birds may remain but attempt to source food and water from other areas and to return to the nest and to their eggs or young. Compounding these concerns is the supposition that seabirds will tend to select colonial nesting locations where habitats are inundated, and spawning fish are plentiful. The Murray-Darling system, in particular, includes a number of irrigation reservoirs with marshland borders that remain inundated regardless of the season and these provide a nesting refuge for migratory seabirds during lower-flow seasons when alternative natural wetlands and floodplains are not inundated. An outbreak of CyHV-3 within an irrigation reservoir has the potential to be aggressive, as carp may also be present in high numbers, and this may in turn trigger a widespread cyanobacterial bloom. Conversely, an aggressive outbreak, and a widespread cyanobacterial bloom resulting from it, are less likely within an inundated natural wetland during a high-flow season as the density of spawning carp will be much lower in that setting.

Overall, it is plausible that, in some settings, a widespread cyanobacterial bloom associated with an outbreak of CyHV-3 would result in a long-term decrease in the size of a population of seabirds or interfere with its recovery. This is particularly the case during a lower-flow season, when large colonies of nesting seabirds are likely to have taken refuge in the fringes of permanent waterbodies such as irrigation reservoirs. In this context, a population of migratory seabirds is taken to be a geographically separate part of the broader population, a significant proportion of whose members cyclically and predictably cross one or more national jurisdictional boundaries. It is also plausible that a widespread cyanobacterial bloom would disrupt the breeding cycle of one or more seabird species and, thus, recruitment. Although a bloom of this character would substantially modify important habitat for these migratory species, there is not a real chance or possibility that these effects would persist in the longer term and result in a reduced area of occupancy.

Shorebirds (order Charadriiformes), including the stilts, snipes, sandpipers, plover and oystercatchers (the waders), as well as gulls and terns. These birds feed by probing in the mud, or picking items off the surface, in both coastal and freshwater environments. The waders are not generally colonial nesters, although individual nests may be congregated within a loosely-defined area. Gulls and terns may nest singly or within colonies. Opportunities for the exposure of shorebirds to the effects of widespread cyanobacterial blooms arising from the decomposition of carp carcasses are similar to those for seabirds (above), in the sense that both groups spend time in or on the water and feed on aquatic biota. The risks faced by shorebirds, however, are likely to be mitigated to some extent by the fact that they nest individually and do not tend to exist in, or behave as, a single unified colony. In this way, stresses are more likely to be exerted on individuals, or certain groups of individuals, than on the population as a whole, and individuals may be more able or likely to take action to avoid such stressors – including seeking alternative nesting sites. Four shorebirds whose distribution overlaps with that of carp are listed under the EPBC Act as critically endangered, three are endangered and a further species is vulnerable. All but two of these threatened species are also listed under international agreements for the protection of migratory species to which Australia is a party. This list contains 12 additional migratory shorebirds whose habitat may overlap with carp.

Overall, it is plausible that, in some settings, a widespread cyanobacterial bloom associated with an outbreak of CyHV-3 would result in a long-term decrease in the size of a population of shorebirds or interfere with its recovery. The likelihood of this event would not differ markedly between high-flow and lower-flow seasons. It is also plausible that a widespread cyanobacterial bloom would disrupt the breeding cycle of one or more shorebird species and, thus, recruitment. Although a bloom of this character would substantially modify important habitat for these migratory species, there is not a real chance or possibility that these effects would persist in the longer term and result in a reduced area of occupancy.

Waterfowl, including ducks, geese and swans (order Anseriformes) and grebes, do not tend to nest in colonies although, like the shorebirds (above), individual nests may be congregated within a loosely-defined area. None of the waterfowl whose distribution overlaps with that of carp are listed under the EPBC Act as threatened or listed under international agreements for the protection of migratory species to which Australia is a party.

Geese and swans are herbivorous and may be exposed to cyanotoxins through their contact with affected water and consumption of aquatic plants. Dabbling ducks feed in shallow water and are more likely to have a diet with more aquatic plants and insects, while diving ducks feed deeper in the water and typically feed on small fish or crustaceans. The diet of grebes consists mainly of small fish and aquatic invertebrates. Collectively these members of the waterfowl group may be exposed to cyanotoxins both as a result of their contact with affected water, and through the consumption of affected aquatic biota. All waterfowl will be exposed to the broader impacts of a widespread cyanobacterial bloom on water quality and food webs. Although some waterfowl in the northern hemisphere migrate to escape harsh winters, the same does not occur in Australia. Waterfowl are, however, mobile and may move through a landscape to locate suitable habitat and to some extent may be able to avoid an area affected by a significant cyanobacterial bloom.

Overall, the risks posed to waterfowl through a widespread cyanobacterial bloom associated with an outbreak of CyHV-3 are likely to be very similar to those posed to shorebirds (above). Geese and swans may be slightly less exposed as a result of being herbivorous. Collectively, it is plausible that, in some settings, a bloom would result in a long-term decrease in the size of a population of waterfowl or interfere with its recovery. The likelihood of this event would not differ markedly between high-flow and lower-flow seasons. It is also plausible that a widespread cyanobacterial bloom would disrupt the breeding cycle of one or more waterfowl species, although there is not a real chance or possibility that these effects would persist in the longer term and result in a reduced area of occupancy.

<u>Large waders</u> (order Ciconiiformes), including the storks, herons, egrets, ibises, spoonbills and others, are predominantly migratory and colonial nesters. They are a carnivorous order, with a diet that may include fish, reptiles, amphibians, crustaceans, molluscs and aquatic insects. In these respects, the exposure of large waders to the risks associated with a widespread cyanobacterial bloom is likely to be very similar to that of the seabirds (above). The Australasian bittern is the single threatened species of large wader whose distribution overlaps with that of carp.

It is plausible that, in some settings, a widespread cyanobacterial bloom associated with an outbreak of CyHV-3 would result in a long-term decrease in the size of a population of large waders or interfere with its recovery. This event is more likely during a lower-flow season, when colonies of nesting birds (excepting the Australasian bittern, which is not a colonial nester) are likely to have taken refuge in the fringes of permanent waterbodies such as irrigation reservoirs. It is also plausible that a widespread cyanobacterial bloom would disrupt the breeding cycle of one or more species of large wader and, thus, recruitment. Although a bloom of this character would substantially modify important habitat for these migratory species, there is not a real chance or possibility that these effects would persist in the longer term and result in a reduced area of occupancy.

<u>Gruiformes</u>, including cranes rails, crakes, coots, moorhens and waterhens, are a diverse group of non-migratory, omnivorous birds that occupy the habitat between marshland and terrestrial habitats. The Gruiformes are not colonial nesters. Some species nest and forage in wetland environments while others prefer drier environments adjacent to a waterbody. Species that

occupy wetlands feed on a range of animal biota, including molluscs, frogs, small fish and insects, as well on aquatic plants. The exposure of these species to the effects of a widespread cyanobacterial bloom would be similar to that of many shorebirds and would arise largely from contact with affected water and the consumption of cyanotoxins in aquatic biota. The exposure of Gruiformes that occupy a more terrestrial habitat might be lower, although many of these will nevertheless nest close to a waterbody and are likely to take some prey from it. All Gruiformes would be exposed to the broader impacts of a widespread cyanobacterial bloom on water quality and food webs. Like the shorebirds, exposure of the Gruiformes may be mitigated to some extent by the fact that they are not colonial nesters and thus more able to take individual action to avoid affected areas.

Overall, is plausible that a widespread bloom would result in a long-term decrease in the size of a population of Gruiformes or interfere with its recovery. The likelihood of this event would not differ markedly between high-flow and lower-flow seasons. It is also plausible that a widespread cyanobacterial bloom would disrupt the breeding cycle of one or more species of Gruiformes, although there is not a real chance or possibility that these effects would persist in the longer term and result in a reduced area of occupancy.

<u>Kingfishers</u> (order Coraciiformes, family Alcedinidae) may be exposed to cyanotoxin as a result of feeding on affected aquatic biota or drinking contaminated water. They may also be exposed to the broader impacts of a widespread cyanobacterial bloom on water quality and food webs. Kingfishers are not colonial nesters, however, and their nests are not close to water. Although they are not migratory, they would generally be able to move away from an affected area. Overall, there is not a real chance or possibility that a widespread bloom would result in a long-term decrease in the size of a population of kingfishers (including kookaburras) or interfere with its recovery. This would not differ markedly between high-flow and lower-flow seasons. There is also not a real chance or possibility that a widespread cyanobacterial bloom would disrupt a breeding cycle, or that its effects would persist in the longer term and result in a reduced area of occupancy.

<u>Songbirds</u> (order Passeriformes, the passerine birds) are the largest order of birds. Their diet varies amongst species, and may include seeds, insects and small crustaceans. Some passerine birds, such as the clamorous warbler, are true waterbirds. Others may be present in wetlands and other water-focussed ecosystems, whether permanently or as an opportunistic means by which to gain refuge from drought and other environmental stresses. These two groups of passerine birds may be exposed to cyanotoxin as a result of drinking affected water or consuming affected aquatic biota. Passerine birds that live in close association with waterbodies, and are considered waterbirds, would be additionally exposed through contact with affected water. These birds may also be relatively less able to move away from an affected area. All passerines are likely to be exposed to the broader impacts of a widespread cyanobacterial bloom on water quality and food webs.

Overall, the risks faced by the passerine waterbirds is likely to be similar to those faced by the Gruiformes (above). That is, it is plausible that, in some settings, a widespread bloom would result in a long-term decrease in the size of a population of passerine waterbirds or interfere with its recovery. This would not differ markedly between high-flow and lower-flow seasons. It is also plausible that a widespread cyanobacterial bloom would disrupt the breeding cycle of one or more species, although there is not a real chance or possibility that these effects would persist in the longer term and result in a reduced area of occupancy.

Bush-birds are an eclectic mixture of species that roost, forage and nest in riparian or floodplain forests but are not generally considered to be true waterbirds. Although some of these birds may consume insects contaminated with cyanotoxin, their exposure will in general be limited to drinking water. Some of the bush-birds nest in close proximity to water, although most will be able to move away from an affected area. Except in unusual circumstances, they would be considered one of the less exposed of the bird functional groups. Overall, it is plausible that, in some settings, a widespread bloom would result in a long-term decrease in the size of a population of bush-birds or interfere with its recovery. This would not differ markedly between high-flow and lower-flow seasons. It is also plausible that a widespread cyanobacterial bloom would disrupt a breeding cycle, although there is not a real chance or possibility that these effects would persist in the longer term and result in a reduced area of occupancy.

Aquatic raptors (in this assessment) include the Accipitriformes (hawks, goshawks, kites, eagles and ospreys), Falconiformes (falcons and kestrels) and Strigiformes (owls) that inhabit riverine, lacustrine and palustrine ecosystems. The red goshawk, whose distribution overlaps with that of carp, is listed under the EPBC Act as vulnerable. As a group, the exposure of these birds to cyanotoxins would be limited to drinking affected water and consuming affected animals or insects. Raptors in the proximity of widespread cyanobacterial bloom are also likely to be exposed to the broader impacts on water quality and food webs. They are in general, however, both mobile and able to hunt over a relatively large area and these attributes would help them to avoid areas affected by a cyanobacterial bloom. There are also two species of raptor whose diet regularly includes fish: the eastern osprey and white-bellied sea eagle. Although these are primarily coastal species, both also inhabit wetlands and lakes. The eastern osprey’s diet is principally fish, while the sea eagle may take carrion as well as waterbirds. The eastern osprey is listed under international agreements for the protection of migratory species to which Australia is a party.

The exposure of raptors to cyanotoxins would include drinking affected water and (depending on species) consuming affected animals or insects. Importantly, this may extend to carrion. As cyanotoxins can bioaccumulate, repeated low-dose exposures may also result in exceeding the toxic threshold. The two raptors that consume fish (eastern osprey and white-bellied sea eagle) are likely to be relatively more exposed to cyanotoxins. For these species, it is plausible that, in some settings, a widespread cyanobacterial bloom associated with an outbreak of CyHV-3 would result in a long-term decrease in the size of a (generally limited) local population or interfere with its recovery. This is particularly the case during a lower-flow season when a local population of eastern ospreys or white-breasted sea eagles may be less able to locate alternative unaffected habitat. It is also plausible that a widespread cyanobacterial bloom would disrupt the breeding cycle of ospreys or sea eagles. A bloom of this character will substantially modify important habitat for the migratory osprey and, given generally low size of raptor populations, it is plausible that this effect would persist in the longer term and result in a reduced area of occupancy for either species.

(c) Frogs

Frogs occupy a wide variety of habitats, and many have developed highly-specialised adaptations to particular climates, ecological sites and communities (Part II: Section 3.3). Of the 208 identified species of frog in Australia, two have a distribution that overlaps with that of carp and are listed under the EPBC Act as critically endangered, five species are endangered and a further five species are vulnerable. As most frog species are susceptible to dehydration, proximity to water is a general prerequisite. Preferred habitats can include rivers, lakes and wetlands. Exposure to cyanotoxins as a result of a widespread bloom could arise from consuming affected insects or from contact with and consumption of affected water. Frogs will also be intimately exposed to the poor water quality associated with the decomposition of a cyanobacterial bloom. Although frogs have some ability to move between waterbodies, it is relatively unlikely that they would be able to avoid a widespread cyanobacterial bloom. Most frogs spawn in spring and summer, and this is likely to coincide with the spawning of carp and an outbreak of CyHV-3. Both eggs and tadpoles may thus be exposed to cyanotoxins in the event of a widespread bloom.

Overall, it is plausible that, in some settings, a widespread cyanobacterial bloom associated with an outbreak of CyHV-3 would result in a long-term decrease in the size of a local frog population or interfere with its recovery. This is particularly the case during a lower-flow season when a local population may be less able to locate alternative unaffected habitat. A widespread cyanobacterial bloom may also disrupt the breeding cycle and substantially modify important habitat and, given the small size of the extant populations of some frog species, and their fragmentation, it is plausible that this effect would persist in the longer term and result in a reduced area of occupancy.

(d) Freshwater turtles

Australia is home to 23 species of freshwater turtle, of which one (the Bellinger River snapping turtle) has a distribution that overlaps with that of carp and is listed under the EPBC Act as critically endangered, while another (the Bell's turtle) is vulnerable (Part II: Section 3.4). Freshwater turtles inhabit riverine, lacustrine and palustrine environments and only come onto land to lay eggs, in spring and summer. Hatchlings immediately migrate to the closest water. Some species can also survive for months in a dormant state (aestivation) buried in soil or dry lake beds. Freshwater turtles are either carnivorous (including insects, tadpoles, small fish and crustaceans) or omnivorous, and some species may also feed on carrion.

Although there are marked differences in lifecycles, the extent of exposure of most freshwater turtles to cyanotoxins associated with a widespread of multifocal cyanobacterial bloom is likely to be similar to that of most frog species (above). With the exception of brief periods when seeking a suitable nesting site, or moving between habitats, turtles spend their lives within a waterbody and feed on aquatic biota. The species that consume carrion might also be exposed to cyanotoxin that has accumulated within the carcass of a dead fish or bird. Turtles would also be exposed to the broader impacts of a widespread cyanobacterial bloom on water quality and food webs. Although they have a greater ability than frogs to move away from an affected area, and source clean water, the high rate of dehydration, predation and death through other causes is likely to mean that this would not greatly increase the survivability of a local population.

Overall, it is plausible that, in some settings, a widespread cyanobacterial bloom associated with an outbreak of CyHV-3 would result in a long-term decrease in the size of a local population of freshwater turtles or interfere with its recovery. This is particularly the case during a lower-flow season when a local population may be less able to locate alternative unaffected habitat. A widespread cyanobacterial bloom may also disrupt the breeding cycle and substantially modify important habitat, and for some small and fragmented populations, it is plausible that this effect would persist in the longer term and result in a reduced area of occupancy.

(e) Wetland and marshland ecological communities and ecosystems

As noted in the evaluation of the exposure pathway for low DO (Section 11.1), wetland and marshland communities include the assemblage of native flora, fauna and micro-organisms associated with and dependent upon the floodplain and river wetland ecosystem. Communities extend to both aquatic and terrestrial ecosystems of interconnected environmental subunits, and may include river channels, lakes and estuaries, tributaries and streams, floodplain wetlands and marshes, woodlands, and groundwater. In ecological terms, these complex communities may be considered landscape-scale ecological meta-ecosystems whose hydrological connectivity is recognised as being essential to their long-term health.

The impact of a widespread cyanobacterial bloom on such communities is likely to be significant and may exceed the comparative impact of a low DO event. This is because a cyanobacterial bloom can harm an ecological community in four key ways: (a) through the effects of cyanotoxin (if the strain involved is cyanotoxic); (b) through the precipitous drop in DO that will follow from collapse of the bloom; (c) through the decline in water quality that will follow from the death and decomposition of cyanobacteria and aquatic biota; and (d) through the ecosystem-wide impacts of these processes on food webs. These effects extend beyond the immediate aquatic environment to all parts of the integrated wetland ecological community. The intensity of effects is likely to be particularly marked during a lower-flow season, when the likelihood of an aggressive outbreak in carp is also highest. During a high-flow season the ecological community will be significantly wider-reaching, and richer in biodiversity, although the biomass density of carp will be lower and the opportunity for carp carcasses to precipitate a widespread cyanobacterial bloom will be less.

Overall, it is plausible that a widespread cyanobacterial bloom would result in a reduction in the extent of a wetland or marshland ecological community, or cause a substantial change in its species composition – the latter including: (a) harm to the habitat or lifecycle of native species, including invertebrate fauna and fish species dependent upon the wetland being seriously affected; and (b) a decline or loss of functionally important species as a result of a substantial and measurable change in the water quality. It is also plausible that a widespread bloom would result in areas of the wetland being destroyed or substantially modified. These effects are more likely in the context of an aggressive outbreak of CyHV-3 that occurred within a part of a wetland or marsh that remained inundated (naturally or through environmental watering) during a lower-flow carp spawning season.

(f) Forests and woodland, and grassland, communities

Both forest and woodland, and grassland, ecological communities may be exposed to the effects of a widespread cyanobacterial bloom if those communities rely upon a waterbody such as river or lake as a source of drinking water and of the various components of the community's food web. In this situation, forest and woodland, and grassland, ecological communities may be exposed to the same harmful processes as wetland communities (above), albeit to a lesser degree given the greater degree of integration that a waterbody has within a water-focussed wetland community. This is likely to mean that a widespread cyanobacterial bloom that occurs within a forest and woodland, and grassland, ecological community, would cause stress to that community with less emphasis on actual and direct harm. Bird life and terrestrial animals may move to unaffected parts of the community, or away from the community altogether. To the extent that it is possible, aquatic life may also attempt to move away from the bloom. As a result, the richness and diversity of biota is likely to be lower within the community while the bloom remains, and until its decomposition is complete, and the water quality is able to improve.

Overall, it is plausible that a widespread cyanobacterial bloom would result in a reduction in the extent of a forest and woodland, or grassland, ecological community, or cause a substantial change in its species composition – the latter including a decline or loss of functionally important species as a result of a substantial and measurable change in the water quality. It is also plausible that a widespread bloom would result in areas of the community being destroyed or substantially modified. These effects are more likely in the context of an aggressive outbreak of CyHV-3 that occurred within a part of a forest and woodland, or grassland, that remained inundated (naturally or through environmental watering) during a lower-flow carp spawning season.

(g) Public health

Exposure to cyanobacteria and their toxins may occur by accidental or deliberate ingestion or inhalation of toxin-contaminated water, or dermal contact during recreational activities (for example, swimming or water-skiing). Cyanobacteria and cyanotoxins have been shown to cause a wide range of acute inflammatory effects or illnesses.[61] Exposure to cyanobacterial cells while in recreational waters may cause skin irritations, including rashes, hives, swelling or skin blisters. Ingestion of cyanotoxins can also cause more severe health effects such as liver or kidney damage, depending on the cyanotoxin and the magnitude, duration and frequency of the exposure. For example, short-term exposures to microcystins could cause liver damage, while kidney damage is a key health effect for cylindrospermopsin. The mitigating factor in this scenario is that the visible effects of a widespread cyanobacterial bloom will in general be apparent, and that both this and the odour of decomposing biota and (later in the process) cyanobacteria will deter most water users from undertaking recreational activities or taking fish, crustaceans or drinking water from affected areas. There is also likely to be warning signage in places of public amenity, as cyanobacterial blooms resulting from causes other than the death of carp are not uncommon in most Australian waterways.

Overall, although not likely, it is plausible that a person or persons would experience the clinical effects of cyanotoxins resulting from a widespread cyanobacterial bloom.

(h) Livestock

Livestock and companion animals may also be exposed to cyanotoxins if they drink water from toxin-contaminated waterbodies, lick their coats after swimming in such waters, or consume toxin-containing algal scum or mats. The effects of cyanotoxin are as varied in animals as they are in people, with visible symptoms including vomiting, diarrhoea, seizures and death.

The exposure of livestock in Australia may not be improbable in some settings, as livestock are frequently husbanded in such a way as to depend on a particular source(s) of drinking water. This might be access to a reach of a river or stream, or to a billabong. In dryer seasons, when

[61] See: https: //www.epa.gov/sites/production/files/2017-07/faqs-rec-water.docx

waterbodies have contracted and an aggressive outbreak of CyHV-3 in carp is more likely, such waterbodies may be affected by a cyanobacterial bloom. In this situation, stock within the area may have no choice but to consume affected water – whether directly from that source or when pumped through a storage and distribution system. Importantly, cyanotoxins are also able to accumulate within animal tissues, meaning that repeated low-dose exposures can eventually reach a point within an animal where the toxic threshold is breached and clinical symptoms are observed.

Overall, it is plausible that livestock would experience the clinical effects cyanotoxins as a result of drinking water sourced from a place where a widespread cyanobacterial bloom was occurring.

11.2.3 Conclusions: risks, uncertainty and treatments

The key factor influencing the likelihood that an outbreak of CyHV-3 will result in a widespread cyanobacterial bloom is the flow scenario. Under a high-flow scenario, ephemeral lakes, wetlands, floodplains and marshes will be inundated and ecological communities will be maximally diverse. However, whilst the potential for significant harm is more likely in this setting the likelihood that carp carcasses will accumulate in numbers sufficient to initiate a widespread cyanobacterial bloom, is lower. The converse is true of a lower-flow scenario, or an outbreak within a reasonably contained body of water – including some permanent lakes or and some minor riverine environments with connected billabongs. Overall, it is plausible that an outbreak of CyHV-3 that occurred during a lower-flow season would result in a widespread cyanobacterial bloom, while this scenario is less likely during a high-flow season.

If a widespread cyanobacterial bloom was to develop, then local populations of Murray cod, trout cod and silver perch are again the most likely of the large-bodied native fish species to be at risk and, in some settings, exposure may interfere with their recovery. The area of occupancy of trout cod, some small-bodied native fish and some crustaceans (in particular, the Murray crayfish) might also be threatened, while the breeding cycle for most aquatic species may be disrupted. This risk profile for native fish is similar to the profile associated with low DO concentration (Section 11.1). The risks for native and migratory waterbirds are higher for those functional groups or species whose feeding, foraging, nesting and other behaviours place them in close and regular contact with water. Colonial nesters are also at higher risk, as stressors to these populations are likely to be applied to a large number of birds at a time when harm can fall to adults and the success of recruitment can be threatened. Diet is also important, in particular as cyanotoxins bioaccumulate in animal tissues and regular exposure to affected (living) biota can mean that a toxic threshold is eventually breached. Raptors have an additional layer of exposure inasmuch as their position at the top of the food chain means that they are relatively fewer in number, and that the loss of a small number of individuals could significantly affect the sustainability of a local population. The risks faced by frogs and turtles are reasonably similar, as both spend most of their time in or in proximity to a waterbody and have a limited ability to (safely) avoid affected water. Some individual species within both taxa are also constrained in number, and currently existing in fragmented populations, and the threat to their sustainability is higher. Cyanotoxins affect people and livestock, although people are likely to be protected to a large extent by the innate repulsiveness of a waterbody that contains a significant cyanobacterial bloom, and the signage and other warnings that will generally be given in places of public amenity. Livestock, however, may have less ability to avoid affected drinking water if there are limited or no alternatives. The risks to ecological communities are understandably highest for wetlands and marshlands, although a widespread cyanobacterial bloom affecting a waterbody within or proximal to a forest and woodland, or grassland, ecological community, may also impact on species that utilise that water. Evaluation of the pathway in a range of practical settings is provided in the case studies (Section 12).

Important **areas of uncertainty** encountered in this evaluation again concerned the aggressiveness of an outbreak of CyHV-3 under different scenarios, and the impact that accumulating carcasses may have in respect of precipitating a widespread cyanobacterial bloom. In view of these uncertainties, conservative assumptions were made in respect of the outbreak scenarios considered most likely under high-flow and lower-flow conditions. Conservative assumptions were also made in respect of the vulnerability of key species, functional groups and ecological communities to cyanotoxins and to the effects that collapsing and decomposing blooms may have on DO and, subsequently, on water quality and food webs.

The **treatment of risks** associated with the widespread cyanobacterial bloom that may accompany an outbreak of CyHV-3 will focus on the timely removal of carp carcasses. The extent to which this will be necessary, and practicable, will vary amongst settings and is difficult to quantify. Consideration should be given to the characteristics of a waterbody that will increase its inherent susceptibility to cyanobacterial blooms. These include the connectedness and velocity of water, its temperature, the extent of stratification and the potential for reaeration. Consideration should also be given to characteristics of ecological communities, functional groups and (in some cases) species that may place them at a higher risk. In particular, these include communities with large colonies of colonial-nesting waterbirds, or fragmented populations of threatened species – as noted above. If the physical removal of carcasses is not practicable, then consideration could in some settings be given to flushing carcass components or the bloom itself from a waterbody, or diluting their effect, using environmental watering. This strategy may have complex side-effects at the level of an ecosystem, functional group or individual species, and these should be reviewed by a panel with both ecological expertise and local knowledge prior to the release of environmental water.

11.3 Microorganisms associated with decomposing fish carcasses

This pathway considers the exposure of native aquatic and terrestrial animals, livestock and people to the harmful effects of waterborne microorganisms that may be released as a result of the decomposition of large numbers of carp carcasses.

Key references for the science underpinning this pathway can be found in Part II: Section 6.

11.3.1 Likelihood of exposure

It was explained in Part II: Section 6.3.4 that very little has been published on the topic of the particular waterborne microorganisms that may be associated with the decomposition of fish carcasses in water. This is despite the marked visibility of public health warnings about the need to ensure that fish and other carcass materials are not present in drinking water. It was suggested in a personal communication[62] that carp gut flora and spoilage organisms may proliferate after a fish kill, as might *E. coli*, some *Pseudomonas spp* and other opportunistic microorganisms. Shiga toxin-producing *E. coli* (STEC) has been isolated from bodies of water (such as ponds and streams), wells and water troughs, and has been found to survive for months in manure and water-trough sediments. *Aeromonas spp* have also been found in irrigation water, rivers, springs, groundwater, estuaries and oceans (in particular where water has been contaminated by sewerage) and are of public health concern. These and other waterborne microorganisms may be constrained to waterways directly affected by decomposing carp, or may persist in unaffected waters or in waters that have returned to a healthy state following a fish kill. Pera *et al.* (2019) undertook a mesocosm experiment with carp carcass biomass densities ranging from 250 to 6,000 kg/ha. These authors found that coliforms increased in all mesocosms that contained carp carcasses. Counts fell to around zero after about a week, although increased again at around 25 days in the mesocosms containing lower carp biomass densities. The authors also assessed counts of: (a) signature lake bacteria; (b) environmental copiotroph bacteria; and (c) fish gut signature bacteria. The signature lake bacteria decreased in mesocosms that included the decaying fish. Both environmental copiotroph bacteria and fish gut bacteria increased, although in some cases levels were higher or highest within mesocosm with a lower carp biomass density.

The pathogenic potential of these and other waterborne microorganisms for aquatic animals, while specific to particular genera and species, is unlikely to exceed the ongoing harmful effects of low DO and other water quality parameters. Nevertheless, aquatic animals that remain alive in the face of these larger challenges will be stressed and immunocompromised and may have a diminished resistance to waterborne microorganisms. The pathogenic potential of waterborne microorganisms for terrestrial animals (including waterbirds and amphibians) will arise from consuming contaminated water, or consuming prey or carrion that has been infected or contaminated. Terrestrial animals will not be stressed directly by poor water quality, but may be experiencing a sudden absence of suitable food source or a difficulty in locating unspoiled drinking water. In these situations, terrestrial animals may also be immunocompromised and have a diminished resistance to waterborne microorganisms.

Overall, it is plausible that, in some settings, native aquatic and terrestrial animals, livestock and people will be exposed to waterborne microorganisms that may be released as a result of the decomposition of large numbers of carp carcasses. The key factors affecting this are water temperature and the flow scenario. It was judged that if the water temperature is less than approximately 20C then epidemics of most waterborne microorganisms would be relatively less likely. Likewise, an epidemic would be more probable during a lower-flow scenario when the connectivity of waterbodies and velocity of water is less. Stratification is also more likely under a lower-flow scenario, and this may help to concentrate waterborne microorganisms.

[62] Dr Paul Monis, Adjunct Associate Professor, School of Natural and Built Environments, University of Adelaide and Manager, Research Stakeholders and Planning, South Australia Water

11.3.2 Likely consequences of exposure

A summary of the potential impacts of waterborne microorganisms that may be released into the water following a fish kill is given in Table 12 below. In chief, these are focussed on acute harm, and the effect that this may have on breeding cycles and population sustainability.

Although there is substantial uncertainty (and likely variation) around the pathogens that may be released following a substantial fish kill, and their ability to survive in an aquatic environment and cause harm to aquatic or terrestrial biota, it is reasonable to expect that the relative exposure of communities, functional groups and species will be similar to that obtained for widespread cyanobacterial blooms – albeit with a lower likelihood of realising any of the identified ecological endpoints. The rational for specifying lower likelihoods reflects in part the science – in particular, the barriers faced by waterborne microorganisms in establishing a presence in an ecosystem equivalent to a cyanobacterial bloom – as well as the experience obtained from field observations of Australian freshwater waterways, where substantial fish kills are not uncommon but have not to-date been linked to subsequent epidemics of waterborne pathogens.

Given the above, Murray cod, trout cod and silver perch are the most likely of the large-bodied native fish to be at risk. The area of occupancy of trout cod, some small-bodied native fish and some crustaceans (in particular, the Murray crayfish) might also be threatened, while the breeding cycle for most aquatic species may be disrupted. The risks for native and migratory waterbirds will be higher for those functional groups or species whose feeding, foraging, nesting and other behaviours place them in close and regular contact with water. Colonial nesters are also at higher risk, as stressors to these populations are likely to be applied to a large number of birds at a time when harm can fall to adults and the success of recruitment can be threatened. Diet may be important, and raptors have an additional layer of exposure inasmuch as their position at the top of the food chain means that they are relatively fewer in number, and that the loss of a small number of individuals could significantly affect the sustainability of a local population. The risks faced by frogs and turtles are reasonably similar, as both spend most of their time in or in proximity to a waterbody and have a limited ability to (safely) avoid affected water. Some waterborne microorganisms affect people and livestock, although people are likely to be protected to a large extent by the innate repulsiveness of a waterbody that contains a significant outbreak. Livestock, however, may have less ability to avoid affected drinking water if there are limited or no alternatives. The risks to ecological communities are understandably highest for wetlands and marshlands, although an outbreak affecting a waterbody within or proximal to a forest and woodland, or grassland, ecological community, may also impact on species that utilise that water.

For this and other exposure pathways, the likely consequence provide a preliminary or baseline assessment of the possible impacts of CyHV-3, in the sense that they do not relate to particular places or ecosystems. The site-specific case studies undertaken in Section 12 build on these baseline assessments and give an evaluation of the possible impacts of CyHV-3 on the communities and species that characterise each location.

Table 12 Relevance of waterborne microorganisms to risk assessment endpoints

Endpoints (significant impact criteria)	Relevance
Migratory and non-migratory native species	
Lead to a long-term decrease in the size of a population of a non-migratory native species	✕
Reduce the area of occupancy of a non-migratory native species	✕
Fragment an existing population into two or more populations of a non-migratory native species	✕
Adversely affect habitat critical to the survival of a non-migratory native species	✕
Disrupt the breeding cycle of a population of a non-migratory native species	✓
Modify, destroy, remove, isolate or decrease the availability or quality of habitat to the extent that a non-migratory native species is likely to decline	✕
Result in invasive species that becomes established in a non-migratory native species' habitat	✕
Introduce disease that may cause a non-migratory native species to decline	✓
Interfere with the recovery of a non-migratory native species	✕
Migratory species	
Substantially modify (including by fragmenting, altering fire regimes, altering nutrient cycles or altering hydrological cycles), destroy or isolate an area of important habitat for a migratory species	✕
Result in an invasive species that is harmful to the migratory species becoming established in an area of important habitat for the migratory species	✕
Seriously disrupt the lifecycle (breeding, feeding, migration or resting behaviour) of an ecologically significant proportion of the population of a migratory species	✓
Ecological community	
Reduce the extent of an ecological community	✕
Fragment or increase fragmentation of an ecological community, for example by clearing vegetation for roads or transmission lines	✕
Adversely affect habitat critical to the survival of an ecological community	✕
Modify or destroy abiotic (non-living) factors (such as water, nutrients, or soil) necessary for an ecological community's survival, including reduction of groundwater levels, or substantial alteration of surface water drainage patterns	✕
Cause a substantial change in the species composition of an occurrence of an ecological community, including causing a decline or loss of functionally important species, for example through regular burning or flora or fauna harvesting	✕
Cause a substantial reduction in the quality or integrity of an occurrence of an ecological community, including, but not limited to • Assisting invasive species, that are harmful to the ecological community, to become established, or • Causing regular mobilisation of fertilisers, herbicides or other chemicals or pollutants into the ecological community which kill or inhibit the growth of species in the ecological community, or interfere with the recovery of an ecological community	✕
Wetland ecosystem	
Areas of the wetland being destroyed or substantially modified	✕
A substantial and measurable change in the hydrological regime of the wetland, for example, a substantial change to the volume, timing, duration and frequency of ground and surface water flows to and within the wetland	✕
The habitat or lifecycle of native species, including invertebrate fauna and fish species dependent upon the wetland being seriously affected	✕
A substantial and measurable change in the water quality of the wetland – for example, a substantial change in the level of salinity, pollutants, or nutrients in the wetland, or water temperature which may	✓

Endpoints (significant impact criteria)	Relevance
adversely impact on biodiversity, ecological integrity, social amenity or human health	
An invasive species that is harmful to the ecological character of the wetland being established (or an existing invasive species being spread) in the wetland	✗
Livestock	
Local exposure of livestock to the clinical effects of a pathogenic organism or its toxin	✓
Establishment of a pathogenic microorganism in livestock in areas not previously affected	✗
Public health	
Exposure of individuals to the clinical effects of a pathogenic organism or its toxin	✓
Exposure of groups of individuals or communities to the clinical effects of a pathogenic organism or its toxin	✗

11.3.3 Conclusions: risks, uncertainty and treatments

Although much remains unknown about the risks associated with waterborne microorganisms, the key factors affecting the development of epidemics are likely to be the temperature of the water and the flow scenario. A threshold of approximately 20C broadly denotes 'warm water', and in general terms most waterborne microorganisms are likely to survive or multiply aggressively in these conditions. As was the case for the risks associated with low DO and widespread cyanobacterial blooms (Sections 11.1 and 11.2, respectively), the flow scenario is also likely to be important. Under a lower-flow scenario, carp are likely to be more concentrated and an aggressive outbreak of CyHV-3 is more probable. In this situation, the connectivity of waterbodies – and the extent and velocity of water movement – will be reduced, and waterbodies are more likely to be stratified. These conditions are likely to help concentrate waterborne microorganisms and to increase the exposure of aquatic biota and terrestrial animals. Overall, it is plausible that, in some settings, native aquatic and terrestrial animals, livestock and people will be exposed to waterborne microorganisms that may be released as a result of the decomposition of large numbers of carp carcasses.

The important **areas of uncertainty** encountered in this evaluation concerned the particular species of microorganism likely to be released into a waterbody in substantive numbers following a fish kill, and the virulence and pathogenicity of these organisms for individual aquatic and terrestrial biota. The survivability of particular microorganisms is also important, as is the probable infectious dose for individual aquatic and terrestrial biota. These considerations provide the underpinning for the epidemiology of waterborne disease syndromes that may be linked to fish kills. The absence of much of this information was not considered to be so significant as to invalidate the assessment as: (a) broad inference about the relative exposure of particular communities, functional groups and species could be made from the evaluation of cyanobacterial blooms; and (b) field experience drawn from numerous substantial fish kills in Australian freshwater waterways suggests that a serious epidemic of a waterborne microorganism is an unlikely of an outbreak of CyHV-3.

The **treatment of risks** associated with an epidemic of a waterborne microorganism following an outbreak of CyHV-3 will focus on the timely removal of carp carcasses. The extent to which this will be necessary, and practicable, will vary amongst settings and, in common with the risks associated with a widespread cyanobacterial bloom, is difficult to quantify. Particular consideration should be given to the characteristics of a waterbody that will increase its inherent susceptibility to epidemics of waterborne microorganisms. These include the connectedness and velocity of water and its temperature, and the extent of stratification. Consideration should also be given to characteristics of ecological communities, functional groups and (in some cases) species that may place them at particular risk. In particular, these include communities with large colonies of colonial-nesting waterbirds, or fragmented populations of threatened. If the physical removal of carcasses is not practicable, then consideration could in some settings be given to flushing carcass components from a waterbody, or diluting its effect, using environmental watering. This strategy may have complex side-effects at the level of an ecosystem, functional group or individual species, and these should be reviewed by a panel with both ecological expertise and local knowledge prior to the release of environmental water.

11.4 Removal of carp as a dominant and stable food source

This pathway considers the exposure of ecological communities, functional groups and individual species to the sudden removal of a dominant and stable food source – that is, adult or juvenile carp. One of the key observations from the review of this topic in Part II: Section 8 was the absence of published research examining the food-web effects of fish kills. In the light of this, deductions were made on the basis of a simpler understanding of the underlying ecology and the dynamics of individual predator-prey relationships.

Key references for the science underpinning this pathway can be found in Part II: Section 8.

11.4.1 Likelihood of exposure

Although adult and juvenile carp may be predated upon by a range of aquatic and terrestrial species, this pathway focussed on the exposure of nesting waterbirds as these are considered most likely to be exposed to a substantial risk. The key factor influencing the likelihood that nesting waterbirds will be exposed to the sudden removal of a dominant food source, following from an outbreak of CyHV-3 in carp, is the flow scenario. This is particularly the case for wetland and marshland ecosystems. In a high-flow season, ephemeral wetlands, marshlands and floodplains will be inundated and will be maximally colonised by nesting waterbirds. Carp will spawn in these settings, or in adjacent off-channel waters, and carp larvae and juveniles will be present in very high numbers. Some native fish are also likely to spawn significantly under a high-flow scenario, and adults of many species of (in particular) small-bodied native fish are also likely to move into recently-inundated areas. If at least some exposure of carp to CyHV-3 occurred prior to dispersal to floodplains, then the ensuing outbreak may be aggressive even though the biomass density of carp on the floodplain is likely to be very low. This may have an impact on the spawning success of carp and the survivability of highly-susceptible juveniles. As the summer progresses, the temperature of the water on the floodplain may exceed the threshold for transmission for CyHV-3, and this may slow the outbreak and reduce the exposure of juvenile carp. Once the floodwaters begin to recede, however, juveniles will drift back toward the channels and anabranches where the water temperature is likely to be lower. In this setting, the outbreak may be revived amongst both juvenile and adult carp and this may result in a further decline in the availability of food for nesting waterbirds. This may result in a sudden and precipitous drop in the availability of a staple food source during a time when the demand for food from large numbers of nesting waterbirds is at its peak.

In a lower-flow season, when the floodplain is not inundated, carp are likely to spawn within the river channel and off-channel waters, and juvenile carp will be constrained to these waters. Under these conditions of high biomass density and relatively deeper and cooler water, an aggressive outbreak of CyHV-3 is more likely. Importantly, however, waterbirds will not have congregated within the wetland in such large numbers and the impact of a loss of a stable food source is likely to be substantially less significant in this setting.

Beyond the wetland ecosystem (for example, in riverine environments, lakes or billabongs) an outbreak of CyHV-3 may also have an impact on the food webs of native waterbirds. In these settings, an aggressive outbreak will again be linked to a high biomass density of carp. In some situations, this may be linked to the flow scenario (whether a wet year or a dry year) but in others (for example, some lakes that are also irrigation reservoirs) the water level may be maintained regardless of rainfall or the flow of water within a connected river channel. Indeed, nesting waterbirds may congregate in particularly high numbers in the marshland at the periphery of some permanent waterbodies during lower-flow seasons, when regional ephemeral wetlands and floodplains are not inundated. Carp may also be abundant in these settings, such that an aggressive outbreak of CyHV-3 is likely. This may in turn result in a significant deficit in a staple food source and, potentially, the exposure of a large number of nesting waterbirds.

At a landscape level, the connectedness of a waterway may also influence the extent to which other fish (including carp from unaffected areas) are able to re-colonise an ecosystem that has been depleted by an outbreak of CyHV-3. Colonisation from other areas will, however, be a longer-term adaptation as the immediate effect of a fish kill of sufficient magnitude to result in a stress to food webs is likely to be a negative impact on water quality and an environment that is unattractive or uninhabitable to most aquatic and terrestrial species.

Considering these factors overall, it is plausible that, in some settings, an outbreak of CyHV-3 would result in a significant impact on food webs in a wetland ecosystem. During a high-flow season, this is most likely to occur in an inundated wetland or floodplain ecosystem. During a lower-flow season, a similar outcome may occur within the marshland at the periphery of a permanently-inundated lake.

11.4.2 Likely consequences of exposure

A summary of the potential impacts of the sudden removal of a dominant and stable food source on risk assessment endpoints (Part I: Section 2.2) is given in Table 13. In chief, these impacts focus on direct harm to local populations of waterbirds (including native and migratory waterbirds) and an indirect impact on breeding cycles. There is no clear opportunity for the removal of a dominant food source to have an impact on a species' area of occupancy or habitat, or to result in the establishment of an invasive species or disease. These principles can be carried through from non-migratory species to migratory species, and onwards to ecological communities and wetland ecosystems. There are no direct impacts on livestock or humans.

The likely consequences of the sudden removal of a dominant and stable food for individual functional groups of waterbirds are discussed in part (a) below, while the consequences for wetland ecological communities is discussed in part (b). Evaluation of the pathway in a range of practical settings is provided in the case studies (Section 12).

For this and other exposure pathways, the likely consequence provide a preliminary or baseline assessment of the possible impacts of CyHV-3, in the sense that they do not relate to particular places or ecosystems. The site-specific case studies undertaken in Section 12 build on these baseline assessments and give an evaluation of the possible impacts of CyHV-3 on the communities and species that characterise each location.

Table 13 Relevance of the sudden removal of carp to risk assessment endpoints

Endpoints (significant impact criteria)	Relevance
Migratory and non-migratory native species	
Lead to a long-term decrease in the size of a population of a non-migratory native species	✓
Reduce the area of occupancy of a non-migratory native species	✗
Fragment an existing population into two or more populations of a non-migratory native species	✗
Adversely affect habitat critical to the survival of a non-migratory native species	✗
Disrupt the breeding cycle of a population of a non-migratory native species	✓
Modify, destroy, remove, isolate or decrease the availability or quality of habitat to the extent that a non-migratory native species is likely to decline	✗
Result in invasive species that becomes established in a non-migratory native species' habitat	✗
Introduce disease that may cause a non-migratory native species to decline	✗
Interfere with the recovery of a non-migratory native species	✗
Migratory species	
Substantially modify (including by fragmenting, altering fire regimes, altering nutrient cycles or altering hydrological cycles), destroy or isolate an area of important habitat for a migratory species	✗
Result in an invasive species that is harmful to the migratory species becoming established in an area of important habitat for the migratory species	✗
Seriously disrupt the lifecycle (breeding, feeding, migration or resting behaviour) of an ecologically significant proportion of the population of a migratory species	✓
Ecological community	
Reduce the extent of an ecological community	✗
Fragment or increase fragmentation of an ecological community, for example by clearing vegetation for roads or transmission lines	✗
Adversely affect habitat critical to the survival of an ecological community	✗
Modify or destroy abiotic (non-living) factors (such as water, nutrients, or soil) necessary for an ecological community's survival, including reduction of groundwater levels, or substantial alteration of surface water drainage patterns	✗
Cause a substantial change in the species composition of an occurrence of an ecological community, including causing a decline or loss of functionally important species, for example through regular burning or flora or fauna harvesting	✓
Cause a substantial reduction in the quality or integrity of an occurrence of an ecological community, including, but not limited to • Assisting invasive species, that are harmful to the ecological community, to become established, or • Causing regular mobilisation of fertilisers, herbicides or other chemicals or pollutants into the ecological community which kill or inhibit the growth of species in the ecological community, or interfere with the recovery of an ecological community	✗

Endpoints (significant impact criteria)	Relevance
Wetland ecosystem	
Areas of the wetland being destroyed or substantially modified	✗
A substantial and measurable change in the hydrological regime of the wetland, for example, a substantial change to the volume, timing, duration and frequency of ground and surface water flows to and within the wetland	✗
The habitat or lifecycle of native species, including invertebrate fauna and fish species dependent upon the wetland being seriously affected	✓
A substantial and measurable change in the water quality of the wetland – for example, a substantial change in the level of salinity, pollutants, or nutrients in the wetland, or water temperature which may adversely impact on biodiversity, ecological integrity, social amenity or human health	✗
An invasive species that is harmful to the ecological character of the wetland being established (or an existing invasive species being spread) in the wetland	✗
Livestock	
Local exposure of livestock to the clinical effects of a pathogenic organism or its toxin	✗
Establishment of a pathogenic microorganism in livestock in areas not previously affected	✗
Public health	
Exposure of individuals to the clinical effects of a pathogenic organism or its toxin	✗
Exposure of groups of individuals or communities to the clinical effects of a pathogenic organism or its toxin	✗

Although adult and juvenile carp may be predated upon by a range of aquatic and terrestrial species, this pathway focussed on the exposure of nesting waterbirds as these are considered most likely to be exposed to a substantial risk. An impact on nesting waterbirds may be carried over to an impact on wetland or marshland communities, and these were also considered.

(a) Native and migratory waterbirds

The implications of the sudden removal of a dominant and stable food source for each of nine identified groups of native or migratory waterbirds (Part II: Section 3.2) are outlined below. For ease of reading, parts of this discussion reiterate key points about the ecology of each group that were raised in the assessment of widespread cyanobacterial blooms (Section 11.2).

Seabirds, including pelicans, cormorants and darters, are piscivorous and in general migratory, although none of those whose distribution within Australia overlaps with carp are listed under international agreements for the protection of migratory species to which Australia is a party. These species are colonial nesters and tend to settle within an area as a group and move from it as a group. Stressors applied to colonial nesters will tend to be experienced by a large number of individuals, and a large majority of a local population. Likewise, actions taken to avoid or mitigate stressors will tend to be taken by the colony as a whole, rather than by individuals, and this will necessitate a certain degree of inertia or delay. In the context of food webs, the requirements of a nesting colony are likely to be significant and any adaptations from one source of food to another will require the second source to be both substantive and relatively reliable. The extent to which any category of waterbird predates upon adult or juvenile carp is not well understood, although it seems very likely that coincidence of large numbers of nesting birds with highly-fecund spawning carp will mean that carp juveniles, in particular, are likely to provide an important source of food for hatchlings. Given the dominance of carp in many waterways and waterbodies, it is also likely that most piscivorous species would have adapted to feeding upon carp.

Overall, it is plausible that, in some settings, the sudden removal of carp as a dominant and stable food source would result in a long-term decrease in the size of a population of seabirds or interfere with its recovery. This would not differ markedly between high-flow and lower-flow seasons. It is also plausible that the sudden removal of carp as a dominant and stable food source would disrupt the breeding cycle of one or more seabird species and, thus, recruitment.

Shorebirds (order Charadriiformes), including the stilts, snipes, sandpipers, plover and oystercatchers (the waders), as well as gulls and terns. These birds feed by probing in the mud or picking items off the surface in both coastal and freshwater environments. The waders are not generally colonial nesters and most have a diet that is broader than fish alone (some species may not feed on fish at all). Gulls and terns are an exception within the shorebirds category, as both may nest in colonies and both feed principally on fish. The gulls and terns whose distribution overlaps with carp, however, are not a large component of the shorebird functional group. Overall, the shorebirds are likely to be less exposed to population-level stress with the sudden removal of carp as a dominant and stable food source. Four shorebirds whose distribution overlaps with that of carp are listed under the EPBC Act as critically endangered, three are endangered and a further species is vulnerable. All but two of these threatened species are also listed under international agreements for the protection of migratory species to which Australia is a party. This list contains 12 additional migratory shorebirds whose habitat may overlap with carp. Most of these migratory species do not breed in Australia.

Overall, it is not plausible that the sudden removal of carp as a dominant and stable food source would result in a long-term decrease in the size of a population of shorebirds or interfere with its recovery. This would not differ markedly between high-flow and lower-flow seasons. It is also implausible that the sudden removal of carp would disrupt the breeding cycle of one or more shorebird species and, thus, recruitment.

Waterfowl, including ducks, geese and swans (order Anseriformes) and grebes, do not tend to nest in colonies although, like the shorebirds (above), individual nests may be congregated within a loosely-defined area. None of the waterfowl whose distribution overlaps with that of carp are listed under the EPBC Act as threatened or listed under international agreements for the protection of migratory species to which Australia is a party. Geese and swans are herbivorous and would not be exposed through this pathway. Dabbling ducks feed in shallow water and are more likely to have a diet with more aquatic plants and insects, while diving ducks feed deeper in the water and typically feed on small fish or crustaceans. The diet of grebes consists mainly of small fish and aquatic invertebrates. Collectively these omnivorous members of the waterfowl group may be exposed to the sudden removal of carp as a dominant and stable food source although the diversity of their diet is likely to mitigate much of the stress. Although some waterfowl in the northern hemisphere migrate to escape harsh winters, the same does not occur in Australia. Waterfowl are, however, mobile and are able to move through a landscape to locate suitable habitat and to some extent may be able to avoid an area affected by the sudden removal of carp.

Overall, it is not plausible that the sudden removal of carp as a dominant and stable food source would result in a long-term decrease in the size of a population of waterfowl or interfere with its recovery. This would not differ markedly between high-flow and lower-flow seasons. It is also implausible that the sudden removal of carp would disrupt the breeding cycle of one or more waterfowl species and, thus, recruitment.

Large waders (order Ciconiiformes), including the storks, herons, egrets, ibises, spoonbills and others, are predominantly migratory and colonial nesters. They are a carnivorous order, with a diet that may include fish, reptiles, amphibians, crustaceans, molluscs and aquatic insects. The exposure of large waders to the risks associated with the sudden removal of carp as a dominant and stable food source is likely to be similar to that of the seabirds (above), albeit mitigated to some extent by the greater dietary diversity.

Overall, it is plausible, although not likely, that the sudden removal of carp as a dominant and stable food source would result in a long-term decrease in the size of a population of large waders or interfere with its recovery. This would not differ markedly between high-flow and lower-flow seasons. It is also plausible that the sudden removal of carp as a dominant and stable food source would disrupt the breeding cycle of one or more species and, thus, recruitment.

Gruiformes, including cranes rails, crakes, coots, moorhens and waterhens, are a diverse group of non-migratory, omnivorous birds that occupy the niche between marshland and terrestrial habitats. The Gruiformes are not colonial nesters. Some species nest and forage in wetland environments while others prefer drier environments adjacent to a waterbody. Species that occupy wetlands feed on a range of animal biota, including molluscs, frogs, small fish and insects, as well on aquatic plants. The exposure of these species to the effects of the sudden removal of carp as a dominant and stable food source is likely to be very similar to that of the omnivorous subgroups of waterfowl (above).

Overall, it is not plausible that the sudden removal of carp as a dominant and stable food source would result in a long-term decrease in the size of a population of Gruiformes or interfere with its recovery. This would not differ markedly between high-flow and lower-flow seasons. It is also implausible that the sudden removal of carp would disrupt the breeding cycle of one or more species and, thus, recruitment.

Kingfishers (order Coraciiformes, family Alcedinidae) have a diet that is more diverse than their name implies, and commonly includes crustaceans and invertebrates – and for some species may also include other small animals. Kingfishers are not colonial nesters and are not migratory. Overall, there is not a real chance or possibility that the sudden removal of carp as a dominant and stable food source would result in a long-term decrease in the size of a population of kingfishers (including kookaburras) or interfere with its recovery. This likelihood would not differ markedly between high-flow and lower-flow seasons. Likewise, there is not a real chance or possibility that the sudden removal of carp would disrupt the breeding cycle of one or more species and, thus, recruitment.

Songbirds (order Passeriformes, the passerine birds) are the largest order of birds. Their diet varies amongst species, and may include seeds, insects and small crustaceans. Some passerine birds, such as the clamorous warbler, are true waterbirds. Others may be present in wetlands and other water-focussed ecosystems, whether permanently or as an opportunistic means by which to gain refuge from drought and other environmental stresses. Passerine birds have negligible exposure to harm as a result of the sudden removal of carp.

Bush-birds are an eclectic mixture of species that roost, forage and nest in riparian or floodplain forests but are not generally considered to be true waterbirds. Except in very unusual circumstances, their exposure to harm as a result of the sudden removal of carp is negligible.

Aquatic raptors are (in this assessment) include those Accipitriformes (hawks, goshawks, kites, eagles and ospreys), Falconiformes (falcons and kestrels) and Strigiformes (owls) that inhabit riverine, lacustrine and palustrine ecosystems. The red goshawk, whose distribution overlaps with that of carp, is listed under the EPBC Act as vulnerable. There are also two species of raptor whose diet regularly includes fish: the eastern osprey and white-bellied sea eagle. Both inhabit coastal regions as well as wetlands and lakes. The eastern osprey's diet is principally fish, while the sea eagle may take carrion as well as waterbirds. The eastern osprey is listed under international agreements for the protection of migratory species to which Australia is a party. Both species are, however, both mobile and able to hunt over a relatively large area and these attributes would help to mitigate to a negligible level any harm that might otherwise result from the sudden removal of carp as a dominant and stable food source.

(b) Wetland and marshland ecological communities

Wetland and marshland communities include the assemblage of native flora, fauna and micro-organisms associated with and dependent upon the floodplain and river wetland ecosystem. Communities extend to both aquatic and terrestrial ecosystems of interconnected environmental subunits, and may include river channels, lakes and estuaries, tributaries and streams, floodplain wetlands and marshes, woodlands, and groundwater. In ecological terms, these complex communities may be considered landscape-scale ecological meta-ecosystems whose hydrological connectivity is recognised as being essential to their long-term health.

The impact of the sudden removal of carp on wetland communities might be significant, both in respect of the effect this may have on species that feed on adult or juvenile carp (this exposure pathway) and in respect of a compensatory increased predation on native aquatic species – including fish, crustaceans and frogs (Section 11.5). The intensity of the effect is likely to be particularly marked during a high-flow season, when the assemblage of biota within the wetland (in particular, colonial-nesting waterbirds) will be maximal.

Overall, it is plausible that the sudden removal of carp as a dominant and stable food source would cause a substantial change in species composition of a wetland community. This might occur through an impact on the lifecycle of native species, and might result in a decline or loss of functionally important species.

11.4.3 Conclusions: risks, uncertainty and treatments

It is plausible that an outbreak of CyHV-3 would result in a significant impact on food webs in a wetland ecosystem. During a high-flow season, this is most likely to occur in an inundated wetland or floodplain ecosystem. During a lower-flow season, a similar outcome may occur within the marshland at the periphery of a permanently-inundated lake. Colonial-nesting piscivorous waterbirds are most likely to be affected by the sudden removal of a dominant and stable food source, and these effects are most likely to be observed within wetland or marshland settings. In a high-flow season, this would include ephemeral wetlands and floodplains. In a lower-flow season, nesting will move to the marshland at the periphery of permanently-inundated lakes – including those that are maintained as irrigation reservoirs.

The key **area of uncertainty** encountered in this evaluation was in relation to the importance of adult and juvenile carp to the diet of many waterbird species. It was noted in Part II: Section 8.2 that much of the current supposition about this has followed from fieldwork undertaken in the 1980s. Since that time, the population of carp has expanded geographically, and its dominance in many Australian freshwater waterways has increased. Given that, it is likely that both adult and juvenile carp now represent a larger proportion of the diet of many piscivorous, carnivorous and generalist consumers, as well as detritivores and scavengers.

The **treatment of risks** associated with the sudden removal of a dominant and stable food source might focus on the strategic release of the virus within particular catchments at times when its impact is least likely to result in stress to piscivorous waterbirds. This might include consideration of where particular species of colonial-nesting piscivorous waterbirds are likely to be nesting during high-flow and lower-flow seasons. It would also be beneficial to consider the reliance that piscivorous birds are likely to have on carp in particular settings, given the populations of both waterbirds and carp and the breadth of alternative prey species (below). Supplementation of local populations of native fish and crustaceans may also be beneficial, and this is discussed within the context of prey-switching (below).

11.5 Increased predation as a result of prey-switching

This pathway considers the exposure of native fish, crustaceans, amphibians and other aquatic animals to increased predation as a result of prey-switching with the removal of carp. It was noted in the evaluation of the effects of removing a dominant and stable food source (Section 11.4) that there is very little published research about the food-web effects resulting from major fish kills. In the light of this, deductions were made on the basis of a simpler understanding of the underlying ecology and the dynamics of individual predator-prey relationships.

Key references for the science underpinning this pathway can be found in Part II: Section 8.

11.5.1 Likelihood of exposure

The likelihood that aquatic species other than carp would be exposed to increased predation following an outbreak of CyHV-3 is conditioned on the likelihood that waterbirds will be affected by the sudden removal of a stable food source (Section 11.4 above). If waterbirds are affected in this way, then they will inevitably turn to alternative food sources, and this will include juvenile large-bodied native fish, adult and juvenile small-bodied native fish, crustaceans, frogs and possibly turtle eggs and hatchlings. The emphasis in this evaluation was placed on the likely consequences of exposure.

11.5.2 Likely consequences of exposure

A summary of the potential impacts of prey-switching on risk assessment endpoints (Part I: Section 2.2) is given in Table 14. In chief, these impacts focus on direct harm to local populations of native fish, frogs, crustaceans and turtles and an indirect impact on breeding cycles. There is no clear opportunity for prey-switching to have an impact on a species' area of occupancy or habitat, or to result in the establishment of an invasive species or disease. These principles can be carried through from non-migratory species to migratory species, and onwards to ecological communities and wetland ecosystems. There are no direct impacts on livestock or humans.

The likely consequences of prey-switching for individual taxa or functional groups are discussed in parts (a) to (f) below, while the consequences for wetland ecological communities is discussed in part (g). Evaluation of the pathway in a range of practical settings is provided in the case studies (Section 12).

For this and other exposure pathways, the likely consequence provide a preliminary or baseline assessment of the possible impacts of CyHV-3, in the sense that they do not relate to particular places or ecosystems. The site-specific case studies undertaken in Section 12 build on these baseline assessments and give an evaluation of the possible impacts of CyHV-3 on the communities and species that characterise each location.

Table 14 Relevance of prey-switching to risk assessment endpoints

Endpoints (significant impact criteria)	Relevance
Migratory and non-migratory native species	
Lead to a long-term decrease in the size of a population of a non-migratory native species	✓
Reduce the area of occupancy of a non-migratory native species	✗
Fragment an existing population into two or more populations of a non-migratory native species	✗
Adversely affect habitat critical to the survival of a non-migratory native species	✗
Disrupt the breeding cycle of a population of a non-migratory native species	✓
Modify, destroy, remove, isolate or decrease the availability or quality of habitat to the extent that a non-migratory native species is likely to decline	✗
Result in invasive species that becomes established in a non-migratory native species' habitat	✗
Introduce disease that may cause a non-migratory native species to decline	✗
Interfere with the recovery of a non-migratory native species	✓
Migratory species	
Substantially modify (including by fragmenting, altering fire regimes, altering nutrient cycles or altering hydrological cycles), destroy or isolate an area of important habitat for a migratory species	✗
Result in an invasive species that is harmful to the migratory species becoming established in an area of important habitat for the migratory species	✗
Seriously disrupt the lifecycle (breeding, feeding, migration or resting behaviour) of an ecologically significant proportion of the population of a migratory species	✗
Ecological community	
Reduce the extent of an ecological community	✗
Fragment or increase fragmentation of an ecological community, for example by clearing vegetation for roads or transmission lines	✗
Adversely affect habitat critical to the survival of an ecological community	✗
Modify or destroy abiotic (non-living) factors (such as water, nutrients, or soil) necessary for an ecological community's survival, including reduction of groundwater levels, or substantial alteration of surface water drainage patterns	✗
Cause a substantial change in the species composition of an occurrence of an ecological community, including causing a decline or loss of functionally important species, for example through regular burning or flora or fauna harvesting	✓

Endpoints (significant impact criteria)	Relevance
Cause a substantial reduction in the quality or integrity of an occurrence of an ecological community, including, but not limited to • Assisting invasive species, that are harmful to the ecological community, to become established, or • Causing regular mobilisation of fertilisers, herbicides or other chemicals or pollutants into the ecological community which kill or inhibit the growth of species in the ecological community, or interfere with the recovery of an ecological community	✕
Wetland ecosystem	
Areas of the wetland being destroyed or substantially modified	✕
A substantial and measurable change in the hydrological regime of the wetland, for example, a substantial change to the volume, timing, duration and frequency of ground and surface water flows to and within the wetland	✕
The habitat or lifecycle of native species, including invertebrate fauna and fish species dependent upon the wetland being seriously affected	✓
A substantial and measurable change in the water quality of the wetland – for example, a substantial change in the level of salinity, pollutants, or nutrients in the wetland, or water temperature which may adversely impact on biodiversity, ecological integrity, social amenity or human health	✕
An invasive species that is harmful to the ecological character of the wetland being established (or an existing invasive species being spread) in the wetland	✕
Livestock	
Local exposure of livestock to the clinical effects of a pathogenic organism or its toxin	✕
Establishment of a pathogenic microorganism in livestock in areas not previously affected	✕
Public health	
Exposure of individuals to the clinical effects of a pathogenic organism or its toxin	✕
Exposure of groups of individuals or communities to the clinical effects of a pathogenic organism or its toxin	✕

(a) Large-bodied native fish

The effects on large-bodied native fish of prey-switching following the sudden removal of carp will largely focus on the survivability of juveniles and the ability of local populations to recruit.

Murray cod utilise a diverse range of habitats from clear and rocky upland streams to slower-flowing, turbid lowland rivers and billabongs (Part II: Section 3.1.1). The species is a main-channel specialist, with only limited use of minor tributaries and inundated floodplain habitats. Murray cod migrate upstream prior to spawning over 4 to 5 weeks in late spring or early summer, when day length is increasing, and water temperature reaches 16 and 21C. The adhesive eggs are laid on solid substrates, and larvae then drift downstream before settling out in suitable protected channel habitat. Juveniles become territorial at a young age and remain aggressively so as adults. Adult Murray cod are solitary and return to their territory following spawning.

The exposure of juvenile Murray cod to predation as a result of prey-switching is likely to be highest within permanent lakes or irrigation reservoirs, or riverine impoundments. Populations in these settings may be less able to migrate to and from natural spawning grounds and may therefore spawn within the waterbody in which the young will then grow out and mature. These populations may be augmented through the addition of farmed fingerlings. Conversely, the young of Murray cod moving freely within the main river systems are likely to have relatively less overlap with juvenile carp and are thus less likely to be exposed to predation if a high proportion of juvenile carp are removed from those settings.

Overall, it is plausible, although not likely, that prey-switching following the sudden removal of carp would result in a long-term decrease in the size of the population of Murray cod or interfere with its recovery. It is also plausible that prey-switching might disrupt the breeding cycle of Murray cod in some settings and, thus, recruitment, although Murray cod are long-lived, and their sustainability does not rely heavily on individual recruitment years.

<u>Trout cod</u> occupy comparatively deep and rapidly moving water (Part II: Section 3.1.1). The single naturally-occurring population is restricted to a small (approximately 120 km) stretch of the Murray River from Yarrawonga Weir to the Barmah-Millewa Forest and, occasionally, beyond to Gunbower. Trout Cod pair to spawn in late September to late October, when water temperatures are between 14 and 22C – often about 3 weeks prior to Murray cod. Trout cod display high site fidelity, with a small home range, and do not routinely undertake migratory movements for spawning other than to move from the main channel to off-channel branches or to floodplains in the event of a significant flood.

The reach of the Murray River below Yarrawonga Weir, and the Barmah-Millewa Forest, are case studies and are examined in detail in Sections 12.3 and 12.1, respectively. In brief, the reach of the Murray River below Yarrawonga Weir is a lentic and highly-regulated channel environment. By contrast, Barmah-Millewa Forest includes an ephemeral floodplain that is a focus for the recruitment of carp during high-flow seasons. In lower-flow seasons, carp will spawn in, and remain constrained to, in the permanently-inundated off-channel waters. An outbreak of CyHV-3 is likely to be most aggressive in the Barmah-Millewa Forest during a lower-flow season when carp are concentrated and opportunities for close contact are maximal.

Overall, it is plausible that prey-switching following the sudden removal of carp would result in a long-term decrease in the size of the population of trout cod in some settings or interfere with its recovery. It is also plausible that prey-switching might disrupt the breeding cycle of trout cod in some settings and, thus, recruitment.

<u>Silver Perch</u> are strongly migratory, travelling long distances upstream to spawn (Part II: Section 3.1.1). Larvae and eggs then drift downstream. Silver perch can spawn in lower-flow and high-flow settings, and at relatively cool water temperatures, although a flood event does maximise spawning and recruitment. Spawning occurs in warm water (generally above 23C) and in faster-flowing areas of the river – generally over gravel or rock-rubble substrates. The only sizeable and self-sustaining population of silver perch is in the middle reaches of the Murray River (including the Edwards/Wakool anabranches), from the base of Yarrawonga Weir to the Torrumbarry Weir, and then to the Euston Weir and downstream. This population may also extend down to the South Australian border and up to the lower Darling River. The species occupies a larger reach of the Murray-Darling Basin than trout cod (above), including the lower turbid reach of the Darling River, and this may provide a buffer to the effects of prey-switching in the form of a more spatially distributed population.

Overall, it is plausible, although not likely, that prey-switching following the sudden removal of carp would result in a long-term decrease in the size of the population of silver perch in some settings or interfere with its recovery. It is also plausible that prey-switching might disrupt the breeding cycle of silver perch in some settings and, thus, recruitment.

Golden perch are predominantly found in lowland, warmer, turbid, slower-flowing rivers (Part II: Section 3.1.1). Golden perch are nomadic, with schools of fish occupying home ranges of about 100 metres for weeks or months before relocating to another site where a new home range is established. Upstream movements by both immature and adult fish are stimulated by small rises in streamflow and may extend as far as a 1,000 km if fish are able to navigate weirs and other barriers. Spawning occurs in temperatures ranging from 17 to 25. The large eggs semi-buoyant and drift downstream.

The wide distribution, mobility and resilience of golden perch to adverse conditions likely to provide a buffer to the effects of prey-switching. Because juvenile golden perch can be found in both channel and inundated floodplain habitats, it is not plausible that prey-switching following the sudden removal of carp would result in a long-term decrease in the size of the population of golden perch or interfere with its recovery. It is also implausible that prey-switching might disrupt the breeding cycle of golden perch in some settings and, thus, recruitment.

Macquarie perch is a schooling species closely related to Golden perch, although adapted to be an upland riverine specialist that prefers clear deep water and rocky holes (Part II: Section 3.1.1). Spawning occurs just above riffles (shallow running water), where rivers have a base of rubble (small boulders, pebbles and gravel). Some fish use the same river each year for spawning. Migrations are undertaken by fish resident in lakes, but not otherwise.

The predilection of Macquarie perch for upland riverine environments where the biomass density of carp is lower, and where carp juveniles do not exist in the high numbers that attract waterbirds, mean that there is not a real chance or possibility that prey-switching following the sudden removal of carp would result in a long-term decrease in the size of its population or interfere with its recovery. There is also a negligible likelihood that prey-switching would disrupt the breeding cycle of Macquarie perch.

Freshwater catfish is a benthic species that prefers slower-flowing streams and lake habitats (Part II: Section 3.1.1). Freshwater catfish are relatively sedentary, and do not migrate to spawn, which occurs in the spring and summer when water temperatures are between 20 and 24C. The eggs are comparatively large (approximately 3 mm) and non-adhesive and settle into the interstices of the coarse substrate of the nest in which they are laid. Freshwater catfish within a lacustrine environment may be exposed to the outcomes of an aggressive outbreak of CyHV-3, as carp in these settings may reach a very high biomass density. If the lake is not large, and is partially disconnected, then freshwater catfish could be exposed to the effects of prey-switching.

Overall, it is plausible that, in some settings, prey-switching following the sudden removal of carp would result in a long-term decrease in the size of the population of freshwater catfish in some settings or interfere with its recovery. It is also plausible that prey-switching might disrupt the breeding cycle of freshwater catfish in some settings and, thus, recruitment.

Bony herring are a widespread, abundant and hardy fish, tolerating high temperatures (up to 38C), high turbidity and high salinity (Part II: Section 3.1.1). Bony herring are relatively short-lived (2 to 3 years is common), although are highly-fecund. This combination means that a failure to recruit in a given season may result in a noticeable drop in the population at that location – with rapid a rebound when conditions for breeding are more favourable. Spawning is generally understood to take place in the still waters of shallow, sandy bays in October to February (depending on water temperature). The Character Description for the Barmah-Millewa Ramsar site, however, describes bony herring as a wetland specialist that spawns and recruits in floodplain wetlands and lakes, anabranches and billabongs during in-channel flows (Hale and Butcher, 2011). The disparity may reflect the widespread distribution of bony herring and their versatility within a range of habitats.

The wide distribution and fecundity of bony herring are likely to provide a buffer to the broader effects of prey-switching – which might be intense in a particular location and season. Overall, it is not plausible that prey-switching following the sudden removal of carp would result in a long-term decrease in the size of the population of bony herring in some settings or interfere with its recovery. It is also implausible that prey-switching might disrupt the breeding cycle of bony herring in some settings and, thus, recruitment.

Australian grayling is unique amongst fish in this group in that they are diadromous, spending part of its lifecycle in freshwater and at least part of the larval or juvenile stages in coastal seas (Part II: Section 3.1.1). Adults (including pre-spawning and spawning adults) inhabit cool, clear, freshwater streams with gravel substrate and areas alternating between pools and riffle zones. Spawning occurs in freshwater from late summer to early winter, cued by an increase in river flows from seasonal rains a drop in water temperatures to 12 to 13C. Eggs are scattered over the substrate and newly hatched larvae drift downstream and out to sea, where they remain for approximately six months. Juveniles then return to the freshwater environment around November of their first year, where they remain for the remainder of their lives. Most Australian grayling die after their second year, soon after spawning, however a small proportion reach five years of age. The short life span and high fecundity mean of the species mean that local populations may undergo substantial year-to-year variations in size. The Australian grayling is believed to be absent from the inland Murray-Darling system, occurring only in streams and rivers on the eastern and southern flanks of the Great Dividing Range, from Sydney southwards to the Otway Ranges of Victoria. There is also a Tasmanian population.

The absence of the Australian grayling from the Murray-Darling system and marine environment in which juveniles mature mean that there is not a real chance or possibility that prey-switching following the sudden removal of carp would result in a long-term decrease in the size of the population of or interfere with its recovery. A possible exception to this might be the smaller population of grayling that is endemic to the Gippsland Lakes. There is also a negligible likelihood that prey-switching would disrupt the breeding cycle of Australian grayling.

(b) Small-bodied native fish

Small-bodied native fish are a mixed group that feed principally on aquatic and terrestrial invertebrates (including both insects and small crustaceans) (Part II: Section 3.1.2). They breed in spring, leaving eggs adhered to submerged vegetation or substrate. Low-flow settings are generally more favourable for recruitment, although a small pulse may help to inundate spawning grounds. Two to three cycles of spawning may occur each year. A range of small-bodied native fish have a distribution that overlaps that of carp and are listed as critically endangered, endangered or vulnerable under the EPBC Act. Numerous other small-bodied species are not listed but have a distribution that overlaps with that of carp. The small-bodied native fish are loosely grouped as wetland or floodplain specialists, or foraging generalists, although both groups are likely to move onto the inundated floodplain to feed and spawn and, with the removal of carp juveniles, may be targeted by piscivorous waterbirds. Unlike many large-bodied species (above) exposure may include both adult and juvenile forms of small-bodied fish and this may increase the vulnerability of local populations. Exposure may also occur in a range of settings, although is likely to be highest in a high-flow season when ephemeral wetlands and floodplains are inundated, and large numbers of nesting waterbirds are likely to be present.

Overall, it is plausible that, in some settings, prey-switching following the sudden removal of carp would result in a long-term decrease in the size of a population of small-bodied native fish or interfere with its recovery. It is also plausible that prey-switching might disrupt the breeding cycles of small-bodied native fish and, thus, their recruitment.

(c) Freshwater crustaceans

This category includes the endangered Glenelg spiny freshwater crayfish as well as the Murray crayfish (Part II: Section 3.5) whose population strength is thought to have declined markedly following the widespread and long-lasting blackwater event within the mid-lower Murray River in 2010-11 (Section 6.2.2). Both of these species prefer permanent rivers and large streams where the water flow is moderately fast. A range of other native freshwater crustaceans was also considered, including the yabby and freshwater shrimp. Juvenile crustaceans, and adults of smaller species, are (variably) components of the diet of many waterbird species and may be exposed to increased predation as a result of prey-switching. Exposure is likely to be highest in unregulated riverine reaches during lower-flow seasons, when an outbreak of CyHV-3 in these settings is also likely to be most aggressive. Exposure may be exacerbated by a decline in water quality (including low DO or a widespread cyanobacterial bloom), as many crustaceans will then emerge from the water and be additionally vulnerable to predation.

Overall, it is plausible that, in some settings, in riverine settings during lower-flow seasons prey-switching following the sudden removal of carp would result in a long-term decrease in the size of a local population of crustaceans or interfere with its recovery. It is also plausible that prey-switching would disrupt the breeding cycle of a crustaceans and, thus, their recruitment.

(d) Frogs

Frogs occupy a wide variety of habitats, and many have developed highly-specialised adaptations to particular climates, ecological sites and communities (Part II: Section 3.3). Of the 208 identified species of frog in Australia, two have a distribution that overlaps with that of carp and are listed under the EPBC Act as critically endangered, five species are endangered and a further five species are vulnerable. As most frog species are susceptible to dehydration, proximity to water is a general prerequisite. Preferred habitats can include rivers, lakes and wetlands. Most frogs spawn in spring and summer, and this is likely to coincide with the spawning of carp and an outbreak of CyHV-3. Eggs, tadpoles and adult frogs are (variably) components of the diet of many waterbird species, and each these lifecycle stages may thus be exposed to increased predation as a result of prey-switching. Exposure is likely to be highest within inundated ephemeral wetlands and floodplains during high-flow seasons, and within the marshland at the periphery of permanent lakes and irrigation reservoirs during either high-flow or lower-flow seasons. Alongside some species of small-bodied native fish (above) threatened and fragmented frog populations within these settings are arguably the most exposed of the aquatic native biota to the effects of prey-switching

Overall, it is plausible that, in some settings, prey-switching following the sudden removal of carp would result in a long-term decrease in the size of a population of frogs or interfere with its recovery. It is also plausible that prey-switching would disrupt frog breeding cycles and, thus, their recruitment.

(e) Freshwater turtles

Australia is home to 23 species of freshwater turtle, of which one (the Bellinger River snapping turtle) has a distribution that overlaps with that of carp and is listed under the EPBC Act as critically endangered, while another (the Bell's turtle) is vulnerable (Part II: Section 3.4). Freshwater turtles inhabit riverine, lacustrine and palustrine environments and only come onto land to lay eggs, in spring and summer. Hatchlings immediately migrate to the closest water. The exposure of most freshwater turtles to prey-switching will be limited to eggs and hatchlings, as maturing and adult turtles are relatively protected from predation. The exposure of eggs and hatchlings could, however, have a measurable impact on the sustainability of a local population as both are currently targeted by foxes and other predators.

Overall, it is plausible that, in some settings, prey-switching following the sudden removal of carp would result in a long-term decrease in the size of a local population of freshwater turtle or interfere with its recovery. It is also plausible that prey-switching would disrupt the breeding cycle of a local population of turtle and, thus, their recruitment.

(f) Wetland and marshland ecological communities and ecosystems

These include the assemblage of native flora, fauna and micro-organisms associated with and dependent upon the floodplain and river wetland ecosystem (Part II: Section 3.6). Communities extend to both aquatic and terrestrial ecosystems of interconnected environmental subunits, and may include river channels, lakes and estuaries, tributaries and streams, floodplain wetlands and marshes, woodlands, and groundwater. In ecological terms, these complex communities may be considered landscape-scale ecological meta-ecosystems whose hydrological connectivity is recognised as being essential to their long-term health.

The impact of prey-switching on ecological communities is likely to depend on the extent to which piscivorous waterbirds currently rely upon carp (juveniles in particular) as a stable and dominant source of food and the strength and diversity of native aquatic biota. There is also likely to be a difference in the impact of prey-switching on permanent wetlands or marshlands (such as those that exist at the periphery of some permanent lakes or irrigation reservoirs) and on ephemeral wetlands. The latter are generally typified by a rapid burgeoning of aquatic and terrestrial life following the inundation of wetlands and floodplains, and this ecological process is essential to the longer-term sustainability of the populations of many native species. Because they are ephemeral, these environments may also be largely free from the longer-term impacts of carp and may be markedly more diverse in their biota. Carp are extremely fecund, however, and the presence of many juveniles on the floodplains during high-flow seasons is likely to be attractive to many waterbird species. The sudden removal of a high proportion of these as a result of an outbreak of CyHV-3 could result markedly increased predation upon frogs, small-bodied native fish and the young of these and other aquatic native species.

On balance, it is plausible that an outbreak of CyHV-3 during a high-flow season could result in a degree of prey-switching that led to substantial change in the species composition of a wetland community, including causing a decline or loss of functionally important species. It is also plausible that prey-switching in this setting would impact on the lifecycle of some native aquatic species.

11.5.3 Conclusions: risks, uncertainty and treatments

The likelihood that aquatic species other than carp would be exposed to increased predation following an outbreak of CyHV-3 is conditioned principally on the likelihood that waterbirds will be affected by the sudden removal of a stable food source (Section 11.4).

The likely consequences of exposure – that is, the likelihood of experiencing the endpoints identified in Table 14 – are focussed on juvenile large-bodied native fish, all life stages of small-bodied native fish, crustaceans, frogs and the eggs and hatchlings of freshwater turtles. The exposure of large-bodied native fish is likely to be quite varied amongst the species evaluated, although highest for those species whose juvenile forms are most likely to coexist with juvenile carp and, thus, provide an obvious alternative for nesting waterbirds. The smaller crustaceans (and young of the larger species) are at highest risk in the event of declining water quality, as they are then likely to emerge and be vulnerable to predation. Fragmented populations of threatened frogs are likely to be particularly exposed during high-flow seasons, and within the marshland at the periphery of permanent lakes and irrigation reservoirs during either high-flow or lower-flow seasons. The eggs and hatchlings of freshwater turtles are already under pressure in many freshwater communities, and this pressure might increase measurably as a result of prey-switching. The impacts of prey-switching on ecological communities is likely to depend on the extent to which waterbirds currently rely upon carp (juveniles in particular) as a stable and dominant source of food and the strength and diversity of native aquatic biota.

Notwithstanding these trends, the key **area of uncertainty** encountered in this evaluation and in the evaluation of the effects of removing a dominant and stable food source (Section 11.4) was in relation to the importance of adult and juvenile carp to the diet of many waterbird species. It was noted in Part II: Section 8.2 that much of the current supposition about this has followed from fieldwork undertaken in the 1980s. Since that time, the population of carp has expanded geographically, and its dominance in many Australian freshwater waterways has increased. Given that, it is likely that both adult and juvenile carp now represent a larger proportion of the diet of many piscivorous, carnivorous and generalist consumers, as well as detritivores and scavengers.

The **treatment of risks** associated with prey-switching may be limited to support for partcular local populations of threatened native species through strategic restocking. Although strategic restocking currently occurs for a range of native species – and at a range of locations – additional stocks of threatened native fish and other native aquatic species could be developed in aquaculture settings prior to release of CyHV-3, and guidelines put in place for the prioritisation of restocking through Murray-Darling Basin and elsewhere. Although it is recognised that the results of such releases have not to-date been completely successful (for example, Thiem *et al.*, 2017) it seems likely that some benefit would be obtained in the situation where particular populations within a riverine, wetland or lacustrine ecosystem were likely to sustain a significant and widespread loss. As an embellishment on this approach, consideration may also be given to harvesting adult animals from some existing habitats prior to the release of the virus, and placing them within breeding facilities. This would enable restocking to occur using wild-caught genetics which may be more resilient or competitive in their natural settings than farmed fish.

11.6 Outbreaks of botulism

This pathway considers the exposure of native aquatic and terrestrial animals, livestock and humans to direct or indirect effects of an outbreak of botulism.

One of the key outcomes of the review undertaken in Section 7 was the importance of delineating between type C (and C/D mosaic) and type E botulism in the context of wildlife outbreaks. This delineation reflects, in particular, the different roles that fish and fish carcasses may play in the two forms of botulism outbreak. The delineation is also important as *C. botulinum* type E may not be present in Australia – or may be present at an extremely low prevalence and a restricted geographical distribution.

Key references for the science underpinning this pathway can be found in Part II: Section 7.

11.6.1 Likelihood of exposure

(a) Type C or C/D botulism

Section 7.6.1 provides an overview of key components of the ecology of type C and C/D botulism, and the pathways for exposure of birdlife, terrestrial scavengers and livestock. These pathways are shown pictorially in Figure 31 below.

Initiation of outbreaks of type C or C/D botulism: it is understood that there exists an ongoing baseline level of exposure of wildlife and livestock to outbreaks of type C and C/D botulism, as evidenced through the sporadic events that have been observed in Australia since the 1930s. The scenarios that underpin the pathways considered in this part of the assessment are the concurrence of multiple outbreaks of type C or C/D botulism, or the occurrence of a single very large outbreak affecting a wider range of species and a more significant part of an ecosystem than is generally the case.

These scenarios might arise from a fish kill in one or both of the following ways (Figure 31):

a) The rapid abundance and accumulation of large numbers of fish carcasses, a proportion of which may have been passive carriers of type C or C/D spores. Some of these carcasses are likely to accumulate in shallow water, or at the edges of waterways, while others will sink to the sediment. Fish carcasses provide a suitable micro-environment for the germination of *C. botulinum* spores, and subsequent toxigenesis. Terrestrial or aquatic invertebrates feeding on these carcasses may then concentrate the toxin and vegetative cells and make them available for higher orders of the food chain. This is generally termed the 'carcass-maggot cycle'. In some situations, a critical mass of toxin and vegetative cells enters the food chain and initiates an exponentially expanding outbreak of botulism.

b) The eutrophication of a waterway as a result of a substantial fish kill, and the direct development of hypoxia through aerobic bacteria or the indirect development of hypoxia following the rapid growth and die-off of an algal bloom. Hypoxic conditions may then result in the germination of *C. botulinum* spores within individual carcasses or in the environment, the death of fish or birds from toxicosis and the initiation of the carcass-maggot cycle.

A minor additional pathway that is largely hypothetical in the Australian context concerns the germination of type C or C/D spores within the gastrointestinal tract of living fish that have become stressed through exposure to CyHV-3, with subsequent *in situ* toxigenesis.

The likelihood that can be attached to the scenarios above reflects a balance of two groups of considerations. On the one hand, the demonstrated biological plausibility of the scenarios above and widespread distribution of type C and C/D spores favours the development of concurrent outbreaks – or a single outbreak on a particularly large scale. Offsetting this, however, are the field observations of staff who have responded to fish kills in Australian freshwater waterways and have not reported secondary outbreaks of botulism. The peer-reviewed literature is also effectively silent on the role of large fish kills as initiators of botulism outbreaks in natural settings. Indeed, the single record that was identified during this review was a 2005 media statement in which a range of intrinsic factors within a lagoon in northern New South Wales were thought to have led to a fish kill that subsequently resulted in waterbirds dying from suspected botulism.

On balance, it is possible that a mass mortality of fish would initiate multiple outbreaks of type C or C/D botulism or a single outbreak on a particularly large scale. The key factors affecting this likelihood are water temperature and the type of waterway. If the water temperature is less than approximately 20C then an outbreak would be relatively less likely. Likewise, if a flowing river or estuarine environment was exposed in the manner described above, then an outbreak would be relatively less likely than if a body of standing or very slow-moving water (including lakes, wetlands and billabongs) was exposed.

Exposure of wildlife: the primary route of exposure for non-scavenging wildlife is terrestrial or aquatic invertebrates that contain concentrated type C or C/D botulinum toxin and, in some cases, vegetative cells. As noted above, some fish-eating birds might also be exposed to toxin and vegetative cells by predating upon fish that are carrying type C or C/D spores and have become significantly stressed. The primary route of exposure for terrestrial and avian scavengers is carcass materials from animals that have either died for reasons other than botulism but were passive carriers of type C or C/D spores (either fish or birds) or have died from the effects of type C (or C/D mosaic) botulinum toxin (primarily birds). In the former case, scavengers may be exposed to both vegetative cells and type C (or C/D mosaic) botulinum toxin. In the latter case, scavengers will only be exposed to the toxin.

On balance, the exposure of wildlife co-located with carcasses that contain vegetative cells or toxin is plausible. Diet is ostensibly a key risk factor, such that the exposure of piscivorous birds is relatively less likely than the exposure of birds that feed on macro- and micro-invertebrates and vegetation. The exposure of terrestrial or avian scavengers may be less likely. These considerations notwithstanding, the particular species most commonly affected during a botulism outbreak in wildlife is to some extent site specific, and specific to the characteristics of each outbreak.

Exposure of livestock: livestock may be exposed by either predating upon carcass materials that contain preformed toxin in situations when particular soil minerals are lacking or imbalanced (termed 'pica') or drinking water that contains free preformed toxin or toxin associated with invertebrates or with suspended vertebrate carcass materials. These pathways might occur where fish or bird carcasses have contaminated a natural water source, such a billabong, a wetland or a creek or river. Alternatively, wild bird (and possibly fish) carcasses may contaminate farm dams or a reservoir from which stock water is obtained. Livestock losses have not been associated with past wildlife outbreaks of type C botulism in Australia. That notwithstanding, the co-occurrence of mass fish mortalities in different parts of Australia has not occurred to date. Overall, it is plausible that in some locations the exposure of livestock through carcass materials or drinking water will occur.

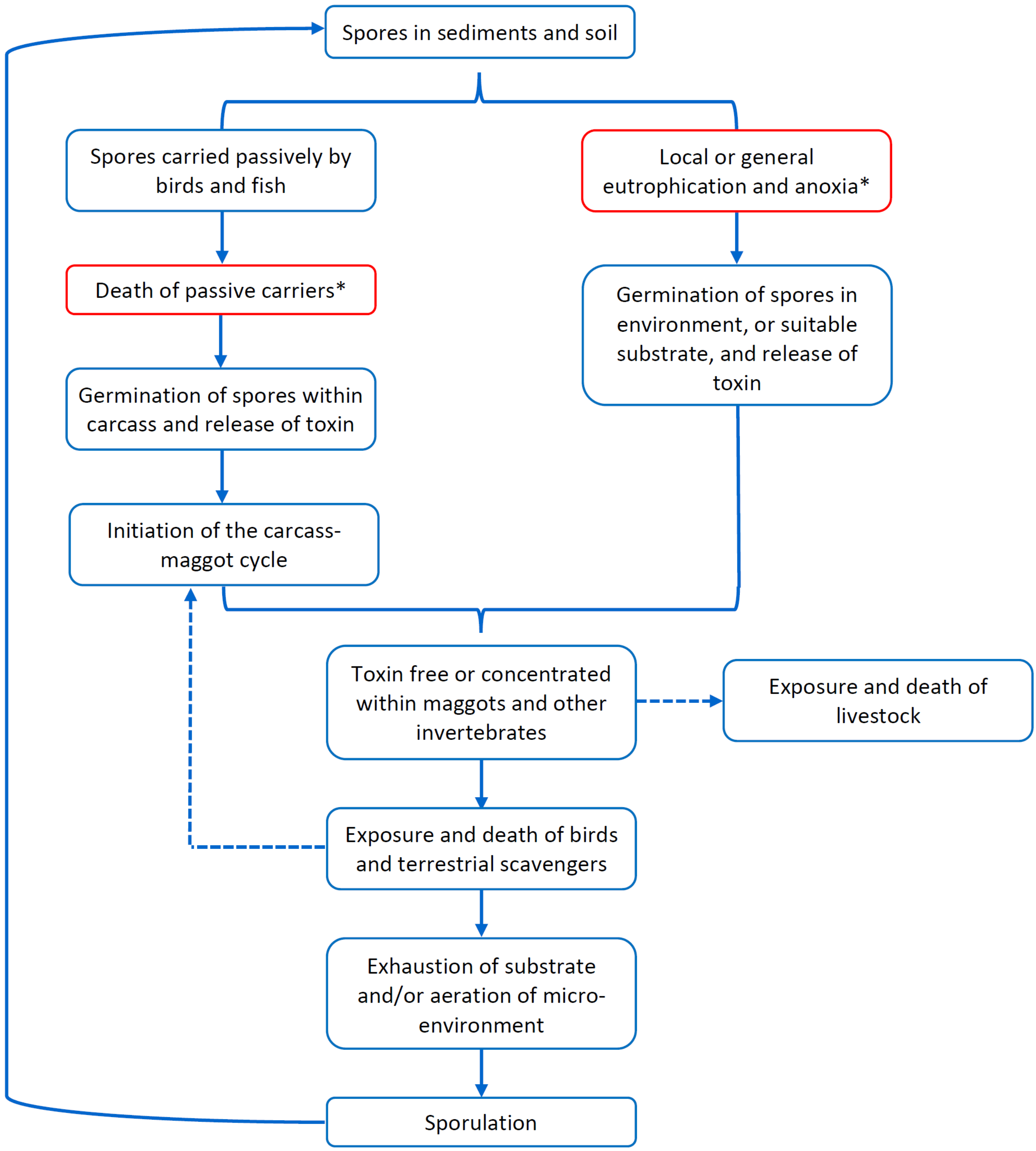

Figure 31 Outbreaks of type C or C/D botulism in wildlife

* Parts of the cycle that may be augmented through fish kills

(b) Type E botulism

Section 7.6.2 provides an overview of key components of the ecology of type E botulism, and the pathways for exposure of birdlife, terrestrial scavengers and humans. These pathways are shown pictorially in Figure 31.

Initiation of an outbreak of type E botulism: the scenario that underpins the pathways considered in this part of the assessment concerns the sudden abundance of large numbers of carcasses of fish, a proportion of which may have been passive carriers of *C. botulinum* type E spores. As noted above, some of these carcasses are likely to accumulate in shallow water at the edge of waterways while others will sink to the surface of the sediment. Germination of spores can take place in the detritus at the top layer of the sediment, with release of type E toxin. Macro- and micro-invertebrates may then concentrate the toxin and vegetative cells.

The likelihood attributed to this scenario was driven principally by the distribution of type E botulism spores across different parts of Australia (that is, its local presence or absence) and, where it is endemic, the prevalence of passive carriers of type spores within local populations of carp. Aside from a single case in France, outbreaks of type E botulism do not appear to have occurred in wildlife beyond the Great Lakes ecosystem in North America. A review of human cases of botulism in Australia between 2002 and 2017 did not identify type E. Even in the situation of mass mortalities of fish, the likelihood that an outbreak would be initiated in Australia is considered to be negligible. A detailed evaluation of type E botulism was, however, included here as: (a) type E is a dangerous zoonosis; and (b) there was no concrete evidence, or even a definitive expert opinion, to effect that type E is exotic to Australian freshwater waterways.

Exposure of wildlife: the primary routes of exposure of small benthic fish to type E botulinum toxin and vegetative cells are detritus in the sediment (including dead and decaying fish carcasses) and aquatic macro- or micro invertebrates. Depending on their diet and foraging habits, larger fish may be exposed in the same way, or may predate upon smaller living moribund fish. The primary route of exposure for wild birds is either aquatic macro or micro-invertebrates that contain concentrated toxin (non-piscivorous birds), moribund fish (piscivorous birds) or dead fish (scavenger birds). The carcass-maggot cycle is not a dominant feature of outbreaks of type E botulism.

If germination and toxigenesis were to occur in fish carcasses as a result of the mass mortality of carriers, then the subsequent exposure of other fish or birds would be almost certain. Diet is, however, a key risk factor. The exposure of non-piscivorous birds, whose diet may include macro- and micro-invertebrates and, in some cases, vegetation, was considered less likely. The exposure of avian scavengers was also considered less likely.

Exposure of people: the primary route of exposure of people to type E botulinum toxin is the consumption of affected fish or aquatic macro-invertebrates (including mussels and crustaceans). If the exposure of fish throughout the aquatic food chain in a particular location were to occur, then the subsequent exposure of humans would be plausible although not likely. This is because fish and crustaceans will not generally be taken for human consumption from places where mass fish mortalities and type E botulism are occurring. Inevitably there may some exceptions to this, but these events will be uncommon and those concerned are likely to be protected to a large extent by the predilection of type E toxin for parts of fish that are not routinely eaten by people. As meals are generally consumed by families or other groups (as opposed to individuals), the likelihood of exposure for individuals and groups will be similar.

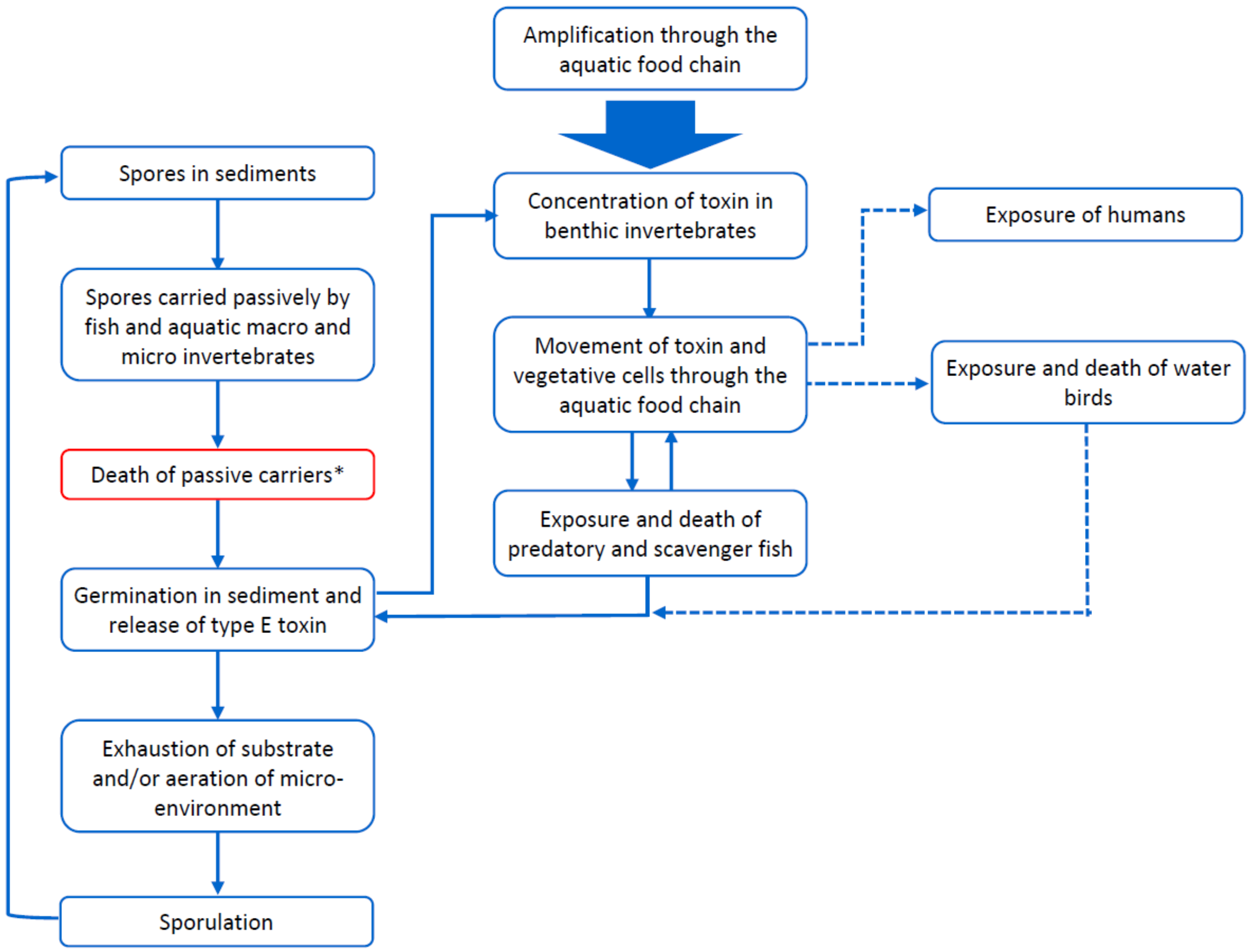

Figure 32 Outbreaks of type E botulism in wildlife

* Parts of the cycle that may be augmented through fish kills

11.6.2 Likely consequences of exposure

A summary of the potential impacts of botulism on risk assessment endpoints (Part I: Section 2.2) is given in Table 15. In chief, the impacts of type C (or C/D mosaic) botulism focus on direct harm to susceptible aquatic and terrestrial species and their communities. An outbreak of botulism that followed from extensive carcass decomposition is likely to be a relatively short-term event. This means that while the size of a local population, or its area of occupancy, might be affected, these populations are less likely to be fragmented. An outbreak of botulism is also unlikely to have a direct impact on habitat, nor result in the establishment of a disease or invasive species. An outbreak of botulism might impact on the lifecycle of a migratory species, although would not be likely result in the establishment of a harmful invasive species. Similar logic was applied across ecological communities and wetlands.

Susceptible local populations will include aquatic native biota, such as large-bodied and small-bodied fish and waterbirds (including native and migratory waterbirds). A single reference was identified to substantiate the impact of type C botulinum toxin on turtles. No references were identified to substantiate the disease in frogs, although some early papers about the physiology of botulinum toxin included reference to frog skeletal muscle paralysis. Crustaceans may be contaminated with botulinum toxin and thus play an important role in the epidemiology of botulism outbreaks, but no references were identified linking type C botulism with clinical disease in crustaceans. Type C (or C/D mosaic) botulinum toxin affects livestock but does not affect people. Because there is a negligible likelihood that type E *C. botulinum* exists within Australian freshwater waterways – or exists at a prevalence that would correlate with a meaningful risk in respect of an outbreak of CyHV-3 – type E was not taken further in this assessment.

Exposure to botulinum toxin may result from consuming affected or contaminated water, plant materials, insects, animals or carcass materials. Where an outbreak of botulism has developed as a result of the germination of environmental spores, then most species and functional groups will be at risk. Fish-eating birds are usually associated with type E botulism, although pelicans, herons and others have died in in large numbers in type C (or C/D mosaic) outbreaks in North America and may also be exposed. Fish themselves are less sensitive to type C toxin than they are to type E, and their role in type C (or C/D mosaic) outbreaks is more likely to be as a source of spores or as a vector for moving vegetative cells and toxin through the food chain to piscivorous birds. Where an outbreak has developed through the carcass-maggot cycle, then the diet and foraging behaviour of particular groups of waterbirds may make them more or less vulnerable. Filter-feeding and dabbling waterfowl, such as mallards, teal, and shovelers, are at high, as are probing shorebirds, such as avocets and stilts. Shorebird species that feed near the surface of wetland soils and sediments may be at higher risk than those that probe deeply into the substrate for food. Carrion-eating raptors may be relatively less susceptible or may just be more likely to die at a distance from an affected waterbody and, for that reason, their carcasses may not be so commonly discovered. The level of unpredictability in respect of the functional groups or species that will be most affected by an outbreak of botulism reflects the very complex and highly-probabilistic nature of its initiation and is borne out by the fact that, while the organism exists in spore form in most Australian freshwater waterways, outbreaks in wildlife are only infrequently observed.

For this and other exposure pathways, the likely consequence provide a preliminary or baseline assessment of the possible impacts of CyHV-3, in the sense that they do not relate to particular places or ecosystems. The site-specific case studies undertaken in Section 12 build on these baseline assessments and give an evaluation of the possible impacts of CyHV-3 on the communities and species that characterise each location.

Table 15 Relevance of an outbreak of type C or C/D botulism to risk assessment endpoints

Endpoints (significant impact criteria)	Relevance
Migratory and non-migratory native species	
Lead to a long-term decrease in the size of a population of a non-migratory native species	✓
Reduce the area of occupancy of a non-migratory native species	✓
Fragment an existing population into two or more populations of a non-migratory native species	✕
Adversely affect habitat critical to the survival of a non-migratory native species	✕
Disrupt the breeding cycle of a population of a non-migratory native species	✓
Modify, destroy, remove, isolate or decrease the availability or quality of habitat to the extent that a non-migratory native species is likely to decline	✕
Result in invasive species that becomes established in a non-migratory native species' habitat	✕
Introduce disease that may cause a non-migratory native species to decline	✓
Interfere with the recovery of a non-migratory native species	✓

Endpoints (significant impact criteria)	Relevance
Migratory species	
Substantially modify (including by fragmenting, altering fire regimes, altering nutrient cycles or altering hydrological cycles), destroy or isolate an area of important habitat for a migratory species	✗
Result in an invasive species that is harmful to the migratory species becoming established in an area of important habitat for the migratory species	✗
Seriously disrupt the lifecycle (breeding, feeding, migration or resting behaviour) of an ecologically significant proportion of the population of a migratory species	✓
Ecological community	
Reduce the extent of an ecological community	✓
Fragment or increase fragmentation of an ecological community, for example by clearing vegetation for roads or transmission lines	✗
Adversely affect habitat critical to the survival of an ecological community	✗
Modify or destroy abiotic (non-living) factors (such as water, nutrients, or soil) necessary for an ecological community's survival, including reduction of groundwater levels, or substantial alteration of surface water drainage patterns	✗
Cause a substantial change in the species composition of an occurrence of an ecological community, including causing a decline or loss of functionally important species, for example through regular burning or flora or fauna harvesting	✓
Cause a substantial reduction in the quality or integrity of an occurrence of an ecological community, including, but not limited to • Assisting invasive species, that are harmful to the ecological community, to become established, or • Causing regular mobilisation of fertilisers, herbicides or other chemicals or pollutants into the ecological community which kill or inhibit the growth of species in the ecological community, or interfere with the recovery of an ecological community	✓
Wetland ecosystem	
Areas of the wetland being destroyed or substantially modified	✗
A substantial and measurable change in the hydrological regime of the wetland, for example, a substantial change to the volume, timing, duration and frequency of ground and surface water flows to and within the wetland	✗
The habitat or lifecycle of native species, including invertebrate fauna and fish species dependent upon the wetland being seriously affected	✗
A substantial and measurable change in the water quality of the wetland – for example, a substantial change in the level of salinity, pollutants, or nutrients in the wetland, or water temperature which may adversely impact on biodiversity, ecological integrity, social amenity or human health	✓
An invasive species that is harmful to the ecological character of the wetland being established (or an existing invasive species being spread) in the wetland	✗
Livestock	
Local exposure of livestock to the clinical effects of a pathogenic organism or its toxin	✓
Establishment of a pathogenic microorganism in livestock in areas not previously affected	✗
Public health	
Exposure of individuals to the clinical effects of a pathogenic organism or its toxin	✗
Exposure of groups of individuals or communities to the clinical effects of a pathogenic organism or its toxin	✗

11.6.3 Conclusions: risks, uncertainty and treatments

Because there is a negligible likelihood that type E *C. botulinum* exists within Australian freshwater waterways – or exists at a prevalence that would correlate with a meaningful risk in respect of an outbreak of CyHV-3 – type E was not taken further in this assessment.

It is plausible that an outbreak of type C (or C/D mosaic) will be initiated by an outbreak of CyHV-3 and the death of large numbers of carp. This assessment takes into consideration both the historical paucity of botulism outbreaks arising from fish kills, and the observation that CyHV-3 may result in a series of coincident fish kills that is unprecedented in respect of its occurrence across the breadth of Australian freshwater waterways and waterbodies. Once initiated, aquatic and terrestrial wildlife are likely to be will be exposed and it is plausible that this exposure will extend to livestock.

The likely consequences of exposure reflect the likelihoods of observing each of the identified risk assessment endpoints. In the context of botulism in wildlife, it was not feasible to attribute a greater or lesser likelihood to particular tax, functional groups, species or endpoints, as the character of each botulism outbreak is likely to differ in ways that have historically been difficult to predict. It is possible that, once initiated, an outbreak of botulism would result in each of the identified endpoints being observed.

The key **area of uncertainty** encountered in this evaluation concerned the occurrence and distribution of type E *C. botulinum* within Australia. Although there is no evidence that it exists within Australia, there also no evidence of a systematic search for type E in Australian waterways and no experts have been willing to state that type E does not exist within Australia. The importance of type E is twofold: (a) it is primarily a disease of fish (although waterbirds are severely impacted), and type E botulism is ostensibly more likely to arise from a widespread and multifocal fish kill; and (b) it is highly-toxic to humans. The other key uncertainty encountered in the evaluation concerns the likely character and key drivers of an outbreak of type C (or C/D mosaic) botulism in Australian wildlife that followed from a fish kill. It is somewhat surprising that outbreaks linked to fish kills have not been widely reported in Australia or overseas, although the epidemiology of the disease would support this aetiology. In view of this uncertainty, the likely consequences of an outbreak of type C (or C/D mosaic) botulism in Australian wildlife were rated as possible for all of the identified ecological endpoints and across all taxa, functional groups and species.

The **treatment of risks** associated with the initiation of an outbreak of type C (or C/D mosaic) botulism arising from a widespread fish kill will focus on minimising the likelihood that the carcass-maggot cycle will be initiated and minimising the likelihood of significant hypoxic or anoxic events. In both cases, the key action will be the removal of carcasses – that is, the carcasses of carp that have died from CyHV-3, and the carcasses of carp and other aquatic animals or waterbirds that have died from other processes linked to an outbreak of CyHV-3. If the physical removal of carcasses is not practicable in a given setting, then consideration may also be given to flushing carcasses or decomposed carcass materials from a waterbody, or diluting their effects using environmental watering. Whichever approach is taken, priority should be given to scenarios where the ecosystem at risk includes a high number of waterbirds, as well as threatened aquatic or terrestrial species or communities.

11.7 Direct pathogenic effects of CyHV-3 on native species

This pathway considers the exposure of native fish and other aquatic animals to the pathogenic effects of CyHV-3.

The species specificity of CyHV-3 was noted in Part II: Section 5.1 and was reviewed in further detail in Section 5.3.1. It was explained that in contrast to herpesviruses, which are generally restricted to their natural host species or closely related species, some alloherpesviruses are able to induce subclinical disease in a broader range of hosts. It was also explained that while the clinical disease caused by CyHV-3 has only been associated with carp and carp hybrids, there is some disagreement within the literature as to whether the virus may subclinically infect other host species. This notwithstanding, there is good agreement within the literature as to the specificity of the *pathogenic* effects of CyHV-3, which have only been observed in carp and carp hybrids. None of the experimental work undertaken globally, nor observations from aquaculture or outbreaks in wild fish, have reported pathogenic effects in other species. This includes aquatic species other than fish.

The OIE (World Organisation for Animal Health) Manual of Diagnostic Tests for Aquatic Animals (the Aquatic Manual), the sister publication to the OIE Aquatic Animal Health Code (the Aquatic Code), states that, "*Species that fulfil the criteria for listing as susceptible to infection with KHV according to Chapter 1.5. of the Aquatic Animal Health Code (Aquatic Code) include: All varieties and subspecies of common carp (Cyprinus carpio), and common carp hybrids (e.g. Cyprinus carpio × Carassius auratus)*". The role that other species may play as vectors of the disease is noted within the Aquatic Manual. This is consequently the default position held by nations trading in live fish and fish products under the World Trade Organisation (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement).

The matter of the host-specificity of CyHV-3 is currently under investigation by the NCCP. A systematic scientific review is in train and may result in a recommendation for further experimental work to be undertaken by the CSIRO Australian Animal Health Laboratory.

CSIRO's position given the current state of knowledge is to defer to the OIE, but to withhold any judgement or assessment of risk until the Australian situation can be clarified. This pathway was not considered further.

11.8 Direct pathogenic effects of a strain of CyHV-3 with altered specificity

This pathway considers the exposure of native fish and other aquatic animals to the pathogenic effects of a mutated strain of CyHV-3 with an altered species specificity.

The phylogeny of CyHV-3 and the global emergence of disease in carp were reviewed in Part II: Sections 5.1 and 5.2, respectively. The disease associated with CyHV-3 first appeared in 1997 and since then has evolved into two major lineages; European and Asian. Under this classification, the 'European' lineage includes the strains thought to have arisen in the United States, Israel and China, while the 'Asian' lineage includes the Indonesian and Japanese strains (Figure 9). The Indonesian isolate KHV C07 is the strain of CyHV-3 that is being investigated for release in Australia. Although these strains differ to a minor degree in their pathogenicity and other epidemiological characteristics, the virus itself has not mutated to the extent that its species specificity has been altered – and neither has such a significant change been observed with other alloherpesviruses. This outcome is despite the exposure of a wide range of species worldwide, and the passage of the virus through a wide range of high-density farmed and wild carp populations.

In view of this natural history, there is not likely to be a real chance or possibility that native species will be exposed to the pathogenic effects of a mutated strain of CyHV-3 with altered species specificity. This result applies to each of the ecological endpoints (Part I: Section 2.2), and there are no risk factors that would elevate the likelihood in different circumstances.

This notwithstanding, CSIRO's position given the current state of knowledge is, once again, to withhold judgement or definitive assessment of risk until the Australian situation can be clarified. This pathway was not considered further.

11.9 Direct pathogenic effects of CyHV-3 for humans

This pathway considers the exposure of humans to the pathogenic effects of CyHV-3, through drinking contaminated water or through consuming contaminated fish or fish products.

In a systematic review of the literature, Roper and Ford (2018) concluded that there was no published evidence to indicate that the virus has or will develop zoonotic capability. In support of this, the authors also pointed out that the permissive temperature range for the transmission of CyHV-3 is approximately 18 to 28C (Section 5.3.5) while human body temperature is approximately 36.1 to 37.2C.

Accordingly, the likelihood that humans would be exposed to harm as a result of the release of CyHV-3 was considered negligible. This pathway was not taken further.

12 Case studies

Six case studies were selected for the assessment. The locations of these sites are shown in Figure 33. Cursory treatment of three additional case studies (the Macquarie Marshes, Kow Swamp and the upper Glenelg Catchment) is given in the Discussion in Part III: Section 13.

Figure 33 Case study sites

The case studies were chosen to correlate as far as was practicable with the case studies undertaken by the NCCP project teams: (a) modelling the epidemiology of CyHV-3 outbreaks in an Australian context; and (b) evaluating the possible impacts of these outbreaks on water quality. In following this strategy, care was also taken to ensure that the case studies provided a broad geographical coverage and coverage of a wide range of ecosystems and ecological values. Each of the case studies represents a site where carp have colonised with negative consequences for the local ecosystem(s). To a variable extent, and with variable outcomes, the control of carp has also been active at each of the selected sites.

1	Barmah-Millewa Forest	This case study considers outbreak scenarios and possible impacts of CyHV-3 in a large regulated Ramsar wetland within the mid Murray River. The Barmah-Millewa Forest includes a range of threatened species and ecological communities and is also one of the places within the Murray-Darling Basin where carp recruit most successfully.
2	Chowilla Floodplain	This case study was included alongside the Barmah-Millewa Forest as it has a unique character arising from the placement of the Chowilla regulator and associated works. These structures enable the hydrology of the Chowilla Floodplain to be controlled, with environmental watering strategies targeted very specifically at particular physical or species assets. The Chowilla Floodplain has experienced a long-standing altered hydrology as a result of locks on the Murray River, and this has led to widespread salination and other environmental degradations. The regulator and associated works were commissioned in 2012 and seek to halt and remedy this degradation in the face of decreasing water flows within the river channel.
3	Mid-Murray River (Lake Mulwala to Tocumwal)	This case study considers the reach from Lake Mulwala (above Yarrawonga Weir) to Tocumwal (at the Newell Highway bridge). The reach includes exits to major irrigation channels to the north (Mulwala channel) and south (Yarrawonga main channel) of Lake Mulwala. The reach represents a highly-regulated riverine impoundment and release environment, and lotic downstream segment of the mid Murray River (mirroring many other lotic waterbodies downstream from locks), and includes some important environmental assets (for example, remnant breeding populations of the endangered trout cod: *Maccullochella macquariensis*).
4	Moonie River Catchment	This case study examines the Moonie River Catchment. The Moonie River is representative of a seasonally-disconnected and highly-turbid Queensland waterway. The characteristics of the ecosystem and ecological communities within this setting – and the stresses placed upon them by carp – are quite different from southern rivers.
5	Lower lakes and Coorong	The Lower Lakes and Coorong form a regulated and heterogeneous Ramsar wetland ecosystem that includes estuarine waters, coastal brackish or saline lagoons, permanent freshwater lakes and marshes and seasonally-flooded agricultural land. The Lower Lakes

		and Coorong also support a range of threatened species and significant ecological communities.
6	Upper Lachlan River	This case study represents a relatively fast-flowing riverine environment with lower winter and summer temperatures than those experienced in either mid-lower Murray River or the seasonally-disconnected waterways in western New South Wales and Queensland. The carp biomass density is also relatively lower in this reach, with a higher proportion of larger (older) fish.

12.1 Case study 1: Barmah-Millewa Forest

12.1.1 Physical description of the Barmah-Millewa Forest case study site

The Barmah-Millewa Forest (including the Barmah Forest Ramsar wetland[63] and the Barmah and Moira lakes) is a large, complex floodplain wetland system in the middle reaches of the Murray River (Figure 34). It is an area of river red gum forest that is subject to periodic inundation.

The southern (Victorian) section of the Barmah-Millewa Forest lies within the Barmah National Park while the northern (New South Wales) section forms the Murray Valley National Park. Together, the Barmah-Millewa Forest spans 70,000 ha (170,000 acres) of national park and includes the Barmah-Millewa Murray River Icon Site.[64]

The Barmah Forest Ramsar site lies within the Victorian side of the larger Barmah-Millewa area (Figure 35) and features a variety of permanent and temporary wetlands, including lakes, swamps, lagoons and flooded forest. These wetlands provide habitat for a large number of bird species. The Barmah Forest was originally nominated as a Wetland of International Importance under the Ramsar Convention in 1982.

[63] See: http: //www.environment.gov.au/cgi-bin/wetlands/ramsardetails.pl?refcode=14

[64] See: http: //www.mdba.gov.au/discover-basin/environment/significant-environmental-sites/icon-sites-along-river-murray

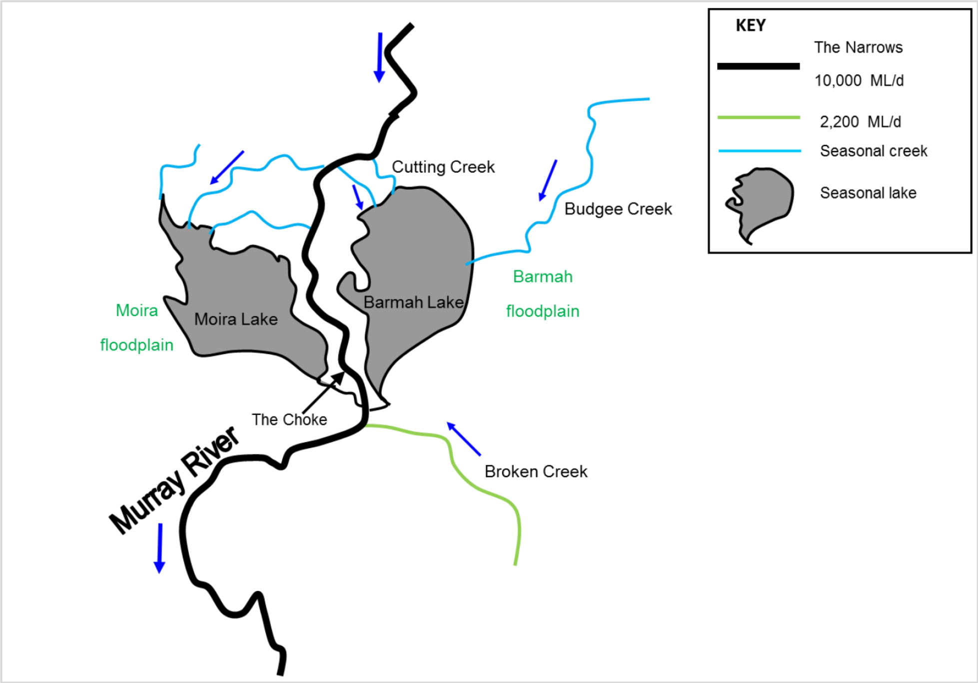

Figure 34 Barmah-Millewa (incl. Barmah Forest Ramsar wetland and Barmah-Moira lakes)

Source: Koehn *et al.* (2016)

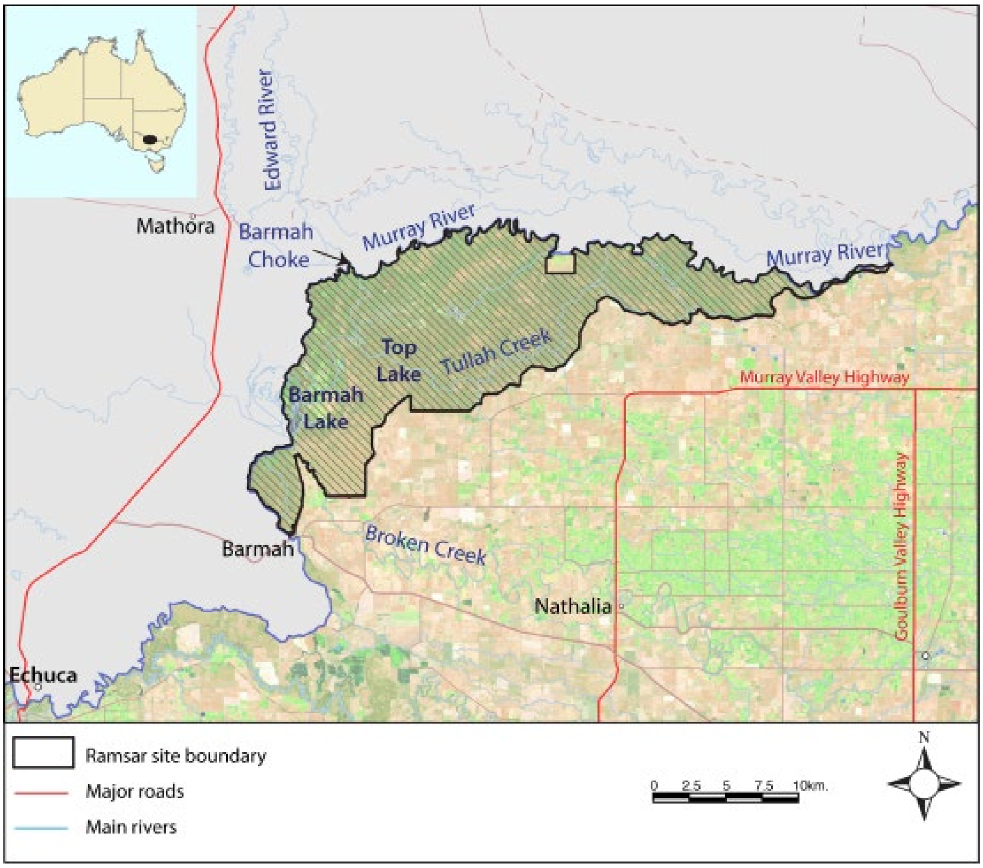

Figure 35 Barmah Forest Ramsar site

Source: Hale and Butcher (2011)

12.1.2 Hydrology of the Barmah-Millewa Forest

The Barmah Forest Ramsar Site Ecological Character Description (Hale and Butcher, 2011) outlines the hydrology of the area, which is defined by flow in the Murray River. Although the Barmah Forest Ramsar site is restricted to the southern (Victorian) portion of the larger Barmah-Millewa Forest, the detailed and systematic Ecological Character Description provides detail in respect of hydrology and ecology that can be extrapolated to the northern side of the Murray River. Much of the baseline information about the site and its assets (Sections 12.1.1 to 12.1.3.7) has been adapted directly from that report.

River regulation began in about 1915 and continued through to 1974 with the construction of dams, locks and weirs. Regulators were constructed in the 1930s and 1940s on streams within both the New South Wales and Victorian parts of the Barmah-Millewa Forest, and hydrology within the site has been regulated and managed since that time. The character of the Barmah Forest Ramsar site at the time of listing was strongly influenced by river regulation, and this continues to be the case.

Surface water inflows into the Barmah-Millewa Forest are controlled by releases from the Yarrawonga Weir, which is located approximately 200 kilometres upstream. There are two types of surface water flows (MDBA, 2005):

- In-channel flow, which includes inundation of effluent streams, channels and floodplain depressions connected at the pool level
- Overbank flow as water moves laterally from channels and spreads across the broader floodplains.

In-channel flow is controlled by the large number of water-regulating structures within the forest, whose operation also determines inundation extent, frequency and duration. The original procedures for regulator operation, developed in the 1950s and 1960s, were aimed at preventing loss of regulated flow into the forest, which can occur at river flows as low as 6,000 ML/day. Regulators were generally closed to exclude floodwaters from the forest at flows that did not exceed channel capacity (approximately 10,400 ML/day) during the 10 months of the irrigation season (August to May). This was so that the maximum volume of water could be delivered downstream for consumptive use. It also prevented unseasonal flooding in summer and autumn.

When flows in the Murray River downstream of Yarrawonga exceed the capacity of the Barmah Choke (approximately 10,400 ML/day), the regulators are progressively opened to allow water to enter the forest. At flows between 10,400 and 16,000 ML/day, channels, swamps and other low-lying areas, including about 16 percent of the forest, are inundated. Larger floods of over 35,000 to 45,000 ML/day are required to inundate about 60 percent of the forest and it is only at flows of greater than 60,000 ML/day that inundation of most of the river red gum forest and substantial proportions of the black box communities occurs.

Operation of the regulators influences the movement of water through the forest and, given the number of regulators, there are many possible inundation scenarios depending on which regulators are opened and closed and at what time. The results of modelled inundation scenarios (30-day steady inflows and all Victorian regulators open) provide an indication of flood extents within the Barmah Forest under a number of flow thresholds (Figure 36). A comparison with inundation under a similar 30-day inundation scenario, but with all the New South Wales regulators open (Figure 37) highlights the significant effect of regulator operation. By opening Victorian regulators a large area in the centre of the Ramsar site is inundated at 13,000 ML/day, as opposed to 25,000 ML/day when the Victorian regulators are closed and those in New South Wales are open. This is not surprising, given that the Ramsar site lies on the southern side of the Murray River. As flows increase, the ability to control water movement diminishes. This is illustrated by the two modelled scenarios (Figure 36 and Figure 37) which show very little difference in inundation above about 35,000 ML/day.

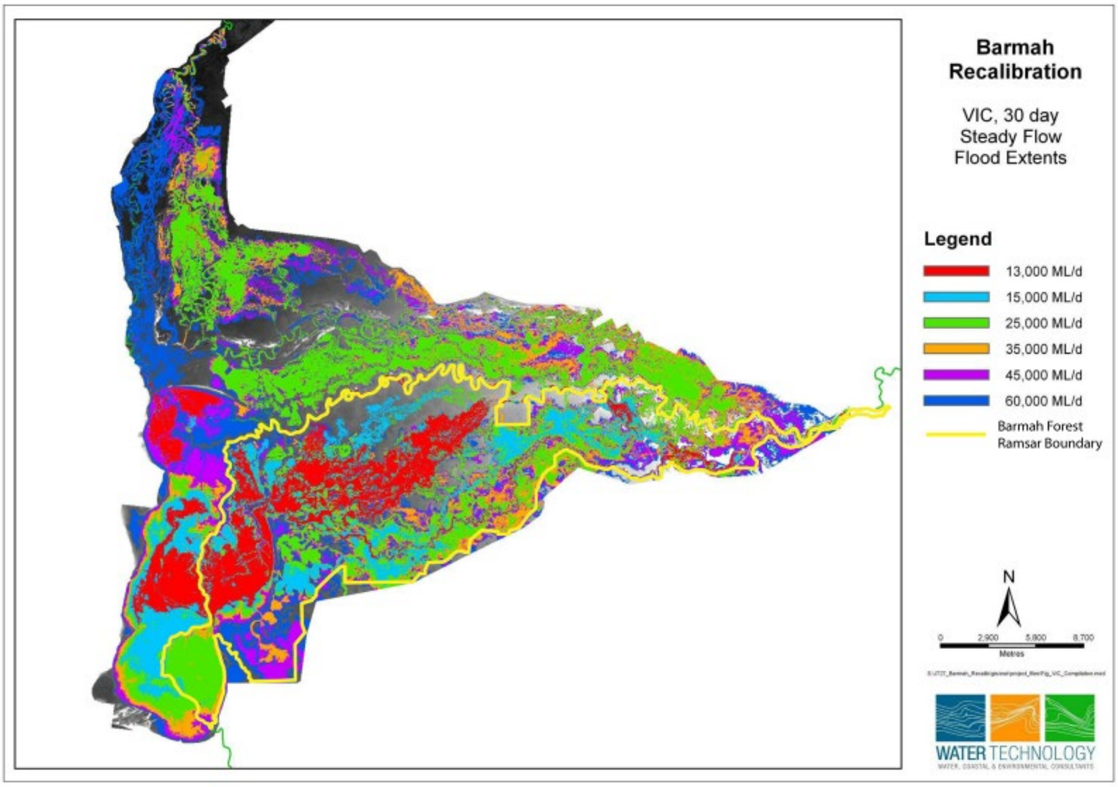

Figure 36 Inundation of Barmah Millewa Forest with Victorian regulators open

Source: Hale and Butcher (2011)

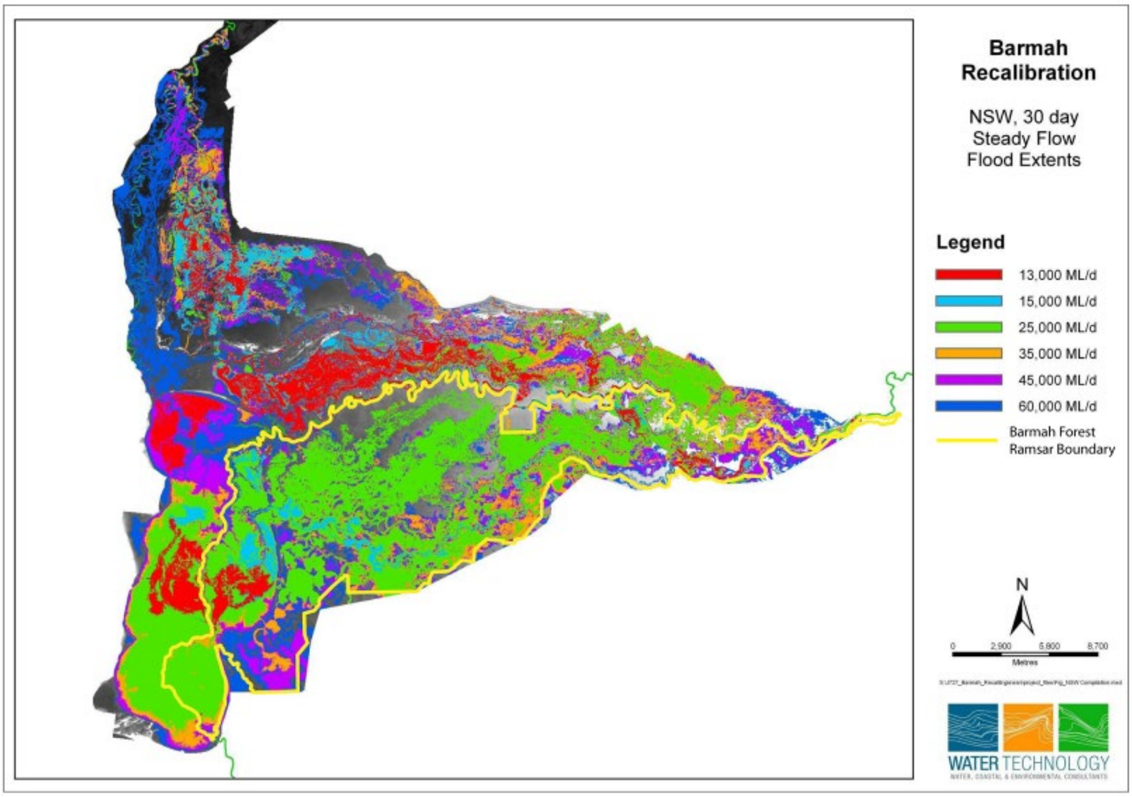

Figure 37 Inundation of Barmah Millewa Forest with New South Wales regulators open

Source: Hale and Butcher (2011)

12.1.3 Ecological values of the Barmah-Millewa Forest

The Barmah-Millewa Forest provides for the following six key groups of ecological services (Hale and Butcher, 2011):

- Supports a diversity of wetland types
- Supports biodiversity
- Provides physical habitat for waterbird feeding and breeding
- Supports threatened wetland species
- Maintains ecological connectivity for fish spawning and recruitment
- Organic carbon cycling.

12.1.3.1 Diversity of wetlands

The Barmah-Millewa Forest encompasses three key habitat types (Figure 38) (Hale and Butcher, 2011):

- Freshwater tree-dominated wetlands, including river red gum forest and woodland (approximately 23,300 hectares)
- Intermittent freshwater marshes, including freshwater marshes and open water (approximately 960 hectares)

- Permanent and intermittent rivers and streams, including permanent pools and instream habitats.

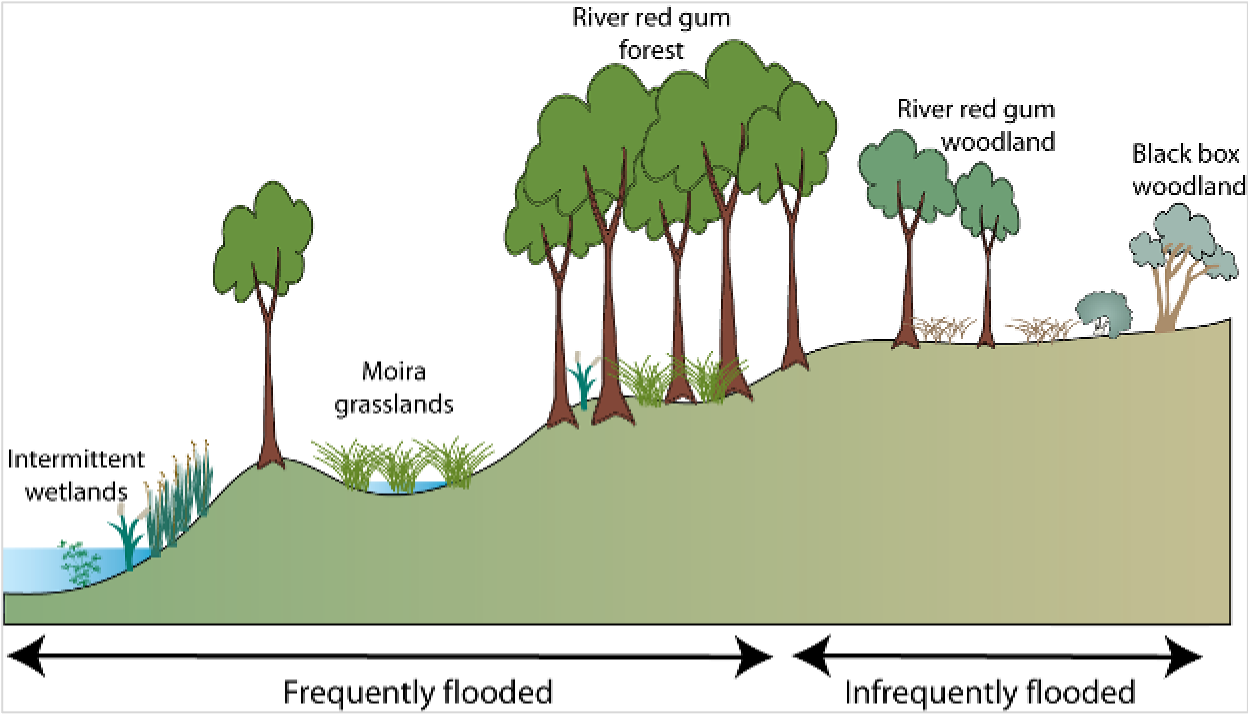

Figure 38 Vegetation associations, geomorphic setting and flood regime

Source: Hale and Butcher (2011)

This diversity of habitat is brought about by the interactions between geomorphology, hydrology and vegetation. Water regime is the single biggest determinant of wetland vegetation, with different groups of species having different morphological adaptations to patterns of inundation. The water regime requirements for different wetland types are provided in Table 16.

Table 16 Water requirements of select vegetation communities within the Barmah forest

Vegetation	Frequency	Duration	Timing
Giant rush	Seven to 10 years in 10	Nine to 12 months	May to January
Moira grass	Ten years in 10	Five to 7 months	July to December
River red gum forest	Seven to 10 years in 10	One to 18 months	August to December
River red gum woodland	Three years in 10	One to 18 months	August to December
Black box woodland	One year in 10	One month (range of 1 to 4 months)	August March

Source: Hale and Butcher (2011)

12.1.3.2 Diversity of native species

Eleven species of native fish are believed to occupy the Barmah Forest Ramsar site (Hale and Butcher, 2011), and this can reasonably be extrapolated to the Barmah-Millewa Forest more broadly. Five of these are the large-bodied Murray cod, trout cod, silver perch, golden perch and freshwater catfish. Three of these (Murray cod, silver perch and trout cod) are listed as threatened under the EPBC Act. Six small-bodied species have also been recorded, Australian smelt, carp gudgeons, dwarf flathead gudgeon, Murray-Darling rainbowfish, southern pygmy perch and unspecked hardyhead. None of these species is listed as threatened under the EPBC Act.

A further 11 native fish species are thought likely to occur within the Barmah-Millewa Forest (Hale and Butcher, 2011), but have not been definitively recorded within surveys. These include bony herring, climbing galaxias, flathead galaxias, flathead gudgeon, mountain galaxias, Murray hardyhead, purple-spotted gudgeon, olive perchlet, river blackfish and the short-headed lamprey. Of these, the flathead galaxias and Murray hardyhead are listed as threatened under the EPBC Act.

A total of 60 species of wetland birds have been recorded within the Barmah Forest Ramsar site (Hale and Butcher, 2011), and again this can be extrapolated to the Barmah-Millewa Forest more broadly. The list includes two species that are considered as nationally or internationally threatened (superb parrot and Australasian bittern). There are also eight species listed as migratory under the EPBC Act, seven of which are also listed under international migratory bird agreements to which Australia is a party (including CAMBA, JAMBA or ROKAMBA), although some of these species (for example, the eastern great egret, glossy ibis, cattle egret and the white-bellied sea eagle) are considered to be resident in Australia – that is, they are not known to undertake international migrations.

Table 17 Native and migratory bird species recorded within the Barmah Forest Ramsar site

Category	Common name	Scientific name	EBPC Act Listing
Seabird	Australian darter	*Anhinga novaehollandiae*	Not listed
Seabird	Australian pelican	*Pelecanus conspicillatus*	Not listed
Seabird	Great cormorant	*Phalacrocorax carbo*	Not listed
Seabird	Little black cormorant	*Phalacrocorax sulcirostris*	Not listed
Seabird	Little pied cormorant	*Microcarbo melanoleucos*	Not listed
Seabird	Pied cormorant	*Phalacrocorax varius*	Not listed
Shorebird	Black-fronted dotterel	*Elseyornis melanops*	Not listed
Shorebird	Black-winged stilt	*Himantopus himantopus*	Not listed
Shorebird	Common greenshank	*Tringa nebularia*	Migratory
Shorebird	Latham's snipe	*Gallinago hardwickii*	Migratory
Shorebird	Masked lapwing	*Vanellus miles*	Not listed
Shorebird	Red-kneed dotterel	*Erythrogonys cinctus*	Not listed
Shorebird	Red-necked stint	*Calidris ruficollis*	Migratory
Shorebird	Silver gull	*Chroicocephalus novaehollandiae*	Not listed
Shorebird	Whiskered tern	*Chlidonias hybrid*	Not listed
Songbird	Clamorous reed-warbler	*Acrocephalus stentoreus*	Not listed
Waterfowl	Australasian grebe	*Tachybaptus novaehollandiae*	Not listed
Waterfowl	Australasian shoveler	*Anas rhynchotis*	Not listed
Waterfowl	Australian shelduck	*Tadorna tadornoides*	Not listed
Waterfowl	Australian wood duck	*Chenonetta jubata*	Not listed

Category	Common name	Scientific name	EBPC Act Listing
Waterfowl	Black swan	*Cygnus atratus*	Not listed
Waterfowl	Blue-billed duck	*Oxyura australis*	Not listed
Waterfowl	Chestnut teal	*Anas castanea*	Not listed
Waterfowl	Freckled duck	*Stictonetta naevosa*	Not listed
Waterfowl	Great crested grebe	*Podiceps cristatus*	Not listed
Waterfowl	Grey teal	*Anas gracilis*	Not listed
Waterfowl	Hardhead duck	*Aythya australis*	Not listed
Waterfowl	Hoary-headed grebe	*Poliocephalus poliocephalus*	Not listed
Waterfowl	Musk duck	*Biziura lobate*	Not listed
Waterfowl	Pacific black duck	*Anas superciliosa*	Not listed
Waterfowl	Pink-eared duck	*Malacorhynchus membranaceus*	Not listed
Waterfowl	Plumed whistling-duck	*Dendrocygna eytoni*	Not listed
Large wader	Australasian bittern	*Botaurus poiciloptilus*	Endangered
Large wader	Australian little bittern	*Ixobrychus dubius*	Not listed
Large wader	Australian white ibis	*Threskiornis molucca*	Not listed
Large wader	Brolga	*Grus rubicunda*	Not listed
Large wader	Cattle egret	*Ardea ibis*	Migratory
Large wader	Eastern great egret	*Ardea modesta*	Migratory
Large wader	Glossy ibis	*Plegadis falcinellus*	Migratory
Large wader	Intermediate egret	*Ardea intermedia*	Not listed
Large wader	Little egret	*Egretta garzetta*	Not listed
Large wader	Nankeen night-heron	*Nycticorax caledonicus*	Not listed
Large wader	Royal spoonbill	*Platalea regia*	Not listed
Large wader	Straw-necked Ibis	*Threskiornis spinicollis*	Not listed
Large wader	White-faced heron	*Egretta novaehollandiae*	Not listed
Large wader	White-necked heron	*Ardea pacifica*	Not listed
Large wader	Yellow-billed spoonbill	*Platalea flavipes*	Not listed
Gruiformes	Australian spotted crake	*Porzana fluminea*	Not listed
Gruiformes	Ballion’s crake	*Porzana pusilla palustris*	Not listed
Gruiformes	Black-tailed native-hen	*Tribonyx ventralis*	Not listed
Gruiformes	Buff-banded rail	*Gallirallus philippensis*	Not listed
Gruiformes	Dusky moorhen	*Gallinula tenebrosa*	Not listed
Gruiformes	Eurasian coot	*Fulica atra*	Not listed
Gruiformes	Purple swamphen	*Porphyrio porphyrio*	Not listed
Gruiformes	Spotless crake	*Porzana tabuensis*	Not listed
Kingfisher	Azure kingfisher	*Alcedo azurea*	Not listed
Kingfisher	Sacred kingfisher	*Todiramphus sanctus*	Not listed

Category	Common name	Scientific name	EBPC Act Listing
Bush-bird	Superb parrot	*Polytelis swainsonii*	Vulnerable
Raptor	Swamp harrier	*Circus approximans*	Not listed
Raptor	White-bellied sea eagle	*Haliaeetus leucogaster*	Migratory

In addition to native fish and waterbirds (above), three mammalian, nine amphibian and four reptilian native species have been recorded within the Barmah Forest Ramsar site (Hale and Butcher, 2011). The Barmah Forest Ramsar site also supports the swamp yabby (*Cherax sp. C*) a burrowing crayfish that, although widely distributed and locally abundant, is poorly understood.

Water rat (*Hydromys chrysogaster*)	Spotted marsh frog (*Limnodynastes tasmaniensis*)
Large-footed myotis (*Myotis macropus*)	Peron's tree frog (*Litoria peronii*)
Platypus (*Ornithorhynchus anatinus*)	Common spadefoot (*Neobatrachus sudelli*)
Plains froglet (*Crinia parinsignifera*)	Brown toadlet (*Pseudophryne bibronii*)
Common froglet (*Crinia signifera*)	Broad-shelled river turtle (*Macrochelodina expansa*)
Sloanes froglet (*Crinea sloanei*)	Eastern long-necked turtle (*Chelodina longicollis*)
Barking marsh frog (*Limnodynastes fletcheri*)	Murray turtle (*Emydura macquarii*)
Pobblebonk (*Limnodynastes dumerili*)	Yellow-bellied water skink (*Eulamprus heatwolei*)

12.1.3.3 Physical habitat for waterbird feeding and breeding

The Barmah-Millewa Forest provides a range of habitats that support the feeding and breeding of wetland birds (Figure 39). As noted above, 60 species of wetland birds have been recorded within the Ramsar site and this represents a wide variety of species that rely on a range of different habitats. In many instances, birds that breed within the site utilise different habitats for foraging, roosting and breeding and a network of different habitat types is required to meet all of their needs (Hale and Butcher, 2011).

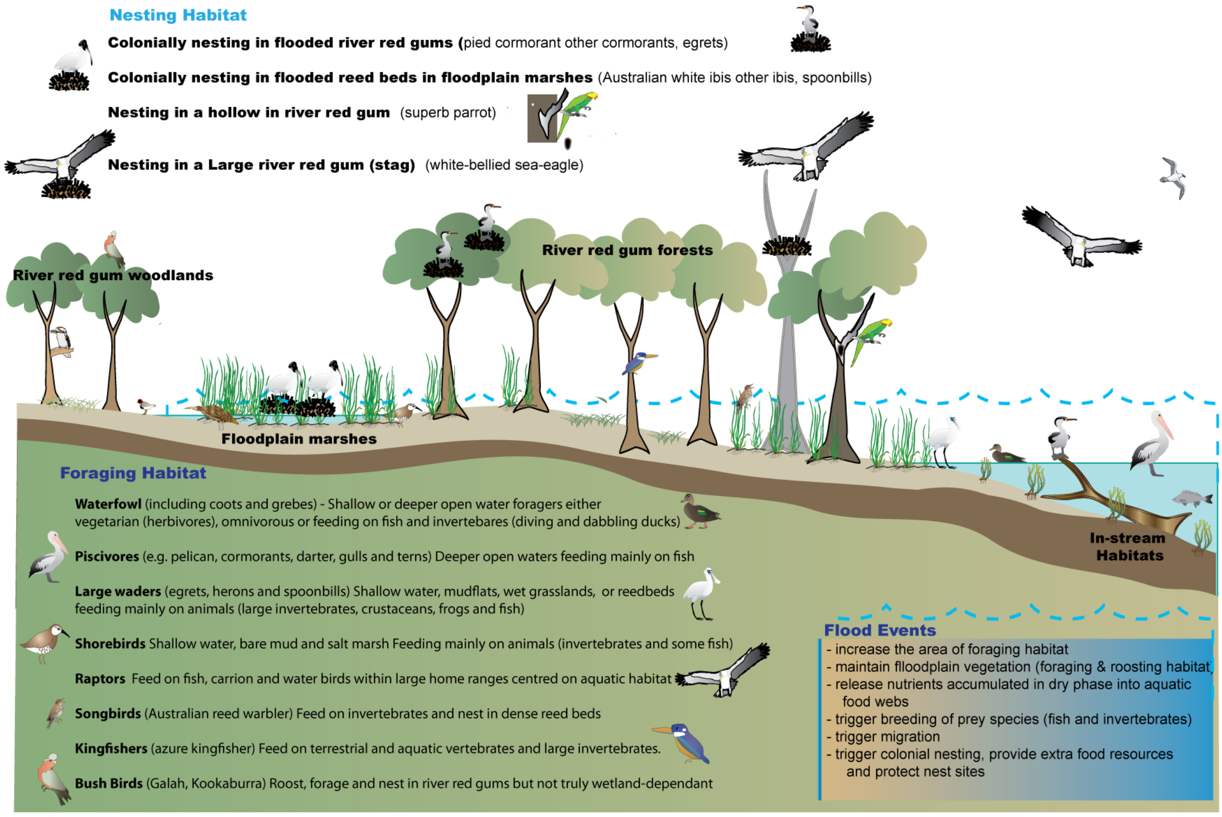

Figure 39 Habitats for wetland birds within the Barmah Forest Ramsar site

Source: Hale and Butcher (2011)

12.1.3.4 Support for threatened wetland species

Seven threatened species are considered critical to the ecological character of the Barmah Forest Ramsar site (Hale and Butcher, 2011).

- **Australasian bittern** is a shy and cryptic wading species of wetland bird that prefers permanent, densely vegetated freshwater wetlands. It forages mainly at night in shallow water up to 30 centimetres deep and feeds on frogs, fish and invertebrates as well as occasionally plant material. Permanent and intermittent freshwater marshes with emergent vegetation provide habitat for this species within the Ramsar site.
- **Superb parrot** generally inhabits box-gum, box-cypress pine and boree woodlands and river red gum forest. It nests in hollows in small colonies in mature river red gum, often with more than one nest in a single tree. It forages up to 10 km from nesting sites, primarily in grassy box woodland, feeding mainly on grass seed and herbaceous plants, fruits, berries, nectar, buds, flowers, insects and grain.
- **Murray cod**, **trout cod** and **silver perch** are large-bodied native fish that utilise predominantly flowing environments within the site. Murray cod and trout cod prefer deep holes in rivers, with instream cover such as rocks, snags and undercut banks, while silver perch are found mostly in lowland, turbid and slower-flowing rivers. Barmah Forest also provides temporary habitat for all three species when floods link the normal riverine habitat of these fish species with the floodplains.

- **Mueller daisy** (*Brachyscome muelleroides*) is a small annual herb restricted to the mid-Murray and Murrumbidgee Rivers region in New South Wales South and Victoria. The species occurs in seasonally wet depressions in the landscape such as shallow depressions and around the margins of swamps, lagoons and claypans. It is thought that sufficient autumn rainfall that results in localised soil waterlogging, or periodic flooding, is required to initiate seed germination and plant growth.
- **Swamp wallaby grass** (*Amphibromus fluitans*) is a slender, up to 1m tall aquatic or semi-aquatic native grass. It inhabits intermittent wetlands and the littoral zone of permanent wetland systems, as well as occurring as the understorey in river red gum forests following inundation.

12.1.3.5 Maintenance of ecological connectivity for fish spawning and recruitment

Juvenile and larval native fish species have been recorded in wetland, lake and creek habitats within the Barmah-Millewa Forest and fish that are known to spawn in river channels (such as Murray cod) are thought to utilise inundated floodplains and creek systems to feed (Hale and Butcher, 2011). Native fish have been recorded moving large distances along the Murray River from the Ramsar site (up to 1,000 km upstream and 900 km downstream), which is indicative of pre- and post-spawning behaviour. Barmah Forest provides a network of habitats for fish during these long migrations. Floodplain inundation, with its associated boom in productivity, provides both physical habitat and food resources that are important in maintaining regional native fish populations (Hale and Butcher, 2011).

12.1.3.6 Cycling of organic carbon

River red gum forests are important in the cycling of organic carbon in lowland rivers. Organic carbon is a major nutrient in freshwater systems and an important primary source of food in aquatic food webs. In forested catchments, the major terrestrial inputs of carbon to rivers include coarse woody debris (such as logs and branches from riparian and floodplain vegetation), particulate organic matter (such as litter inputs directly from riparian trees or washed from other areas of the floodplains) and DOC released from wetlands and floodplains and carried to the river on return flows (Hale and Butcher, 2011).

12.1.3.7 Importance of seasonal flows and inundation events

The annual cycle of wetting and drying is fundamental to the floodplain ecosystem in that it underpins many of physical, chemical and biological processes and functions and provides cues for the reproductive cycles of flora and fauna (Hale and Butcher, 2011).

The arrival of floodwaters means that dry and aerated sediments rapidly become waterlogged and devoid of oxygen. There is also mineralisation and release of nutrients and carbon from the sediments and floodplain litter. Depending on the quality of source water, velocity of flooding and sediment type, the floodwaters may be highly-turbid (particularly in channels where velocity is greatest) and new sediments may be deposited on the low relief floodplain surface.

The following biological processes are initiated with wetting:

- Microorganisms (bacteria and algae) process mineralised nutrients and a 'boom' of productivity commences
- Egg and seed banks hatch or germinate
- Plant propagules are brought in with the floodwaters from upstream environments
- Fish and invertebrates arrive on the floodplains with the floodwaters
- Aquatic plant growth is stimulated
- Flowering in a number of species such as lignum is stimulated
- The release of nutrients and subsequent increase in productivity act as cues to initiate breeding of waterbirds, frogs, fish and turtles.

When inundated the following ecological processes can be expected:

- Submerged aquatic plants grow and flower, while amphibious aquatic plants exist in their aquatic form
- Aquatic invertebrates occur in both larval (aquatic) stages as well as some emerging into mature aerial forms
- Productivity boom provides important food resources for waterbirds, fish, frogs and turtles as well as insectivorous and nectivorous terrestrial species
- Nesting of waterbirds in a variety of inundated habitats including inundated trees (for example, egrets, ibis and cormorants), shrubs (for example, coots and swamphens) and sedges and rushes (for example, magpie geese and the Australasian bittern)
- Frogs breeding in shallow water and inundated vegetation, tadpoles mature and grow
- Turtles nesting on sandy island habitats, eggs hatch and juveniles feed and grow
- Fish breeding in inundated vegetation and woody debris; larval and juvenile forms within water column.

The recession of floodwaters and subsequent drying of the soil then results in the following ecological processes:

- Nutrients, salts and organic carbon become concentrated in sediment
- Aquatic plants set seed to be stored dormant in the sediment for subsequent floods
- Floodplain plants such as river red gum germinate, and seedlings emerge on the damp soil
- Waterbirds fledge and disperse
- Turtles aestivate or migrate to nearby wet refuges
- Fish return with receding waters to the river or remain in permanent channels.

The seasonality of water flows downstream of Yarrawonga Weir between 1960 and 2018 is shown in Figure 40. On average, water flow peaks in mid-late spring and this corresponds with the spawning of many native and invasive fish and the aggregation of nesting waterbirds. Water flow falls off in late spring / early summer and is then reasonably consistent until it begins to decline again in March. The lowest flows through Barmah Choke are recorded between May and July, which coincides loosely with the period when water demand from downstream users (irrigators and urban water supplies) is lowest.

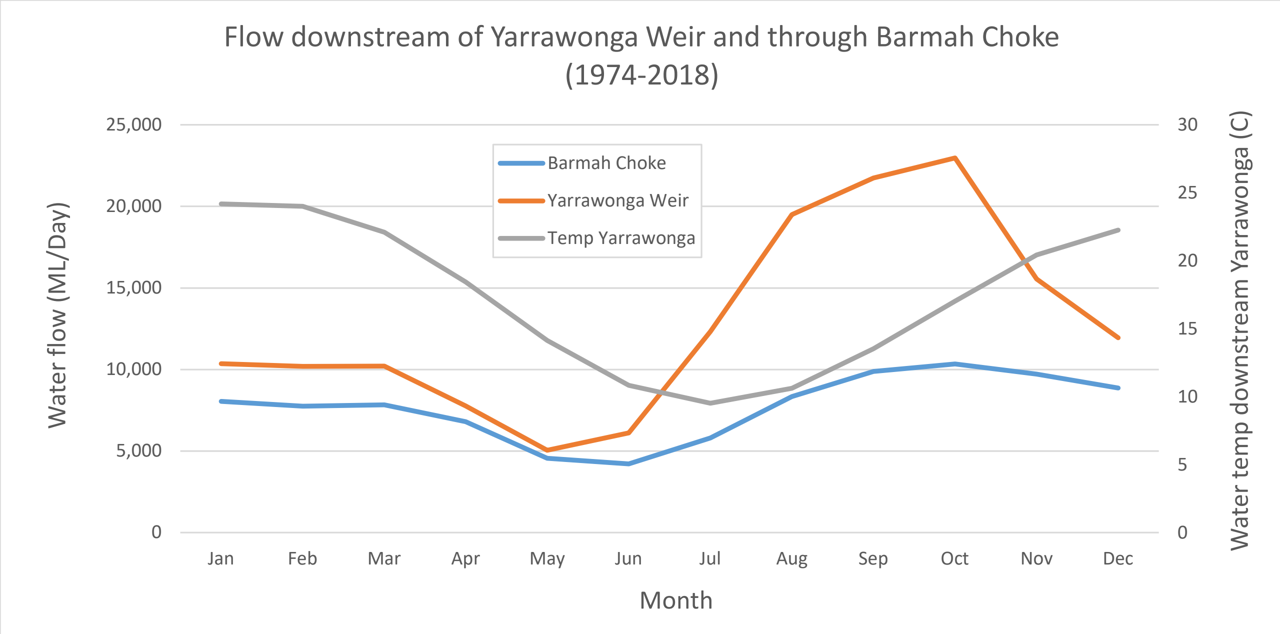

Figure 40 Flow through Barmah Choke and downstream of Yarrawonga Weir (1974-2018)

Water flow (ML/day) and daily average water temperature, aggregated to a monthly average. Data observed downstream of the Yarrawonga Weir and within the Barmah Choke, 1974 to 2018.

Source: raw data obtained from https: //riverdata.mdba.gov.au/

Superimposed on the annual wetting and drying cycle outlined above cycle is the impact of substantially wetter and drier years. The chief factor here is the magnitude of the spring flooding (when it occurs) and its duration. Large scale flooding that inundates the Barmah-Millewa Forest is generally the result of significant rainfall within the greater catchment – that is, as opposed to rainfall at the site itself. Flooding varies in its frequency and duration, with very substantial downstream flows from Yarrawonga (in excess of 100,000 ML/day) recorded in 1964, 1970, 1973, 1974, 1975, 1981, 1990, 1992, 1993, 1996, 2010 and 2016 (Figure 41).

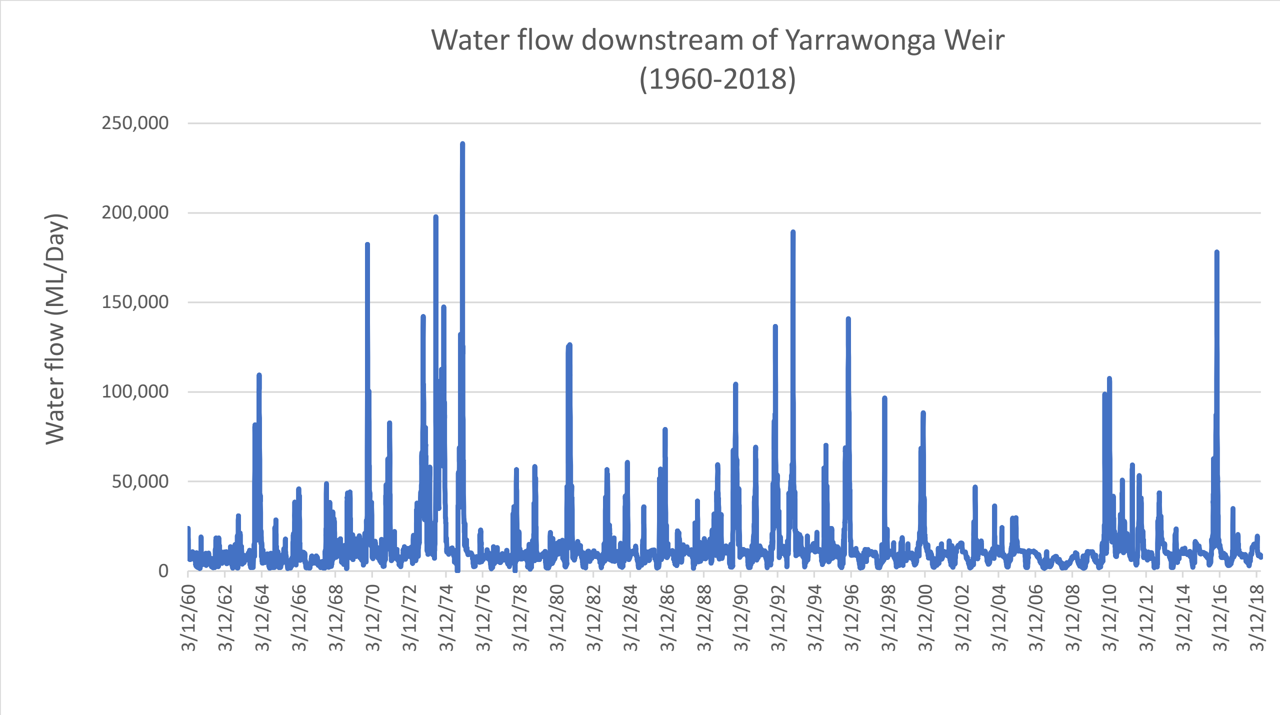

Figure 41 Average daily flow downstream of Yarrawonga from 1960 to 2018

Source: raw data obtained from https: //riverdata.mdba.gov.au/

As noted in Section 12.1.1, at flows between 10,400 and 16,000 ML/day, channels, swamps and other low-lying areas, including about 16 percent of the forest, are inundated. Larger floods of over 35,000 to 45,000 ML/day are required to inundate about 60 percent of the forest and it is only at flows of greater than 60,000 ML/day that inundation of most of the river red gum forest and substantial proportions of the black box communities occurs. The magnitude, frequency and duration of flows downstream from Yarrawonga for the period 1895 to 1984 was modelled by Leitch (1989, as cited in Hale and Butcher, 2011) and this work is reproduced in Table 18. These outcomes were then extrapolated to give the conditions below, which provide the minimum regimens required to maintain the ecological character of the Barmah-Millewa Forest.[65]

- Minimum of 10,400 ML/day no less than 7 years in any 10-year period, with a mean duration no less than 100 days and a maximum interval of 4 years between the flow threshold.
- Minimum of 16,000 ML/day no less than 7 years in any 10-year period, with a mean duration no less than 90 days and a maximum interval of 4 years between the flow threshold.
- Minimum of 35,000 ML/day no less than 10 years in any 20-year period, with a mean duration no less than 60 days and a maximum interval of 10 years between the flow threshold.
- Minimum of 60,000 ML/day no less than 12 years in any 50-year period, with a mean duration no less than 21 days and a maximum interval of 12 years between the flow threshold.

[65] Termed 'limit of acceptable change' (or LAC) in DSEWPC (2011)

Table 18 Modelled flood flow downstream of Yarrawonga for the period 1895 to 1984

Flow (ML/day)	Average frequency (%)	Average duration (months)	Longest dry period (months)	Inundation extent
10,400	78	3.6	45	All low lying areas and channels, floodplain marshes
16,000	70	3	45	Moira grass plains
35,000	57	2.1	116	Sixty percent of river red gum forest and 30% of river red gum woodland
60,000	25	0.7	201	Virtually all river red gum forest, a large proportion of river red gum woodland and some inundation of black box woodland

Source: Hale and Butcher (2011), citing adaptation from Leitch (1989) and Water Technology (2009)

The water quality in permanent and frequently-flooded wetlands on the floodplains can vary considerably between sites and over time and is greatly influenced by floodplain inundation (Hale and Butcher, 2011). Results of monitoring undertaken at Hut Lake and War Plain over a spring inundation event in 1993 (Figure 42) illustrate the variability in water quality in response to inundation and drawdown. Turbidity ranged from 20 NTU[66] to over 150 NTU, dropping following inundation and then rising as water levels receded. A boom in productivity (as indicated by the concentration of chlorophyll-α and DOC) occurred in the months following inundation as nutrients and carbon were released from the inundated floodplains and taken up by phytoplankton.

[66] Nephelometric Turbidity Units

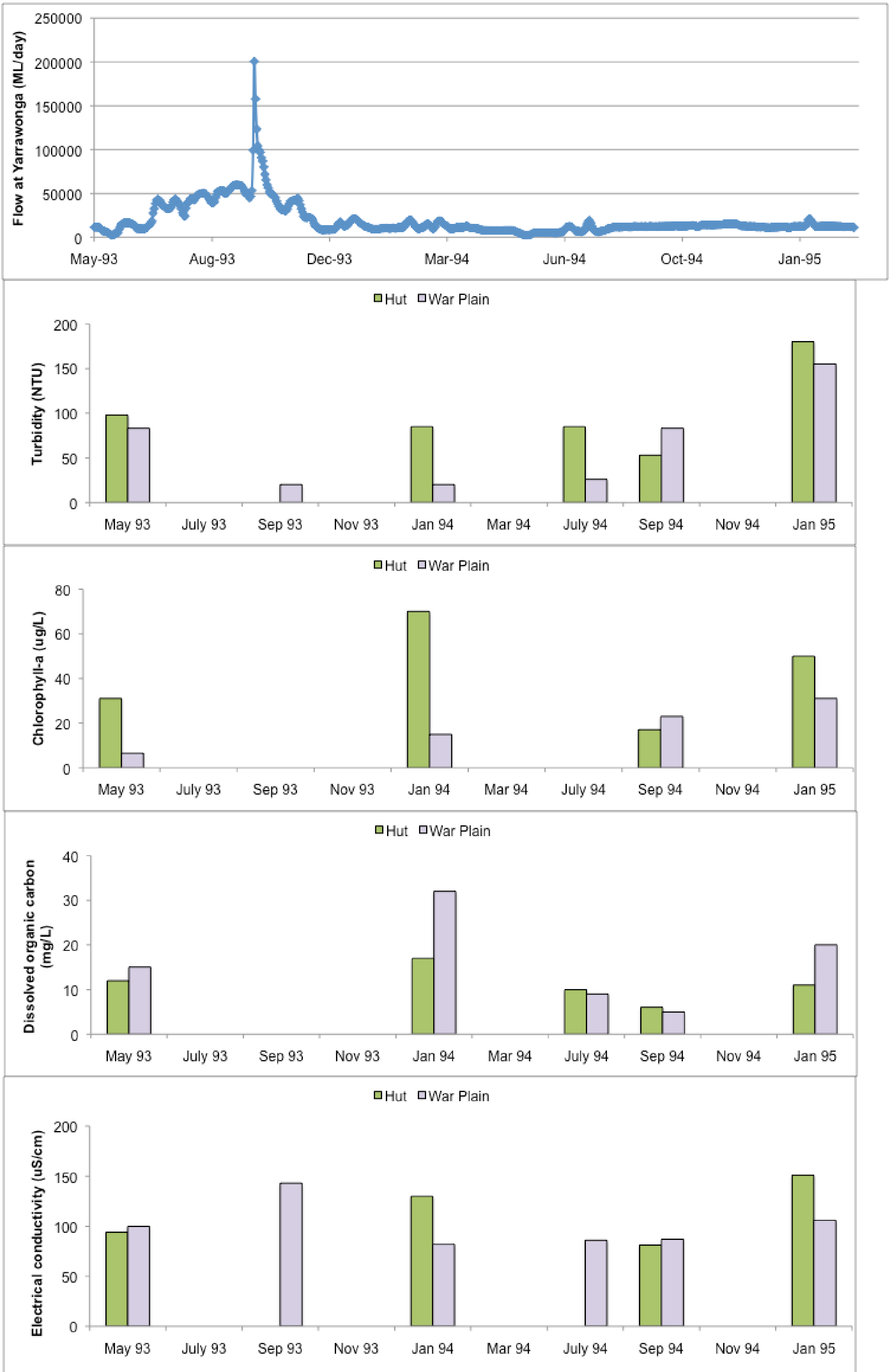

Figure 42 Water quality in Hut Lake and War Plain over a spring inundation event in 1993

Source: Hale and Butcher (2011)

The occurrence and abundance of most of native bird and fish species within the Barmah-Millewa Forest ecosystem is determined by the season and, during the wet season, by the magnitude and duration of flooding. The distribution and abundance of nesting waterbirds, in particular, varies both spatially and temporally in response to flooding. After flood periods, the Barmah Forest Ramsar site is one of Victoria's largest waterfowl breeding areas, supporting ducks (particularly black duck and maned duck), great cormorants, little black cormorants, little pied cormorants, white-faced herons, pacific herons and rufous night herons, yellow-billed spoonbills, crakes and rails. The greatest concentrations of nesting species occur in Barmah Lake, War Plain and Boals Deadwood (Hale and Butcher, 2011). The precise numbers of waterbirds that may gather within the broader Barmah-Millewa Forest during flood events is not well known. Chesterfield *et al.* (1984, as cited by Hale and Butcher, 2011) published an anecdotal report of 100,000 birds during the 1974 floods and there is a record of 55,000 waterbirds from 2005-06. In the opinion of local experts, total counts that include colonial nesting waterbirds as well as waterfowl and other solitary nesters would regularly number greater than 20,000 during floodplain inundation.[67] It is also likely that the Barmah-Millewa Forest may provide a refuge for some native birds (in particular, bush-birds) during times of drought.

The importance of the seasonal wet and dry cycle, and the periodic inundation of floodplains, is also important to the ecology of transitory populations of native fish. In this context, the Barmah-Millewa Forest can be seen as providing an ecosystem service rather than a permanent habitat. Some (for example, golden perch) spawn and recruit during periods of floodplain inundation while others (for example, Murray-Darling rainbowfish) prefer to spawn during lower-flow seasons when in-channel habitats are more suitable. There are also some native species (for example, Murray cod and the freshwater catfish) that spawn and recruit in both lower-flow and high-flow conditions. The number and variety of amphibian species observed within the Barmah-Millewa Forest, on the other hand, is strongly correlated with significant high-flow events and the inundation of floodplains.

12.1.4 Carp biomass and population dynamics within the Barmah-Millewa Forest

The NCCP project team modelling carp biomass density across Australia (Stuart *et al.*, 2019) reported a range in estimates for the Barmah-Millewa Forest between about 80 and 210 kg/ha. The carp biomass density for the Murray River channel passing through the Barmah Choke was approximately 115 and 170 kg/ha. The mapped output from the project, however, showed estimates correlating with inundation of the Victorian side of the forest, but very little on the New South Wales (northern) side of the river channel. It was also unclear whether the estimates were based on low, moderate or high-flow scenarios and the extent to which the dispersion of carp with inundation of wetlands and floodplains was assumed.

Citing Stuart and Jones (2002), Brown *et al.* (2005) noted that recent radio-telemetry studies of carp in the Barmah-Millewa Forest have shown that adult carp make many frequent lateral movements (tens of kilometres) between wetlands and the river channel as well as longitudinally (hundreds of kilometres) both upstream and downstream in the river channel. Aggregations of carp develop during the winter-spring in the Murray River close to access points for the Barmah-Millewa Forest wetlands. Spawning primarily takes place in permanent off-channel still-water

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[67] Personal communications with Keith Ward, Goulburn Broken Catchment Management Authority and Richard Loyn, Arthur Rylah Institute (as cited by Hale and Butcher, 2011)

bodies, including the low-lying, near-permanently-inundated Barmah Lake (Figure 44) (Stuart and Jones, 2006a). Low water levels and small-scale inundation of wetlands restrict the aggregation to these locations (Brown *et al.*, 2005). During raised water levels and sustained wetland flooding, larvae then move to adjacent shallow, recently-inundated warm floodplains. Brown *et al.* (2005) estimated that the average biomass of carp within the Barmah forest in January 2001, at the end of the prolonged flood peak, was approximately 22 kg/ha. When carp were confined to Moira Lake, however, the biomass increased to approximately 190 kg/ha. This is the same value as that provided by the NCCP project team (Stuart *et al.*, 2019) and is likely to be the source of the NCCP estimate.

As floodwaters continue to rise, the warm floodplains become increasingly important as a nursery habitat, and significant recruitment occurs in years when raised water levels provide access to these areas (Brown *et al.*, 2005; Stuart and Jones, 2006). Juveniles originating from the floodplains and wetlands of the Barmah-Millewa Forest move to the river channel when the floodwaters recede. There they drift downstream and, upon reaching a body size that can actively swim against the water current, the young-of-the-year carp swim upstream, dispersing into tributaries as well as the main stem of the Murray River. This upstream migration ceases when the water temperature drops in late summer or early autumn (Mallen-Cooper, 1999).

Stuart and Jones (2006a) observed that the temperature and conductivity of water in Barmah Lake and on the Barmah-Millewa floodplains was higher than in the downstream Murray River, while DO was lower. Noting that carp larvae, unlike the larvae of most native fish, appear to have a higher tolerance to leaf litter toxins and low DO, Stuart and Jones (2006a) postulated that these tolerances may contribute to the ability of carp to form abundant populations within the floodplain habitats alongside the Murray River. In the body of the Murray River, water temperature remained below 15C until mid-September 2000 and then fluctuated between about 15 and 18C until late October. Between late October and late November water temperature ranged between about 21 and 25C, then rose to about 27C at the end of November and remained at that point until early February 2001. By comparison, the water temperature in Barmah Lake was 17.4C in early to mid-September 2000 while the water temperature on the Barmah-Millewa floodplains (measured at Steamer Plain) was consistently warmer than the river body. These results mesh reasonably well with the data obtainable from the Victorian Department of Environment, Land, Water and Planning.[68] An excerpt of this was given in Figure 43, which displays average daily water temperature aggregated to give monthly averages. The two levels of aggregation effectively smooth out peak temperatures, which are likely to be substantially higher – in particular, in mid-to-late summer. Nevertheless, the water temperature recorded at Budgee Creek (at its intersection with Sand Ridge Track, within the Barmah-Millewa Forest) was consistently 1 to 2 degrees higher than the temperature within the river channel at Tocumwal during those months and seasons when Budgee Creek was heavily inundated.

[68] See: http: //data.water.vic.gov.au/

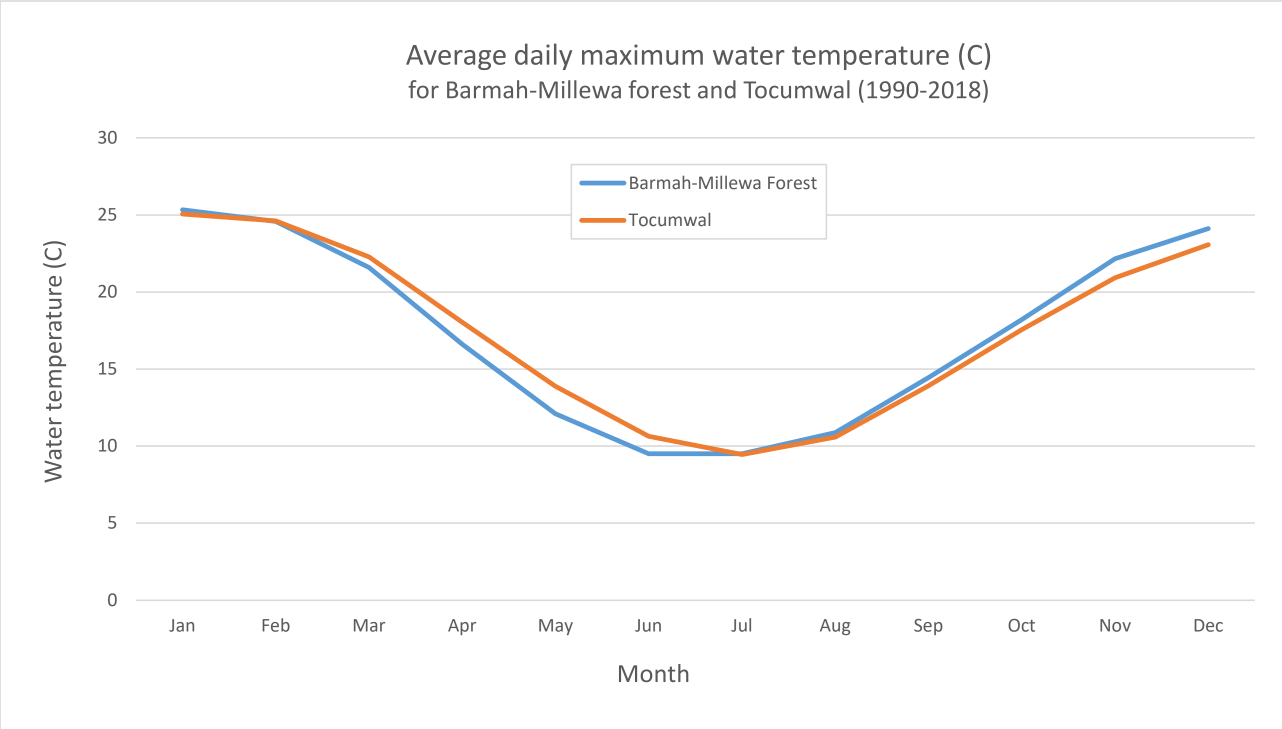

Figure 43 Water temperature in the Barmah-Millewa Forest and at Tocumwal (1990-2018)

Daily maximum water temperature (C) aggregated to a monthly average. Data observed for Budgee Creek within the Barmah-Millewa Forest, and for the Murray River channel at Tocumwal, 1990 to 2018.

Source: raw data obtained from http: //data.water.vic.gov.au/

The floodplains within the Barmah-Millewa Forest are highly-modified by grazing, by the clearing of natural vegetation and through altered flooding regimes. In particular, floods in the higher river red gum areas have almost been eliminated and between the infrequent large floods, there is an accumulation of natural toxins leaching from the leaf litter (Stuart and Jones, 2006a; citing Chesterfield, 1986). Additionally, many more river red gums have regenerated in the low-lying areas of the floodplains since the forest was heavily logged in the 19th century and because of the changed inundation regimes (Bren, 1988; Stuart and Jones, 2006a). Thus, without regular natural winter-spring flushing, there may be elevated concentrations of leaf litter toxins during summer flooding beyond the limits that that native fish can tolerate (Stuart and Jones, 2006a; citing Gehrke 1990). Carp larvae, unlike some native fish, appear to have a higher tolerance to leaf litter toxins and low DO, which may contribute to their ability to form abundant populations within the floodplain habitats alongside the Murray River (Stuart and Jones, 2006a).

Stuart and Jones (2006a) also followed the movements of juvenile carp hatched on the Barmah floodplains. Here they found that the migration of young-of-year carp into downstream river reaches included areas at a considerable distance from the floodplains (Figure 44). For example, in November many small carp appeared in unusually large numbers ascending the Torrumbarry Weir fish-way, about 144 km downstream from the floodplains. This event occurred 1 month after smaller (younger) carp had been observed drifting downstream from the Barmah floodplains. Citing Mallen-Cooper (1999), Stuart and Jones (2006a) noted that the later upstream dispersal of juveniles to reinforce populations above the Barmah-Millewa Forest supports the ‘source-sink’ model for spatial population dynamics within the Murray-Darling system (Part II: Section 4.2.2).

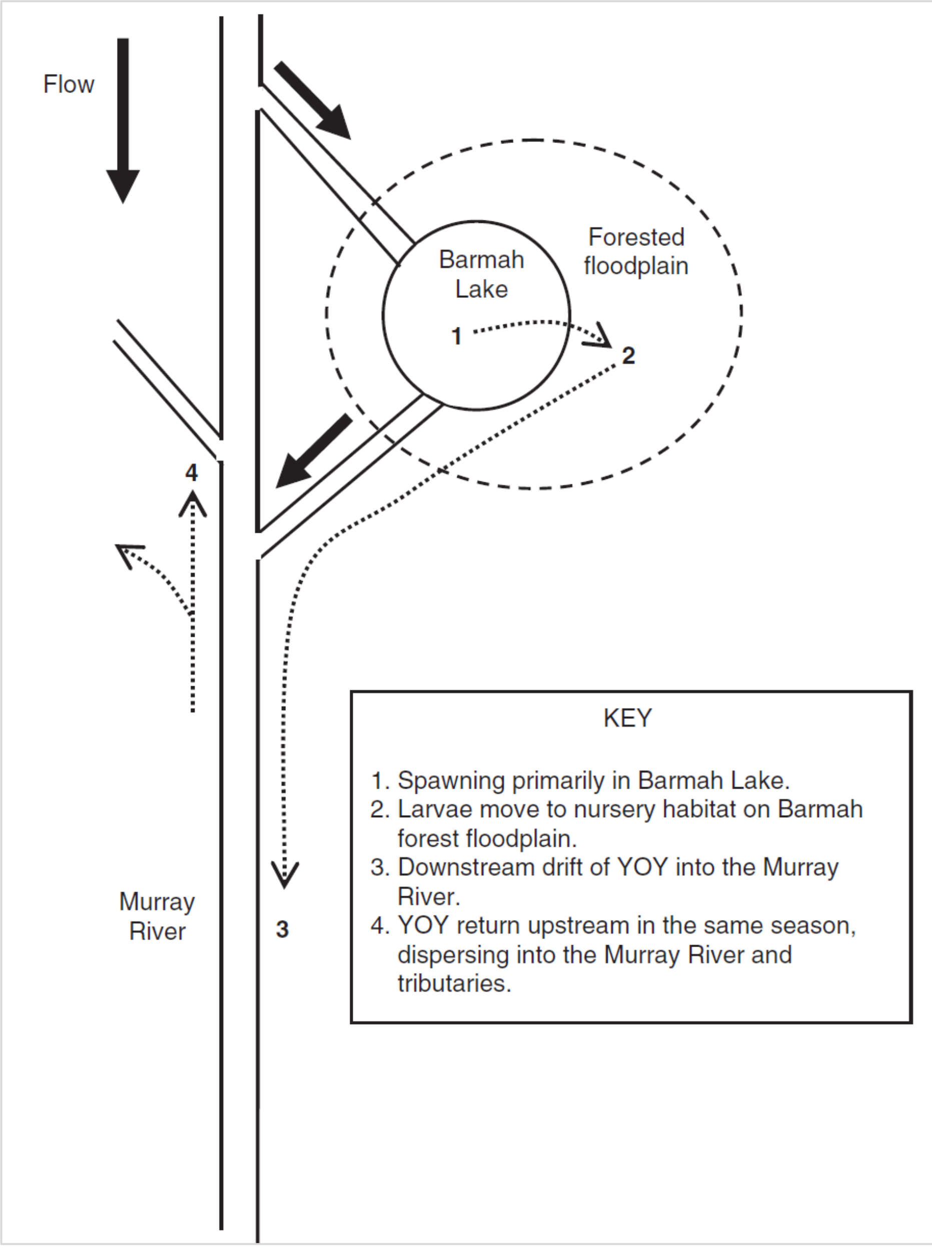

Figure 44 Movement model for young-of-year carp at the Barmah floodplain

Source: Stuart and Jones (2006a)

The spatial population dynamics of carp in the Barmah-Millewa Forest were also examined in the simulation modelling work of Koehn *et al.* (2016). These authors found that when carp were given access to either the Barmah-Moira lakes or the Barmah-Millewa floodplains, the average adult population size and the number of carp available for dispersal significantly increased. From this, Koehn *et al.* (2016) concluded that interaction between the Barmah-Millewa floodplains and the Barmah-Moira lakes would render the management of carp very difficult. They also concluded that the Barmah-Millewa floodplains and the Barmah-Moira lakes are able to produce large numbers of carp for dispersal to other areas of the Murray-Darling system. Annual high irrigation flows during conditions suitable for carp spawning that enable access to the wetlands, and are likely to result in higher numbers of carp in both the Barmah-Millewa area and more broadly.

12.1.5 CyHV-3 outbreak scenarios for the Barmah-Millewa Forest

An outbreak of CyHV-3 that might result in the substantive accumulation of carcasses, and consequent harm to natural assets, livestock or people, is most likely to follow from aggregations of carp and the close contact that occurs between carp during spawning (Part II: Section 5.3.4). The likely characteristics of an outbreak of CyHV-3 within an ephemeral wetland in high-flow and lower-flow seasons were discussed in Section 10.1. These two scenarios are particularly relevant to a wetland-floodplain ecosystem such as Barmah-Millewa Forest.

Under either scenario, aggregations of carp are likely to take place in the river channel and off-channel waters prior to the commencement of spawning. Transmission of CyHV-3 may occur at this time if the water temperature is sufficiently high (approximately 18C). Spawning in the Barmah-Millewa Forest is thought to occur primarily in the lentic and still waters immediately off the main channel – including within Barmah Lake. Transmission at these sites is likely, as the rise in water temperature that triggers spawning is also likely to be sufficient for the transmission of CyHV-3.

The outward visibility of the outbreak that ensues in this setting – and the potential for impacts on water quality – are likely to depend on the carp biomass density.

- In a high-flow season, adult and juvenile carp are likely to move from the off-channel waters with rising floodwaters and out onto the floodplains. Further spawning is then likely to occur on the floodplains. Once this movement occurs, the density of carp in any one location will be significantly reduced. Brown *et al.* (2005), for example, estimated that the average biomass of carp within the Barmah forest in January 2001, at the end of a prolonged flood peak, was as low as approximately 22 kg/ha. When carp were confined to Moira Lake, however, the biomass increased to approximately 190 kg/ha. The low biomass density on the floodplains will mean that opportunities for transmission are likely to be reduced, although individual spawning fish will come into close contact. The lower biomass density of carp on the inundated floodplains will also mean fewer accumulations of carp carcasses and less potential for carcass decomposition to affect water quality. As summer progresses, the temperature of the water on the floodplains is also likely to exceed in many places the maximum temperature for disease transmission (28C). Juvenile carp, however, are very susceptible to CyHV-3 and this may mean that further outbreaks occur as the floodwaters recede and juveniles drift back toward the deeper and cooler waters of the main channel.
- In a lower-flow season, adult and juvenile carp will remain in the off-channel waters and adjacent inundated wetlands as the floodplains will not be inundated. The temperature of these waters during summer may approach the upper limit for transmission, but is unlikely to exceed it substantially. Within the still waters, adult and juvenile carp will be held at a relatively high biomass density and an aggressive outbreak is likely. The high biomass density is also likely to mean that carcass accumulations have a higher potential to impact on water quality. Juvenile carp are particularly sensitive to CyHV-3, and their assemblage in large numbers in close proximity to adults could mean that an outbreak escalates rapidly. An aggressive outbreak of CyHV-3 is likely to be of a relatively short duration in this setting (approximately 3 to 6 weeks) and most carcasses will have decomposed to a large extent within approximately 2 weeks of death. Subsequent outbreaks are also possible.

Under either of these two flow scenarios juvenile carp will return to the river channel toward the end of summer and drift downstream until they reach a body size that enables them to swim against the current (Figure 44) (Section 12.1.4). At this point, the young carp move upstream within the channel and major tributaries. Throughout the period of downstream and upstream movement of young carp, aggregations may occur at certain points in the channel – such as fishways, or in places where water quality is highest or feed most plentiful. In these places, transmission of CyHV-3 may again occur and may lead to minor localised outbreaks. In autumn and winter, the water temperature is likely to drop below the threshold for active transmission (18C). Under these conditions, a low level of infection is possible. Carp infected at this time – and those that were infected earlier but have survived – may enter a latent state (Part II: Section 5.3.6). When the water warms, the disease will again become active and these fish may then seed virus into spawning aggregations in the following spring.

12.1.6 Exposure pathways and risks for the Barmah-Millewa Forest

A generic assessment for the nine identified exposure pathways was undertaken in Section 11. The discussion below interprets the outcomes from Section 11 in the context of the Barmah-Millewa Forest ecosystem and its natural assets.

(a) Exposure of native aquatic species to low DO

Likelihood of exposure: in seasons without substantial flooding, when juvenile and adult carp are confined to the off-channel waters and some wetlands, substantial outbreaks of CyHV-3 are likely. Here carcass accumulations might be significant and, with the concentration of carp in relatively still and stratified waters, it is also likely that significant deoxygenation would result. In parts of the ecosystem that are close to where carcasses are accumulating, DO may reach zero – in particular, overnight. Other parts of the wetland are likely to be less seriously affected. In years with substantial flooding, when adults and juveniles are on the inundated floodplains, it is more likely that outbreaks of CyHV-3 would be localised and that carcass accumulations would be minor and relatively dispersed. Under this scenario, pockets of low or zero DO are possible in relatively disconnected parts of the floodplains, although widespread hypoxia or anoxia is less likely.

Likely consequences of exposure: low DO will primarily affect fish and crustaceans. Air-breathing aquatic animals will not be directly affected by low DO. Indirectly, low DO may also impact on ecological communities and on the integrity of wetlands.

As noted above, 11 species of native fish (five large-bodied and six small-bodied) and the swamp yabby are believed to occupy the Barmah-Millewa Forest. An additional 11 species (one large-bodied and 10 small-bodied) have been reported in Barmah-Millewa and are likely to be present. The large-bodied native fish observed within the Barmah-Millewa Forest include Murray cod, trout cod, silver perch, golden perch and freshwater catfish. Murray cod are sensitive to low DO and, although the sensitivity of trout cod is not known, they are closely related to Murray cod and are likely to be physiologically similar. Trout cod are additionally exposed as their remaining extant sustainable population is focussed largely on the mid-Murray – from Yarrawonga through to the Barmah-Millewa Forest – and harm to the Barmah-Millewa Forest component of this population may have an impact on the ongoing resilience of the species. The population of silver perch is similarly constrained, although this species is likely to be more resilient to low DO than the two cod. Golden perch are physiologically adapted to lowland waterways with poorer water quality and are able to sustain (and to some extent adapt to) low DO. The broader population of golden perch is also more distributed geographically than silver perch or cod, and thus less vulnerable to local hypoxic events. Freshwater catfish are a benthic fish and are considered the most resilient of the large-bodied native fish to low DO.

Overall, trout cod populations are arguably the most exposed of the large-bodied native fish to hypoxic events. Trout cod have a high site fidelity and do not generally undertake migrations for spawning. This means that trout cod identified within the Barmah-Millewa Forest are likely to reside there permanently and may be less likely to move away from an area of poor water quality. Trout cod and other large-bodied native fish identified within Barmah-Millewa Forest spawn in late spring or early summer. Hypoxic events that occur during this time may thus result in a lower recruitment, although the impact of this on the sustainability of local populations may be lower for the long-lived species such as Murray cod and trout cod than for silver perch and freshwater catfish.

The small-bodied native fish (including both the wetland or floodplain specialists and the foraging generalists) are relatively more tolerant to low DO than are most of the larger fish – with the exception of the benthic freshwater catfish. Although there are differences between species, most will begin to use ASR at about 2.5 mg/L and may survive for some time while the oxygen concentration remains above about 1 mg/L. This notwithstanding, they are also generally short-lived and a disturbance to recruitment could have a significant impact on the sustainability of a local population. They may also be more exposed to precipitous drops in DO within shallow wetlands and may be less able to move substantial distances to evade poor water quality. Barmah-Millewa Forest has an abundance of the swamp yabby (*Cherax* sp. *C*) a burrowing crayfish, which is likely to become stressed and emerge when DO falls below approximately 2 mg/L. The emergence of crayfish and yabbies predisposes them to predation from birds and other animals, as well as to desiccation. This particular species is not listed under the EPBC Act, although the Character Description for the Barmah Forest Ramsar site proposed that this ecosystem may contain more than 1 percent of the broader population.

The Barmah-Millewa Forest supports a range of ecological communities, including river red gum forests and woodlands and floodplain marshes. The impact of low DO on these communities is most likely to be realised during high-flow seasons, when these areas are inundated. Although widespread low DO is less probable under the high-flow scenario, pockets of affected water may develop. Where this occurs, small communities – or smaller parts of a broader community – may experience the stress or death of fish and crustaceans. This affect is likely to be short-term (1 to several weeks), and is unlikely to affect the quality of the community when the floodplains are next inundated. The Barmah-Millewa Forest also supports a range of riparian and wetland communities, which may be permanent or semi-permanent. The impact of low DO on these communities may be more marked in the sense that they are not transient, and will not be regenerated with each inundation event. A low DO event could also potentially occur in many of these communities during any season – that is, it would not be dependent on inundation of the floodplains.

Summary of risks: the Barmah-Millewa Forest includes a mixture of permanent or semi-permanent off-channel waterways and wetlands, and periodically-inundated floodplain forests and woodlands. The likelihood and likely consequences of a low DO event in this setting will follow in general terms the discussion in Section 11.1. Environmental assets particularly at-risk include the threatened Murray cod and trout cod, and to a lesser extent the threatened silver perch. A range of small-bodied native fish may also be at risk, although none of the species identified from surveys within the Barmah Forest Ramsar site are listed under the EPBC Act. In addition to these is the swamp yabby, which is locally abundant but may represent an important fragment of the broader population of the species. The floodplain forest, woodland and marshland communities would be affected if hypoxia was to follow from an outbreak of CyHV-3 during a high-flow season, although this scenario is less likely given the spatial distribution (low density) of carp when the broader Barmah-Millewa Forest ecosystem is inundated. Permanent and semi-permanent riparian and wetland communities are likely to be more exposed, given that hypoxia could affect these during most seasons.

Mitigation of risks: the mitigation of risks associated with low DO will focus largely on the timely removal of carp carcasses. The extent to which this will be necessary, and practicable, will vary amongst settings although the overarching goal will be to prevent the DO within a given waterbody falling below about 3 to 4 mg/L or to enable it to return to that level. In high-flow seasons, the likelihood of widespread areas of low DO is less. If such an event were to occur, however, access to the inundated floodplains would be difficult. Conversely, in lower-flow seasons when low DO is more probable access (overland or by water) to areas where carp carcasses have accumulated is likely to be less problematic. Consideration may be given under some circumstances to environmental watering, either to supplement the removal of carcasses or as an alternative. As explained elsewhere (Section 11), however, this option carries its own risks and complications and should only be undertaken after expert consultation.

(b) Exposure of native species, livestock and humans to cyanobacterial blooms

Likelihood of exposure: a substantial outbreak in a year without significant flooding is likely to result in carcass accumulation within still off-channel waters and permanent wetlands, and it is possible that this would precipitate or sustain a widespread cyanobacterial bloom. Under this scenario, the bloom might directly affect most aquatic species within the extant wetland and indirectly affect a wide range of terrestrial species – including amphibians and nesting waterbirds. In high-flow seasons, cyanobacteria will be dispersed throughout the inundated floodplains. The DOC is also likely to be high following significant flood events – principally as a result of leachate from forest leaves. As the flood waters recede, pools of standing water are likely to warm significantly, and these conditions may predispose the wetland to widespread cyanobacterial blooms. Such events, however, are a component of the existing flood cycle within the Barmah-Millewa Forest and are external to the matter of CyHV-3. As the generalised accumulation of large numbers of carcasses is less likely when the floodplains are inundated (because carp themselves are dispersed), it is also less likely that this will precipitate a widespread cyanobacterial bloom. Under either a high-flow or a lower-flow scenario, a widespread cyanobacterial bloom may cause harm through either the direct effect of cyanotoxins (if the strain(s) of cyanobacteria involved are cyanotoxic) or indirectly when the bloom dies off and decomposes, raising the BOD and leading to a precipitous fall in DO.

Likely consequences of exposure: it was concluded within the discussion of this exposure pathway (Section 11.2) that the risks associated with widespread cyanobacterial blooms for large- and small-bodied native fish and crustaceans will mirror those associated with low DO. In addition to these, however, are risks to waterbirds, frogs and turtles. The risks posed by widespread cyanobacterial blooms to ecological communities and wetlands are also different to those posed by low DO.

During high-flow seasons, when the floodplains are inundated, Barmah-Millewa Forest provides ecological services to more than 60 species of waterbird across the nine categories identified in Part II: Section 3.2. Although the risk drivers for each these categories will be the same for birds in the Barmah-Millewa Forest as discussed in a generic sense in the pathway description for cyanobacterial blooms (Section 11.2) the particular birds at highest risk will be those that continue to nest in the Barmah-Millewa Forest during lower-flow seasons when the floodplains are not inundated. In general terms, these will include the waterfowl, the Gruiformes (cranes rails, crakes, coots, moorhens, swamphens and waterhens), the kingfishers, the songbirds and the bush-birds. These birds are not colonial nesters and are not migratory. As a further generalisation, they are also species that may be able to avoid areas of obviously poor quality (for example, malodorous) water. Both waterfowl and Gruiformes closely interact with water, and those that do remain in waterways affected by cyanobacterial blooms may be exposed to cyanotoxins.

Nine species of frog have been recorded within the Barmah-Millewa Forest, and while none of these are listed under the EPBC Act the sustainability of most frog populations is considered to be fragile. The extent of this population is, however, dynamic with significantly higher numbers expected during high-flow seasons when the floodplains are inundated. If a widespread outbreak of cyanotoxic cyanobacteria was to be precipitated during a high-flow season by the decomposition of carp carcasses, then significant numbers of frogs are likely to be exposed. Three species of freshwater turtle are known to inhabit the Barmah-Millewa Forest, and each of these would be exposed to cyanotoxin if a widespread bloom was to occur in either a high-flow or a lower-flow season.

The floodplain forest, woodland and marshland communities would be affected if a widespread bloom was precipitated by the accumulation of carp carcasses during a high-flow season. Permanent and semi-permanent riparian and wetland communities are likely to be more exposed, given that a widespread bloom could be precipitated within these during most seasons. In any affected community, the impacts of a widespread cyanobacterial bloom may include: the direct effect of cyanotoxin (if the strain involved is cyanotoxic) on all faunal species in contact with water, consuming water or consuming biota that is itself affected with cyanotoxins; the effect of the precipitous drop in DO that will inevitably follow from collapse of the bloom; the decline in water quality that will follow from the death and decomposition of cyanobacteria and aquatic biota; and the ecosystem-wide impacts of these processes on food webs.

Livestock and humans are also susceptible to cyanotoxins, and the risk to both were considered in a generic sense within the description of this exposure pathway (Section 11.2). However, the southern (Victorian) section of the Barmah-Millewa Forest lies within the Barmah National Park while the northern (New South Wales) section forms the Murray Valley National Park. Together, Barmah-Millewa Forest spans 70,000 ha (170,000 acres) of national park. None of this land is accessible to livestock species (other than feral horses and pigs), and its waters are not drawn directly for livestock use. Likewise, although recreational fishing is permitted under permit it is very unlikely that drinking water, fish or crustaceans would be taken for human consumption from an area where mass fish mortalities or destructive cyanobacterial blooms were occurring. This likelihood would be further ameliorated through signage. Caution would extend to the use of contaminated waters for swimming and other recreation, although the malodorous nature of a fish kill is likely to be a sufficient deterrent. On balance there are no realistic scenarios under which humans or farmed (c.f. feral) livestock would be significantly exposed to harm as a result of an outbreak of CyHV-3 within the Barmah-Millewa Forest.

Summary of risks: although cyanobacterial blooms are not uncommon in ephemeral wetlands such as the Barmah-Millewa Forest, it is more likely that a widespread bloom would be precipitated during a lower-flow season when the density of carp carcasses is likely to be significantly higher. In this setting, however, many of highest-risk colonial-nesting waterbirds are unlikely to be present – or present in significantly lower numbers than when the floodplains are inundated. Frogs are also likely to be present in lower numbers. During a high-flow season, when the floodplains are inundated, the density of carp is may be as low as approximately 20 kg/ha. At this very low density, some accumulation of carcasses may result from drift from winds or current, but the effects of this on the risk of cyanobacterial blooms are likely to be quite local.

Mitigation of risks: mitigation options for the risk of a widespread cyanobacterial bloom arising from the accumulation of carp carcasses will be the same as those for mitigating the risks associated with low DO – that is, the timely removal of carp carcasses and environmental watering. Again, however, the option of environmental watering carries the caveat that the spectrum of possible follow-on effects be given careful expert consideration.

(c) Exposure of native species, livestock and humans to the pathogens associated with decomposing fish carcasses

Likelihood of exposure: little information is available about the pathogens that may proliferate in the event of a fish kill, and their possible impacts on native species, livestock or humans. This deficit was discussed in the generic description of this pathway (Section 11.3) and in the underpinning review of the literature (Part II: Section 6.3.4). That aside, the scenario of concern is again an aggressive outbreak of CyHV-3 that has been constrained to the shallow and still off-channel waters under a lower-flow scenario. Here it is possible that carp gut flora, spoilage organisms, *E. coli*, *Pseudomonas spp*, *Aeromonas spp* and other opportunistic microorganisms might proliferate and, in doing so, may present a hazard to native aquatic and terrestrial species. If the floodplains are inundated under a high-flow scenario, then it is more likely that microorganisms associated with individual carcasses will be dispersed and, with exposure to ultraviolet light within shallow waters, aquatic microorganisms and other factors, will quickly disappear.

Likely consequences of exposure: it was explained within the generic discussion of this exposure pathway (Section 11.3) that although there is substantial uncertainty (and likely variation) around the pathogens that may be released following a substantial fish kill, and their ability to survive in an aquatic environment and cause harm to aquatic or terrestrial biota, it is reasonable to expect that the relative exposure of communities, functional groups and species will be similar to that obtained for widespread cyanobacterial blooms – albeit with a lower likelihood of realising the identified ecological endpoints. Balancing this, it was also pointed out that the experience obtained from field observations of Australian freshwater waterways is that while substantial fish kills are not uncommon, they have not to-date been linked to subsequent epidemics of waterborne pathogens. Some waterborne microorganisms may affect people and livestock, although people are likely to be protected to a large extent by the innate repulsiveness of a waterbody that contains the detritus of a significant outbreak. Livestock, however, may have less ability to avoid affected drinking water if there are limited or no alternatives. The risks to ecological communities are understandably highest for wetlands and marshlands, although an outbreak affecting a waterbody within or proximal to a forest and woodland, or grassland, ecological community, may also impact on species that utilise that water.

Summary of risks: the likelihood that microorganisms associated with the decomposition of carp carcasses would proliferate following a carp die-off in the Barmah-Millewa Forest and become a threat to native species or communities, or to livestock or people, is higher during a lower-flow season when carp are more concentrated within off-channel waters and permanently-inundated wetlands. During high-flow seasons, when the floodplains are inundated, both carp and their decomposition products will be dispersed and microorganisms will have greater exposure to UV light. Accepting this, the profile of risks associated with waterborne microorganisms for individual species, functional groups, communities, livestock and humans will be reasonably similar to those associated with widespread cyanobacterial blooms – albeit with lower likelihoods.

Mitigation of risks: as for both low DO and widespread cyanobacterial blooms, mitigation of risks associated with waterborne microorganisms will focus on the timely removal of carp carcasses and consideration of environmental watering.

(d) Exposure of native piscivorous species to the removal of a dominant and stable food source

Likelihood of exposure: in high-flow years when the floodplains are inundated, the Barmah-Millewa Forest provides nesting sites for many piscivorous bird fish species, and although undocumented it is likely that these make use of juvenile carp as a stable food source for nesting chicks. An outbreak of CyHV-3 in a high-flow season may be substantial if sufficient transmission occurred during the pre-spawning aggregation and before the adult carp left the off-channel waters. The outbreak may then be sustained through the individual contact that occurs on the floodplains during spawning. An outbreak unfolding in this way may have an impact on the overall spawning success of carp and the survivability of highly-susceptible juveniles. As the summer progresses, the temperature of the water on the floodplain may exceed the threshold for transmission for CyHV-3, and this may slow the outbreak and reduce the exposure of juvenile carp. Once the floodwaters begin to recede, however, juveniles will drift back toward the channels and anabranches where the water temperature is likely to be lower. In this setting, the outbreak may be revived amongst both juvenile and adult carp and this may result in a further decline in the availability of food for nesting waterbirds. This may result in a sudden and precipitous drop in the availability of a staple food source during a time when the demand for food from large numbers of nesting waterbirds is at its peak.

During a lower-flow season, the relative containment of carp in off-channel waters and some permanently-inundated wetlands means that an aggressive outbreak of CyHV-3 is more likely. The highly-susceptible juvenile carp will also be contained, and thus exposed to the virus. This means that the number of juvenile carp available to nesting waterbirds is likely to be significantly reduced. In a lower-flow season, however, many of the colonial-nesting piscivorous waterbirds will inhabit instead the marshes at the fringes of permanent lakes and irrigation reservoirs such as Kow Swamp. Piscivorous birds that are likely to remain within the Barmah-Millewa Forest during a lower-flow season may include some waterfowl, the Gruiformes (cranes rails, crakes, coots, moorhens, swamphens and waterhens) and the kingfishers. These birds are not colonial nesters and are not migratory. While their diet may include small fish, all of these birds also consume other prey (such as crustaceans, frogs or insects) and some are omnivorous.

On balance this suggests that while an outwardly aggressive outbreak resulting in the sudden removal of carp is more likely in a lower-flow season, this would be less likely to result in the widespread exposure of the more vulnerable colonial-nesting and strictly piscivorous waterbird species. These species might be exposed in the event of an aggressive outbreak during a high-flow season, when the floodplains are inundated, although the dispersion of carp under this scenario means that a widespread and aggressive outbreak will be predicated on substantive transmission during pre-spawning aggregation or before the adult carp moved out onto the floodplains.

Likely consequences of exposure: if colonial-nesting and strictly piscivorous waterbird species within the Barmah-Millewa Forest are exposed to the sudden removal of stable and dominant food source (juvenile carp) then they may be able to compensate for this by feeding on alternative waterbodies in the vicinity (for example, Kow Swamp) or by switching to alternative food sources – including frogs, crustaceans, small-bodied native fish and juvenile large-bodied native fish (prey-switching). If these alternatives cannot be achieved adequately, then there may be an impact on the survival rate of chicks and this may in turn impact on the resilience of some populations of piscivorous waterbird species.

Likewise, the impact of the sudden loss of a stable food source (juvenile carp) on ecological communities might be marked in the situation where exposure occurred during a high-flow season when a maximal number and diversity of waterbirds were nesting. The chicks of birds that are unable to source food from another waterbody, or by switching to another prey species, are likely to become stressed and may die. Extrapolated across many colonies of nesting waterbird species, this would have a very marked effect on wetland communities within the Barmah-Millewa Forest ecosystem.

Summary of risks: the food-web effects of freshwater fish kills in Australia and elsewhere are not well understood. As for other pathways, a protective effect is likely to follow from the outbreak scenarios for the Barmah-Millewa Forest, in the sense that an aggressive outbreak resulting in the sudden death of a high proportion of the carp population (in particular, juvenile carp) is more likely to be associated with a lower-flow season when relatively fewer colonial-nesting piscivorous waterbirds will be present. Additionally, those waterbirds that are more likely to be nesting within the Barmah-Millewa Forest during a lower-flow season tend not to be strictly piscivorous – and are less likely to rely absolutely on juvenile carp. A further protective effect may arise from the proximity of alternative waterbodies (such as Kow Swamp and Gunbower), although it is possible under some release scenarios that widespread outbreaks of CyHV-3 would be occurring concurrently within all waterbodies in that part of the Murray-Darling Basin. Overall, while the risks associated with the sudden removal of juvenile carp from Barmah-Millewa cannot be disregarded, they are unlikely to be as significant at this location as at some others where the protective effects outlined above are less readily available.

Mitigation of risks: the key treatment option identified for this pathway relates to the strategy underpinning the release of CyHV-3. It may be possible, for example, to release the virus into the Barmah-Millewa Forest ecosystem a year ahead of releasing it into the Kow Swamp or Gunbower ecosystems, thus minimising the likelihood of concurrent outbreaks and enabling highly-mobile piscivorous species an opportunity to source food from other waterbodies. It may also be possible to release the virus into the Barmah-Millewa Forest during a lower-flow season, when the exposure of colonial-nesting piscivorous waterbirds is likely to be lower.

(e) Exposure of native species to increased predation as a result of prey-switching

Likelihood of exposure: the exposure of small-bodied and juvenile large-bodied native fish, crustaceans and turtle eggs and hatchlings to prey-switching is conditional on the exposure of piscivorous species to the sudden removal of a stable food source – as outlined above. The conditions that might predispose this within the Barmah-Millewa Forest are also the same for the two pathways, and the likelihood that these conditions might be realised is (again) constrained by the fact that aggressive outbreaks of CyHV-3 are more likely in lower-flow seasons when there are also fewer nesting waterbirds.

Likely consequences of exposure: the native species potentially at risk as a result of prey-switching include the juveniles of large-bodied fish, small-bodied fish, crustaceans, frogs, and turtle eggs and hatchlings (Section 11.5).

Trout cod juveniles may be particularly at risk, as this species may spawn on the floodplains in the event of a high-flow season. Murray cod juveniles could also be at risk in some settings, although these will tend to remain within river channels and major anabranches. Silver perch prefer to spawn in faster-flowing parts of the river system, and over gravel or rock substrate, and their juveniles will also be relatively less exposed. Golden perch juveniles may be exposed, although the broader geographic distribution of this species renders it inherently more resilient. Freshwater catfish spawn within an elaborate nest, and juveniles could be exposed in some shallower parts of the Barmah-Millewa Forest ecosystem. All six of the small-bodied native fish known to be present in the Barmah-Millewa ecosystem are likely to be exposed, and although none are listed under the EPBC Act, the impact on some fragmented populations could be substantial. The swamp yabby and all nine species of frog are also likely to be quite exposed, as these species are abundant within the ecosystem and pre-exist as components of the diet of many carnivorous (but not strictly piscivorous) waterbirds. The extent to which the eggs or hatchlings of the three species of freshwater turtle might be targeted is unknown, although it is likely that many carnivorous waterbirds would opportunistically feed on either or both if a large proportion of carp juveniles was removed.

Summary of risks: the risks associated with this pathway are conditional on the realisation of the prior pathway – that is, stress to populations of (in particular) colonial-nesting piscivorous waterbirds as a result of the removal of juvenile carp. This event in itself is not likely in the Barmah-Millewa Forest ecosystem, given that it would require an aggressive outbreak pf CyHV-3 to occur during a high-flow season when the floodplains are inundated. Even then, waterbirds may be able to source juvenile carp from other waterbodies, such as Kow Swamp or Gunbower. Failing this, however, piscivorous waterbirds are likely to switch to other prey species, or increase their dependence on other prey species. Of these, juvenile trout cod, the small-bodied native fish, frogs and swamp yabbies are likely to be most exposed.

Mitigation of risks: the key means by which to support local populations of alternative prey species will be through strategic restocking. This is not likely to be as effective in an open and dynamic ecosystem, such as the Barmah-Millewa Forest, as it might be in a more contained and predictable ecosystem, such as one of the irrigation reservoirs. It is also important to reiterate that this pathway is conditional on the stress of colonial-nesting waterbirds through the sudden removal of carp juveniles, as measures that might further reduce the likelihood of that pathway would subsequently protect native species against prey-switching.

(f) Exposure of native species, livestock and humans to an outbreak of botulism

Likelihood of exposure: an outbreak of type C (or C/D mosaic) botulism might follow directly from the death of carp carrying *C. botulinum* spores, or from the death of waterbirds or fish as a result of low DO, a widespread cyanobacterial bloom or any of the other pathways linked to an outbreak of CyHV-3. Type C (or C/D mosaic) botulism is generally characterised by the establishment of the carcass-maggot cycle (Part II: Section 7.6.1), and it is through this cycle that germinated spores and toxin associated with a small number of individual carcasses can be extrapolated to many thousands of carcasses across an ecosystem. To be initiated and maintained, the carcass-maggot cycle depends on a high density of susceptible species – a high proportion of which must be insectivorous, or otherwise exposed to the toxin through prey or through drinking water. Aggregations of waterbirds are ideal for this scenario, and most outbreaks of type C (or C/D mosaic) botulism in Australia have been associated with waterbird breeding and nesting events in wetlands or at the edges of waterholes, lagoons, billabongs or shallow lakes. Notwithstanding the importance of the carcass-maggot cycle, an outbreak of type C (or C/D mosaic) botulism could also be initiated by a fall in DO and the subsequent germination of (anaerobic) *C. botulinum* spores within individual carcasses or in the environment. A fall in DO might in turn be caused by the decomposition of carp carcasses, or by the death and decomposition of a cyanobacterial bloom.

An outbreak of type C (or C/D mosaic) botulism in the Barmah-Millewa Forest ecosystem arising from the carcass-maggot cycle is more likely to be associated with the inundation of the floodplains during a high-flow season, and the gathering of large numbers of waterbirds. The outbreak would be initiated on a probabilistic basis and, once initiated, would propagate principally within the assemblage of waterbirds. An outbreak of type C (or C/D mosaic) botulism triggered by a fall in DO is more likely to occur during a lower-flow season, when the accumulation of carp carcasses is likely to be more marked and is more likely to result in either a direct effect on DO or the initiation of a widespread cyanobacterial bloom. As a final point, an outbreak of type C (or C/D mosaic) botulism could be triggered by the death of large numbers of animals other than carp. This might again follow from either low DO or a cyanobacterial bloom – or from a combination of the two.

Likely consequences of exposure: the impacts of an outbreak of type C (or C/D mosaic) botulism within the Barmah-Millewa Forest would be strongly dependent on whether the floodplains were inundated, and colonial-nesting waterbirds present in large numbers. Under this scenario, most terrestrial and aquatic species would be at risk, although the exposure of birds and other biota that feed on aquatic and other insects is likely to be higher. This would include, in particular, the waterfowl, large waders and Gruiformes. Insectivorous songbirds and bush-birds would also be at risk, as would frogs and turtles. Neither the New South Wales nor Victorian components of the Barmah-Millewa Forest ecosystem, however, are accessible to farmed livestock (c.f. feral horses and pigs). Humans are not susceptible to type C (or C/D mosaic) botulinum toxin.

Summary of risks: the reasons why outbreaks of type C (or C/D mosaic) botulism in wildlife occur in some settings, and not in others with an ostensibly similar profile of risk factors, are not well understood but are likely to reflect the strongly probabilistic nature of outbreak initiation and expansion. A range of pathways could be relevant to the Barmah-Millewa Forest ecosystem, including the carcass-maggot cycle and the germination of environmental spores as a result of an hypoxic or anoxic event. Accepting this, a widespread outbreak of type C (or C/D mosaic) botulism within the Barmah-Millewa Forest ecosystem during either a high-flow season when the floodplains are inundated, or a lower-flow season, is possible, although the likely consequences of the outbreak would be significantly greater during a high-flow season.

Mitigation of risks: prevention of an outbreak of type C (or C/D mosaic) botulism will rest on the timely removal of carp and other carcasses. The practicality of this is likely to be limited during a high-flow season when the floodplains are inundated and, in that setting, priority should be given to parts of the Barmah-Millewa Forest in which the number and diversity of colonial-nesting and other waterbirds is highest – including Barmah Lake, War Plain and Boals Deadwood (Hale and Butcher, 2011). This may help to minimise the impacts of an outbreak of botulism, although may also help to constrain the probabilistic element of its initiation. In some situations, consideration might also be given to environmental watering as a means by which to disperse and dilute carcasses or help to remove a cyanobacterial bloom from a particularly high-value part of the ecosystem. The impacts of environmental watering are complex however, and this should only be contemplated following the advice of experts.

12.2 Case study 2: Chowilla Floodplain

12.2.1 Physical description of the Chowilla Floodplain case study site

The Chowilla Floodplain contains many natural attributes, including diverse vegetation communities and the largest remaining natural river red gum forests in the lower Murray River.[69] The diversity of natural habitats within the Chowilla Floodplain is supported by over 100 km of anabranch creeks that form permanent and temporary water bodies. During enhanced river flows, the river and creeks in this system spread out to provide water to aquatic habitats, including billabongs, backwaters, temporary wetlands and lakes. The high diversity of terrestrial and aquatic habitats within the Chowilla Floodplain support populations of rare, endangered and nationally-threatened species and it is recognised as an area of significant environmental and cultural importance.

An outline of the physical characteristics and hydrology of the Chowilla Floodplain is given in the Murray-Darling Basin Authority's Chowilla Floodplain Environmental Water Management Plan (MDBA, 2012a) and much of the discussion that follows has been adapted from that source.

The Chowilla Floodplain forms part of the Chowilla and Lindsay-Wallpolla Islands Murray River Icon Site[70], which covers a total area of 43,856 ha. Six Icon Sites were recruited to The Living Murray program[71] which focusses on maintaining the health of these places on account of their

[69] NB: references to support these introductory statements to follow

[70] See: http: //www.mdba.gov.au/discover-basin/environment/significant-environmental-sites/icon-sites-along-river-murray

[71] See: http: //www.mdba.gov.au/publications/brochure/Living-murray-program

national and international environmental and cultural significance. The Chowilla Icon Site comprises four main components: Chowilla Floodplain, and the Lindsay, Mulcra and Wallpolla islands (Figure 45).

The Chowilla Floodplain straddles the South Australia / New South Wales border and covers a total area of 17,781 ha, 74 percent of which lies in South Australia with the remaining 26 percent in New South Wales. The New South Wales component is generally known as Kulcurna. The boundary of the Chowilla Floodplain (Figure 46) is defined by the 1956 flood extent, and the game reserve property boundary immediately to the west of the Chowilla Homestead. The Chowilla Floodplain includes part of the Riverland Wetland Complex Ramsar site.[72]

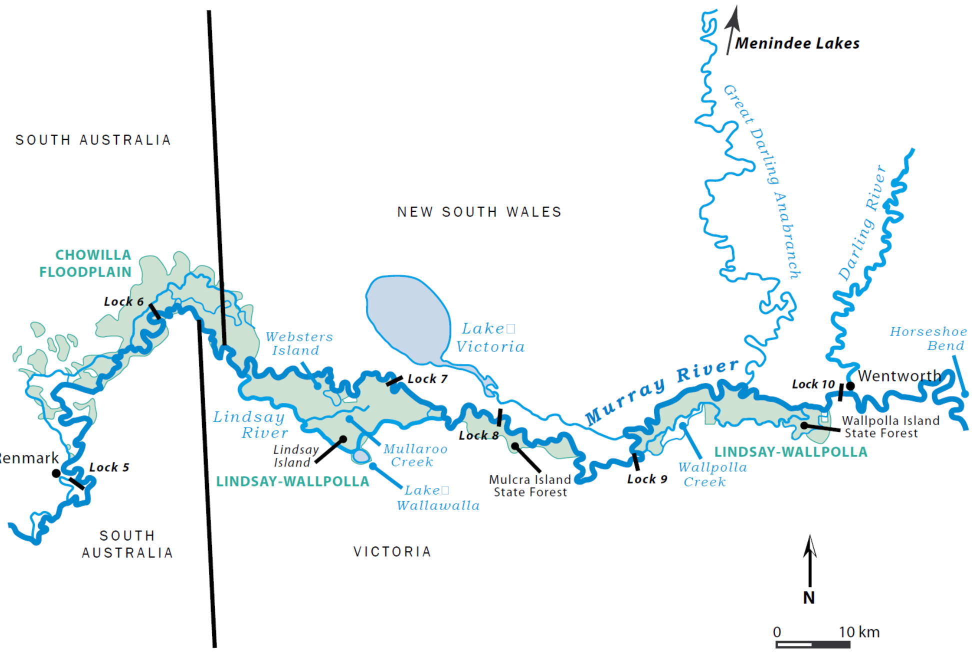

Figure 45 The Chowilla Floodplain and Lindsay-Wallpolla Islands Murray River Icon Site

Source: MDBA (2012a)

[72] See: http: //www.environment.gov.au/cgi-bin/wetlands/ramsardetails.pl?refcode=29

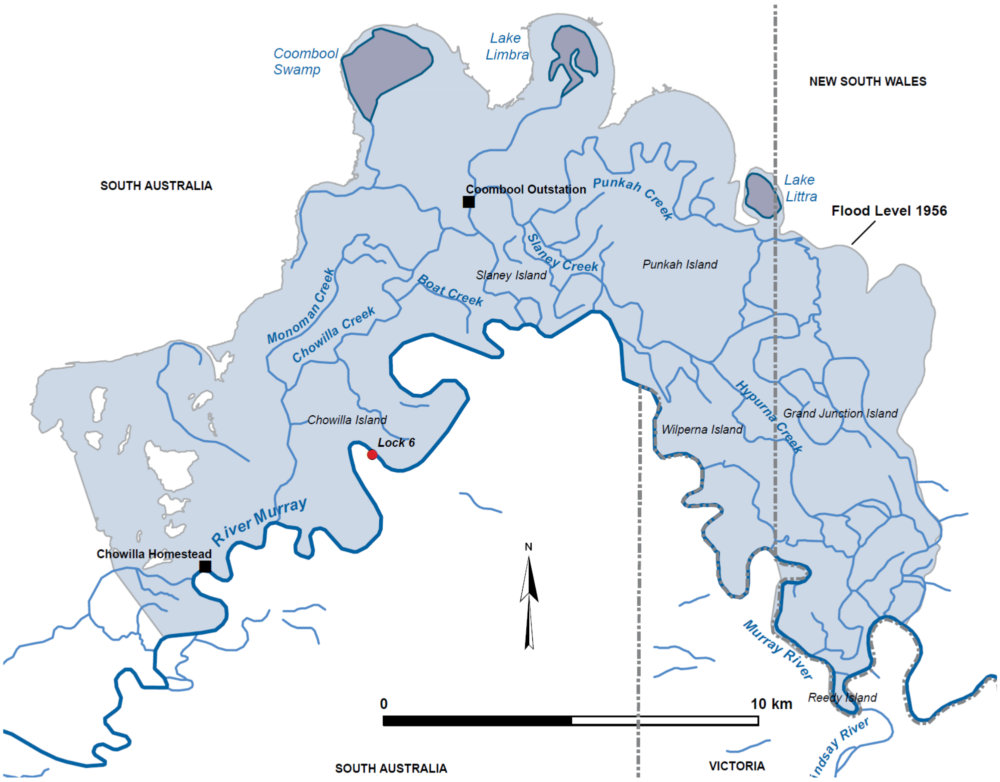

Figure 46 The Chowilla Floodplain boundary

Source: MDBA (2012a)

A range of land tenures apply within the South Australian and New South Wales portions of the Chowilla Floodplain. The South Australian Government is the landowner for the South Australian portion (excluding 17.3 ha of freehold land), which includes:

- **Chowilla Game Reserve**: under the National Parks and Wildlife Act 1972 (SA), 14,620 ha of the Chowilla Floodplain is gazetted as a game reserve (proclaimed in early 1993). The Game Reserve Management Plan guides land management activities over the area and is overseen and implemented by the South Australian Department for Environment and Natural Resources.
- **Chowilla Station**: crown reserve land on Chowilla Station is vested in the South Australian Minister for Environment and Conservation. Robertson Chowilla Pty Ltd has operated Chowilla Station as a wool-growing operation since 1865, and the company is the leaseholder of 12,062 ha of the Chowilla Floodplain. The lease area falls entirely within the game reserve. An agreement was made between the South Australian Department for Environment and Heritage (now Department for Environment and Natural Resources) and Robertson Chowilla Pty Ltd to exclude livestock grazing from 83 percent of the Chowilla Floodplain, effective September 2005.
- **Freehold**: a freehold parcel of 17.3 ha (section 78), historically known as the Chowilla Orangery, is currently a vineyard run by Lonver Pty Ltd.
- **Kulcurna**: the New South Wales portion of the Chowilla Floodplain, covering about 5,192 ha, is owned by the New South Wales Government (excluding 1 ha of freehold land), and vested in the Water Administration Ministerial Corporation on the behalf of the Murray-Darling Basin Authority. Known as ‘Kulcurna’, the area is made up of a mixture of tenures consisting of some freehold parcels and Crown and Western Lands Leases. In addition, two travelling stock routes and a forestry reserve overlay parts of the Western Lands Leases. These tenures have various

implications to land management, public access and resource access and management. There is also a freehold parcel of 1 ha that contains the original Tareena Post Office near Tareena Billabong - the post office itself is currently unoccupied, but is being redeveloped as part of a farm stay and ecotourism enterprise.

While the Murray-Darling Basin Authority plays a key coordination role in the management and delivery of water-related activities within Chowilla Floodplain and other Icon Sites, the Murray-Darling Basin Authority is required to distribute the water resources of the Murray River in accordance with the Murray-Darling Basin Agreement. The South Australian Department for Water is the overall Chowilla Floodplain Icon Site manager, and is responsible for developing policies, plans and actions that focus on improving the health of the Murray River (including the Chowilla Floodplain icon site) through improved operations and management of the river.

Overall, tenure and management of the Chowilla Floodplain is complex.

12.2.2 Hydrology of the Chowilla Floodplain

The natural flow regime of the Murray River has been significantly modified by flow regulation through the operation of a series of weirs and upstream storages. These changes altered the hydrology of Chowilla and other floodplain environments so that they were either permanently inundated, permanently dry, or flooded less frequently and with an altered seasonality. Regulation also resulted in a reduction in the volume of flow, the presence of longer periods of sustained low-flows and an overall reduction in flood frequency and variability.

In pre-regulation times, high-flows were cool, turbid and fast flowing, generally occurring in spring and early summer and gradually changing at the end of summer to lower-flows that were warm, clear and slower moving – in particular, through to autumn and winter (Newall *et al.*, 2009). There was a marked variation between years, and cease-to-flow periods occurred during droughts with some water bodies contracting to saline pools fed by saline groundwater. Local anabranches formerly flowed only during floods or high-flows, with floodplain inundation (and the refilling of disconnected wetlands) determined by flood magnitude, proximity to the river channel and local topography. During drought, the flow would cease and saline pools would form through an interception with underlying saline groundwater.

Following from regulation, the Chowilla's creeks and floodplains experienced significant changes to the seasonal nature of flow regime. These included permanent base flows, which led to permanent inundation of connected wetlands, a delay in flood initiation, and to a reduction in flood duration (Newall *et al.*, 2009). There was also a reduction in the frequency of small-to-moderate-sized flood events and a reduced recharge of local groundwater ('freshwater lens') in semi-permanent wetlands, leaving insufficient water for trees. The river level was raised by approximately 3 metres, which led to permanent inundation of some otherwise-ephemeral wetlands, saline groundwater intrusion into anabranches and floodplain, and widespread tree stress. Regional saline groundwater (30,000 to 40,000 mg/L TDS[73]) flowed into the anabranch creeks. Up to 145 tonnes of salt per day may enter the Chowilla anabranch system following a

[73] Total dissolved solids

major flood, with a baseline of 43 tonnes per day. Saline ground water mounds formed beneath irrigated areas adjacent and downstream of the floodplain. These collective effects were exacerbated by severe and widespread drought during the decade leading up to 2010-11.

Figure 47 Chowilla regulator in operation on Chowilla Creek

Source: South Australian Department of Environment and Water

The upshot of the changes above was an unsustainable trajectory of environmental degradation within the Chowilla Floodplain. Recognition of this resulted in a series of major construction works that centred on the Chowilla regulator. These works commenced in 2010 and were completed in 2012. Approvals for the works rested in turn on rigorous risk assessments which sought to provide an evaluation of the likely impacts of corrective regulation. Operation of the Chowilla regulator and ancillary structures is described within the Murray-Darling Basin Authority's Chowilla Floodplain Environmental Water Management Plan (MDBA, 2012a), which then makes reference to the following stand-alone schedules:

- Chowilla Floodplain event plans and hazard mitigation strategy for operation of the Chowilla Creek regulator and ancillary structures (MDBA, 2014a)
- Chowilla Floodplain operations plan for Chowilla Creek regulator and ancillary structures (MDBA, 2014b)
- Monitoring strategy for operation of the Chowilla Creek regulator and ancillary structures (MDBA, 2014c).

The construction works undertaken are illustrated in Figure 48 and include:

- Construction of an environmental regulator 79 metre wide, with 3 metre head differential and incorporating fishways on Chowilla Creek (the Chowilla regulator, as shown in the photograph in Figure 47)

- Construction of ancillary structures to be operated in conjunction with the Chowilla regulator including,
  - Woolshed Creek south regulator
  - Woolshed Creek east regulator
  - Chowilla Island loop channel and channel regulator
  - Chowilla Island loop regulator
- Upgrade of existing weirs on Pipeclay and Slaney Creeks (major inlet creeks from the Murray River into the Chowilla anabranch) to provide for more flexible operations for environmental management and incorporating fish passage
- Replacement of Bank E with a rock ramp fishway and replacement of Boat Creek Bridge to remove flow restriction, improve fish passage and improve access.

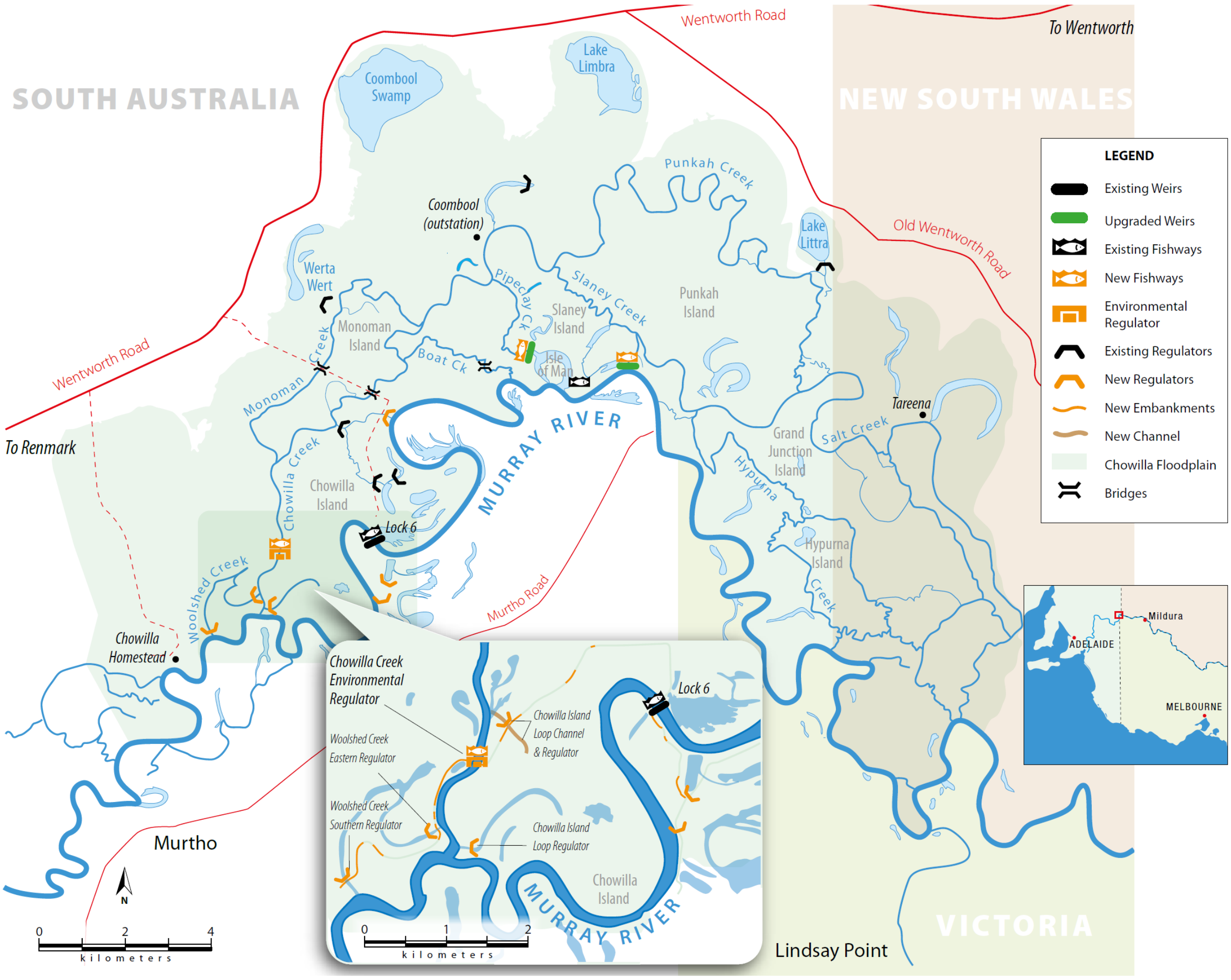

Figure 48 Regulation of flows through the Chowilla Floodplain

Source: MDBA (2012a)

The Chowilla regulator and ancillary structures are operated in conjunction with the Murray River locks and weirs (primarily Lock 6, but also Locks 5 and 7), providing a mechanism to enable large parts of the Chowilla Floodplain to be inundated when the flows in the Murray River would otherwise be insufficient do so. A number of regulators on key wetlands provide further important opportunities for flow and inundation management (Figure 48). Collectively, the existing, new and upgraded structure types, and their spatial configuration, enable a wide range of management actions available including:

- No-action required at the site scale
- Delivery of water to individual wetlands (pumping or gravity)
- Weir pool manipulation
- Pulse flows by way of Pipeclay and Slaney weirs
- In-channel rise
- Managed inundations, including
  - Low elevation
  - Moderate elevation
  - Maximum achievable elevation
- Managed hydrograph recession.

The Chowilla regulator and associated structures enable water level variation within the Chowilla Floodplain to approximately 3.6 metres. They operate under flows ranging from entitlement conditions (3,000 to 7,000 ML/day) up to 50,000 ML/day. The design includes fishways that enable the passage of large-bodied and small-bodied native fish, while the main control regulator is in operation.

Operation of the environmental regulator at full height of 19.87 metres enables the flooding of between 5,630 and 9,000 ha (corresponding to approximately 35 percent and 50 percent of the floodplain) and influences a further area through lateral groundwater freshening. This ensures the maintenance and improvement of 78 percent of the river red gum community and 31 percent of the black box community. The new works also inundate large areas of other floodplain habitats, including 91 percent of wetlands and water courses, 75 percent of river cooba (*Acacia stenophylla*) woodlands and 58 percent of floodplain grasslands.

As well as the vegetation benefits, the regulator provides environmental benefits within and beyond the inundated area, including:

- Increased connectivity between riverine and floodplain habitats
- Freshened groundwater systems
- Improved soil conditions
- Rejuvenated existing wetland habitats
- Establishing new floodplain and wetland plant communities
- Enhanced regional biodiversity
- Increased zooplankton abundance
- Increased habitat and breeding opportunities for waterbirds and frogs
- Provision of additional habitat for small native fish.

12.2.3 Ecological values of the Chowilla Floodplain

Three broad **ecological objectives** were identified as the basis for maintaining the high biodiversity values of the Chowilla Floodplain and Lindsay-Wallpolla Islands Icon Site:

- High-value wetlands maintained
- Current area of river red gum maintained
- At least 20 percent of the original area of black box vegetation maintained.

These broad objectives were expanded to evaluation, as shown in Table 19 below.

Table 19 Refined ecological objecytives for the Chowilla Floodplain Icon Site

Functional group	Objective
Vegetation	
1.	Maintain viable river red gum populations within 70 percent (2,414 ha) of river red gum woodland.
2.	Maintain viable black box populations within 45 percent (2,075 ha) of black box woodland.
3.	Maintain viable river cooba populations within 50 percent of river cooba, and mixed red gum and river cooba woodland areas.
4.	Maintain viable lignum populations in 40 percent of areas.
5.	Improve the abundance and diversity of grass and herblands.
6.	Improve the abundance and diversity of flood-dependent understorey vegetation.
7.	Maintain or improve the area and diversity of grazing sensitive plant species.
8.	Limit the extent of invasive (increaser) species including weeds.
9.	Improve the abundance and diversity of submerged and emergent aquatic vegetation.
Fish populations	
10.	Maintain or increase the diversity and extent of distribution of native fish species.
11.	Maintain successful recruitment of small and large bodied native fish.
Frog populations	
12.	Maintain sustainable communities of the eight riparian frog species recorded at Chowilla.
13.	Improve the distribution and abundance of the nationally listed southern bell frog at Chowilla.
Bird populations	
14.	Create conditions conducive to successful breeding of colonial waterbirds in a minimum of three temporary wetland sites at a frequency of not less than one in three years.
15.	Maintain or improve the diversity and abundance of key bird species.
16.	Maintain the current abundance and distribution of regent parrots
17.	Maintain the current abundance and distribution of the bush stone-curlew (*Burhinus grallarius*)

Source: MDBA (2012a)

To enable these objectives to be measured and monitored adequately, a set of more detailed Ecological Targets were also developed. The Ecological Targets are tested using the monitoring program (MDBA, 2014c), which in turn is linked to an adaptive management regime. Short-term targets were established for achievement by 2020. These provided for the completion of the construction of the Chowilla regulator and initial operation of this and ancillary structures. Longer-term targets have been established for achievement by 2030. Position and trajectory relative to the targets is used as a decision tool to help decide which management actions will be the most appropriate at any given time. Management actions are targeted at maintaining condition of biota and abiotic processes within responsive ranges rather than at a specific target level.

Citing a range of primary and review literature, the Murray-Darling Basin Authority's Chowilla Floodplain Environmental Water Management Plan (MDBA, 2012a) provides a summary of the site's **ecological values**. The following discussion was adapted largely from this Plan.

The Chowilla Floodplain is one of the last remaining parts of the lower Murray floodplain that retains much of the area's natural character and attributes. Significantly, it contains the largest remaining area of natural river red gum forest in the lower Murray River and has highly diverse floodplain vegetation. The region's aquatic habitats include permanent and temporary water bodies, with over 100 km of anabranch creeks. In high-river flows, these creeks spread into a series of temporary wetlands, lakes and billabongs that create an area of outstanding environmental significance.

Flora: Chowilla's vegetation communities are distributed across the floodplain and upland rise, based on environmental watering, soil type and salinity gradients and, in the case of aquatic and littoral vegetation, the velocity of the current. The major vegetation communities of the floodplain are shown in Figure 49, and include:

- River red gum forest and woodlands
- Black box woodlands
- Lignum (*Muehlenbeckia florulenta*) low shrubland.

Black box woodland is the most widespread vegetation class, occupying approximately 5,117 ha (29 percent) of the Chowilla Floodplain. Kulcurna also contains extensive areas of two significant plant communities typical of south-western New South Wales that are otherwise poorly represented elsewhere in that state's reserve system. These are the river red gum/black box woodlands and open rosewood (*Alectryon oleifolius*) and belah (*Casuarina pauper*) mallee mosaic.

Eighteen of the taxa identified at Chowilla Floodplain have been listed as rare in South Australia, five have been listed as vulnerable and one has been listed as endangered. Of taxa of conservation significance, one endangered taxon (*Crassula sieberana* ssp. *tetramera*) and nine of the 18 rare taxa have been recorded or observed since 2004.

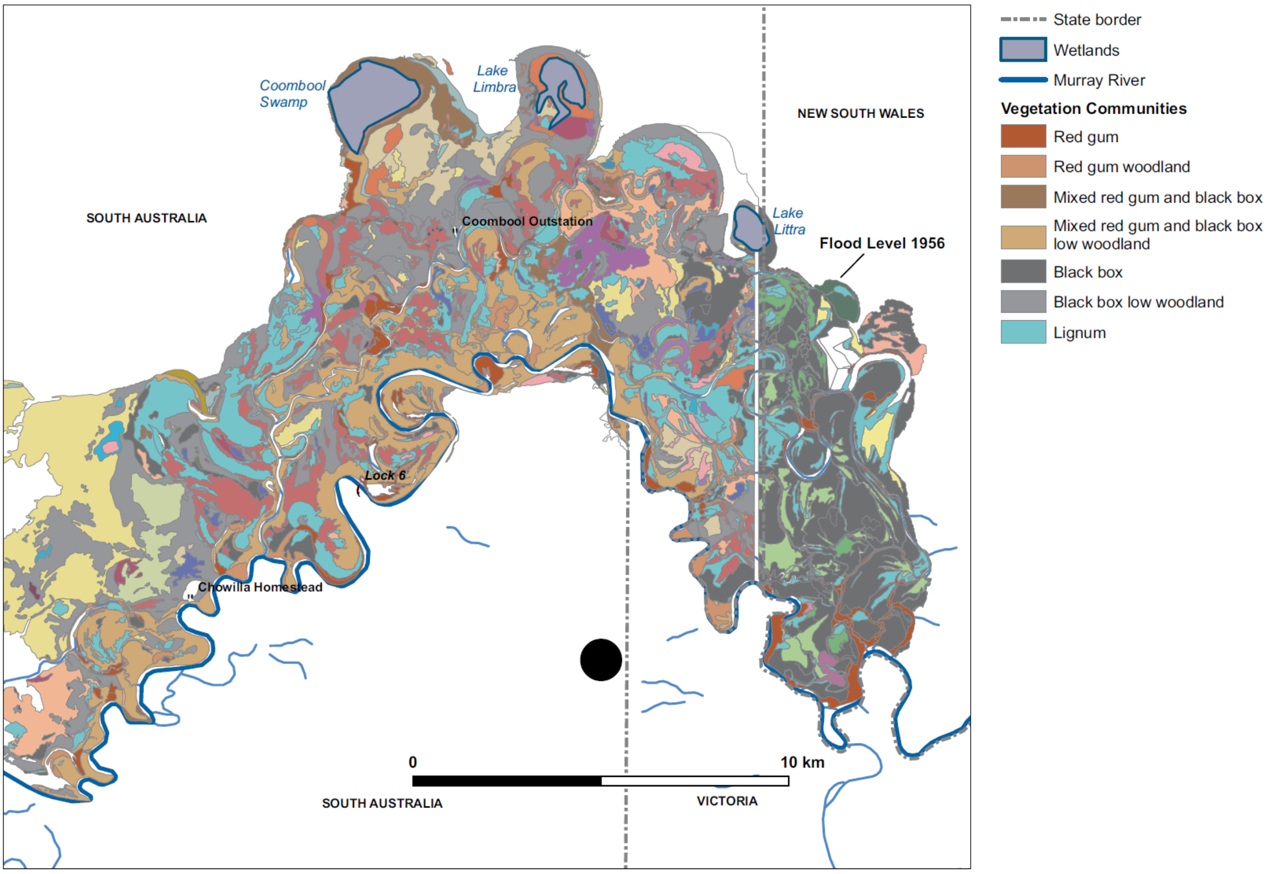

Figure 49 Vegetation communities on the Chowilla Floodplain

Source: MDBA (2012a)

Terrestrial and aquatic animals: the diverse vegetation assemblages and variability of the riverine environments create a mosaic of habitat types that vary in time and space, and in response to changing river flows. This supports distinct assemblages of aquatic and terrestrial fauna, including species listed as threatened at both a national and state level. The wetlands within the Chowilla Floodplain also provide seasonal habitat for a number of migratory birds.

The Chowilla Floodplain is known to contain 17 native mammals, including the feathertail glider (*Acrobates pygmaeus*), Giles' planigale (*Planigale gilesi*) and the fat-tailed dunnart (*Sminthopsis crassicaudata*) as well as eight introduced species. The bat fauna of the Chowilla Floodplain is especially rich — eight species have been recorded and the area within 50 km of Chowilla is identified as a location containing the greatest diversity of bats within South Australia. Eight species of frog have also been recorded in the area, including the southern bell frog, which is listed nationally as vulnerable (below). In addition, the area is known to contain five South Australian-listed reptiles, including the carpet python (*Morelia spilota*) and the broad-shelled turtle (*Macrochelodina expansa*).

The Chowilla anabranch and floodplain system provide flowing water habitats for at least 12 species of native fish, many of which are now poorly represented in the South Australian section of the Murray River (Table 20). This includes the vulnerable Murray cod and the critically endangered silver perch (PIRSA, 2018). In addition to these 12 species, the Riverland Ramsar Site Ecological Character Description (Newall *et al.*, 2009) mentions the endangered Murray hardyhead, the endangered trout cod and the southern pigmy perch.

Table 20 Native fish species identified at the Chowilla Floodplain Icon Site

Species	EPBC Act status
Australian smelt (*Retropinna semoni*)	Unlisted
Bony herring (*Nematalosa erebi*)	Unlisted
Carp gudgeon complex (*Hypseleotris klunzingeri*)	Unlisted
Dwarf flat-headed gudgeon (*Philypnodon macrostomus*)	Unlisted
Flat-headed gudgeon (*Philypnodon grandiceps*)	Unlisted
Freshwater catfish (*Tandanus tandanus*)	Unlisted
Golden perch (*Macquaria ambigua*)	Unlisted
Murray cod (*Maccullochella peelii*)	Vulnerable
Murray hardyhead (*Craterocephalus fluviatilis*)	Endangered
Murray (or crimson-spotted) rainbowfish (*Melanotaenia fluviatilis*)	Unlisted
Silver perch (*Bidyanus bidyanus*)	Critically endangered
Southern pygmy perch (*Nannoperca australis*)	Unlisted
Spangled perch (*Leiopotherapon unicolor*)	Unlisted
Trout cod (*Maccullochella macquariensis*)	Endangered
Unspecked hardyhead (*Craterocephalus fulvus*)	Unlisted

Source: Newall *et al.* (2009) and PIRSA (2018)

Crustaceans: two species of freshwater mussel occur in the wetland complex (Newall *et al.*, 2009). The river mussel (*Alathyria jacksoni*) favours moderate or fast-flowing channels, including the Murray River channel and the larger anabranches. The floodplain mussel (*Velesunio ambiguous*) prefers slow-flowing and still-water habitats, including billabongs, backwaters and impounded areas of the main channels. The river snail (*Notopala hanleyi*) was formerly common in flowing-water habitats within the site prior to listing in pre-regulation times, but has virtually disappeared in South Australia (Newall *et al.*, 2009). The swamp yabby, a burrowing crayfish, is common throughout the Chowilla Floodplain's wetlands, except in fast-flowing water. The freshwater shrimp (*Macrobrachium australiense*) occurs in a range of lotic habitats within the Chowilla Floodplain (Newall *et al.*, 2009).

Waterbirds and woodland birds: in comparison to the larger wetland and floodplain complexes (for example, the Lower Lakes and Coorong – as discussed in Case Study 5: Section 12.5), the Chowilla Floodplain and other parts of the Lower Murray River contain a relatively small amount of habitat to support breeding events and, as such, breeding events in this region tend to be minor with regard to the number of successful recruits (Rogers and Paton, 2008). These minor breeding events may still be important, however, as inundation is generally more regular than it is in the larger floodplain systems. Regular inundation may also provide for the maintenance and survival of adults between breeding seasons. The extent of wetland availability will be an important determinant of the numbers of birds included in a breeding event, as well as the number of birds a wetland supports during non-breeding. However, small-scale breeding events are sensitive to the duration of inundation, with 2 to 6 months required for many colonial species.

The regent parrot is present within the Chowilla Floodplain and is listed under the EPBC Act as vulnerable. The regent parrot is confined to the semi-arid interior of southeastern mainland

Australia. It primarily inhabits riparian or littoral river red gum forests or woodlands and adjacent black box woodlands, with nearby open mallee woodland or shrubland (Newall *et al.*, 2009). In South Australia, the key breeding population occurs in the Murray-Mallee region, centred along the Murray River. Nesting typically occurs in river red gum and occasionally in black box, and usually within 16 metres of permanent water or standing in water. Nest sites may sometimes occur near temporary water sources, such as ephemeral streams or seasonal billabongs, but these are usually within about 60 to 100 metres of permanent water sites. These environmental conditions and tree species are provided within the Chowilla Floodplain.

The vegetation of Chowilla Floodplain also provides important habitat for a range of woodland bird species. Some of these (such as white-plumed and blue-faced honeyeaters – *Lichenostomus penicillatus* and *Entomyzon cyanotis*, respectively) are specialists of riparian and floodplain river red gum woodlands. However, many species that inhabit floodplain woodlands also occur in woodlands that occur on the surrounding semi-arid and arid terrestrial ecosystems – in particular, the mallee ecosystems, sugarwood (*Myoporum platycarpum*) woodlands and black-oak (*Casuarina pauper*) woodlands. These species rely upon the forested floodplain as drought refugia. The most important breeding sites include Pilby Lagoon, Pipeclay Billabong, Lake Littra, Lake Limbra, Slaney Billabong, and Coppermine Complex. In particular, Lake Littra and Lake Limbra are considered the most important breeding sites for colonial nesters, such as Australian white ibis and yellow-billed spoonbill (Rogers and Paton, 2008).

Listed species: as noted above, the following six species endemic to the Chowilla Floodplain have been listed under EPBC Act (1999):

- Murray cod (vulnerable)
- Murray hardyhead (endangered)
- Regent parrot (vulnerable)
- Silver perch (critically endangered)
- Southern bell frog (vulnerable)
- Trout cod (endangered).

12.2.4 Carp biomass and population dynamics within the Chowilla Floodplain

The NCCP project team modelling carp biomass density across Australia reported a wide variation in estimates within the Chowilla Floodplain and adjacent reach of the Murray River channel (Stuart *et al.*, 2019). The lowest estimates (approximately 160 kg/ha) were attributed to some peripheral creeks, although other creeks and waterbodies close by had estimates in the range of 300 kg/ha. The reason for the wide disparity (effectively twice the biomass density) was not clear. Numerous waterways and waterbodies within the Chowilla Floodplain were not included in the biomass layer, although a number of these are likely to be permanently inundated. The highest estimates (approximately 500 kg/ha) were attributed to the Murray River channel adjacent to the Chowilla Floodplain.

Mallen-Cooper *et al.* (2011) provided a (then) prospective analysis of the likely impacts of the Chowilla regulator on native and invasive fish populations within the Chowilla Floodplain. The analysis included discussion of the ecology and population dynamics of carp within the Chowilla

Floodplain, and much of this section was adapted from that work. Further details about the ecology of carp, and the particular relevance of ephemeral wetlands, can be found in Part II: Section 4.2.

Both juvenile and adult carp preferentially occupy stationary or lentic habitats within the waterways of the Chowilla Floodplain, although some lotic anabranch habitats are also used. As for other wetland ecosystems, spawning occurs in spring when the water temperature within spawning habitat reaches approximately 16 to 17C. Spawning in the Chowilla Floodplain ecosystem takes place principally in shallow off-channel lentic habitats and at the edges of channels – usually amongst macrophytes and other submerged aquatic vegetation. Suitable spawning habitat can also include the edges of permanent creeks and recently-inundated creek margins, wetlands adjacent to channels, and the outer lakes of the floodplain. If spawning takes place before the point of maximum inundation, larvae and juveniles may drift out onto the floodplain with rising waters. If inundation has occurred at the time of spawning, then some spawning may also take place on the floodplain itself. Eggs laid become sticky on contact with water and adhere to submerged vegetation. Larvae and juveniles then drift back toward main-stem habitats with receding floodwaters. During low-flow seasons, the drift will be less marked as larvae and juveniles will have been hatched within off-channel lentic habitats, or at the edges of the main channels.

Survival of larvae and juveniles is likely to be high within inundated floodplains, given plentiful refuge and a relative paucity of predators – and even accounting for colonial nesting piscivorous waterbirds. A cyanobacterial bloom or precipitous drop in DO are the most likely events to threaten larvae and juveniles within the floodplain environment. Survival within main-stem habitats will be more variable, as these contain permanent aquatic ecosystems with an established chain of predators. Cyanobacterial blooms and low DO may also occur in these environments. Juveniles will continue to drift with the current that returns water from the floodplain back to the main river channel, by way of the Chowilla regulator, and from there continue to drift downstream. As for other parts of the river system, a proportion of young may return upstream at the end of summer and in autumn. It is also likely that a proportion of juveniles will not drift back to the main-stem habitats, but will remain to grow out and mature in permanently-inundated wetlands and channels within the Chowilla Floodplain ecosystem. A better understanding the movement of adult fish within and from this ecosystem – and the relative importance of recruitment during high-flow and lower-flow seasons – were identified by Mallen-Cooper *et al.* (2011) as key research priorities.

Mallen-Cooper *et al.* (2011) also examined the likely impact of three operations scenarios for the Chowilla regulator, and other structures, on the population dynamics of carp within the Chowilla Floodplain ecosystem.

- The first was effectively a no-operation scenario – that is, continuation of the extant (in 2011) population dynamic for carp without an operating Chowilla regulator. The outcomes for this scenario focussed on continued strong recruitment during high-flow seasons and relatively less recruitment during drought years – although, as noted above, the quantification of this balance is not currently well understood. In parallel with this, the environment itself would continue to degrade as a result of the flow regime dictated by operation of Lock 6 and other structures within the river channel.

- The second scenario described the operation of the Chowilla regulator with more than 75 percent of the lotic habitats retained and some inundation of low-lying floodplains. This scenario provided carp with strong spawning and recruitment opportunities. The local population increased, and dispersed into the Murray River channel. The only constraints to population increase under this scenario concerned: (a) the timing of inundation with respect to spawning; and (b) the extent of the floodplain that is inundated, and the impact this has on spawning and on the survivability of larvae and juveniles.
- The third scenario described the operation of the Chowilla regulator with less than 25 percent of the lotic habitats retained and widespread inundation of floodplains. If timed to coincide with spawning, then this strategy provided the maximum opportunities for recruitment. The outcome of this was a substantial increase in both the local population of carp and, with some delay to allow for maturation of fish, a corresponding increase in the population of carp in other parts of the river system. Similar outcomes were obtained through the modelling studies of Koehn *et al.* (2016). Likewise, Stuart *et al.* (2011) stated that managed inundation will likely result in large numbers of young-of-the-year Carp on the Chowilla Floodplain and recruitment into permanent creeks and the Murray River.

The works of Mallen-Cooper *et al.* (2011) and Stuart *et al.* (2011) were undertaken prior to commissioning of the Chowilla regulator, and helped to clarify some of the risks that might be associated with its operation. The work of Koehn *et al.* (2016) was a modelling study aimed at evaluating the impact of a range of regulation scenarios on carp population dynamics in a range of geographical settings (including the Chowilla Floodplain) and, in doing so, illustrating the utility of computer modelling. The Chowilla regulator and associated structures had been in operation for approximately 7 years at the time that the current risk assessment for CyHV-3 was undertaken, and yet no studies evaluating the actual impact of their operation on the recruitment of carp within the Chowilla Floodplain ecosystem or to other parts of the river system were identified.

12.2.5 CyHV-3 outbreak scenarios for the Chowilla Floodplain

Some aspects of the outbreak scenarios for the Chowilla Floodplain are likely to be quite similar to those that have been described for the Barmah-Millewa Forest (Case study 1: Section 12.1), although the regulation of flows within the Chowilla Floodplain is more controlled than it is within the Barmah-Millewa Forest (following construction of the Chowilla regulator and other structures) and the floodplain ecosystem reflects a range of unique environmental characteristics and stressors – the latter including higher water temperatures, higher salinity and the high turbidity of contributing flows from the Darling River. Importantly, the Chowilla Floodplain also supports a much smaller population of colonial-nesting and other waterbirds than does the Barmah-Millewa Forest.

Accepting these caveats, under the outbreak scenarios for ephemeral wetlands that were laid out in Section 10.1, aggregations of carp are likely to take place in the river channel and off-channel waters prior to the commencement of spawning. The temperature of the water at this time is likely to be sufficiently high (approximately 18C) for transmission to occur. Further transmission is likely to occur during spawning itself, which takes place principally in shallow off-channel lentic habitats, including at the edges of permanent creeks, recently-inundated creek margins, wetlands adjacent to channels and the outer lakes of the floodplain. Toward the end of spring, however, the temperature in these shallow waters is likely to rise above the upper threshold for the transmission of CyHV-3 (approximately 28C) and, where this occurs, the outbreak will diminish.

To illustrate this, Figure 50 compares the average daily water temperature (aggregated to each month of the year) at: (a) two deeper-water locations within the Chowilla Floodplain and upstream of Murray River Lock 6; with (b) similar sites from the Barmah-Millewa Forest. The average daily temperature within the Chowilla waterways is consistently 1 to 2C higher than it is in comparable waterways within the Barmah-Millewa Forest. Considering that these data depict a monthly average of daily average water temperatures (that is, two levels of smoothing), it is likely that daily maximum temperatures within these deeper water locations within the Chowilla Floodplain during the summer months will exceed 28C (the upper threshold for the transmission of CyHV-3). Following from this, it is also likely that the temperature within the shallow wetland or floodplain waters favoured by carp as spawning grounds will be higher again.

Given the above, highly-susceptible juvenile carp drifting back toward the main creeks and river channel are less likely to be exposed until late summer or early autumn, when a resurgence of the original outbreak may take place. As autumn progresses and juveniles continue to drift downstream, minor aggregations may occur at particular places within the channel – including fishways, and places where water quality is highest or feed most plentiful. These aggregations may again stimulate ongoing transmission of CyHV-3 and the continuation of losses amongst juvenile and adult carp. In late autumn and winter, the water temperature is likely to drop below the threshold for active transmission (approximately 18C). A low level of infection remains possible although carp infected at this time – and those that were infected earlier, but have survived – may enter a latent state (Part II: Section 5.3.6). When the water warms, the disease will again become active and these fish may then seed virus into spawning aggregations in the following spring.

The impact of the relatively high water temperature in summer and the effective inundation of some wetlands and floodplains during relatively lower-flow seasons mean that the outward visibility of an outbreak of CyHV-3 (as determined principally by the accumulation of carp carcasses, and their impact on water quality) within the Chowilla Floodplain is likely to be lower than in some other ephemeral wetland settings (such as the Barmah-Millewa Forest). The delineation between outbreaks in high-flow and lower-flow seasons is also likely to be less marked.

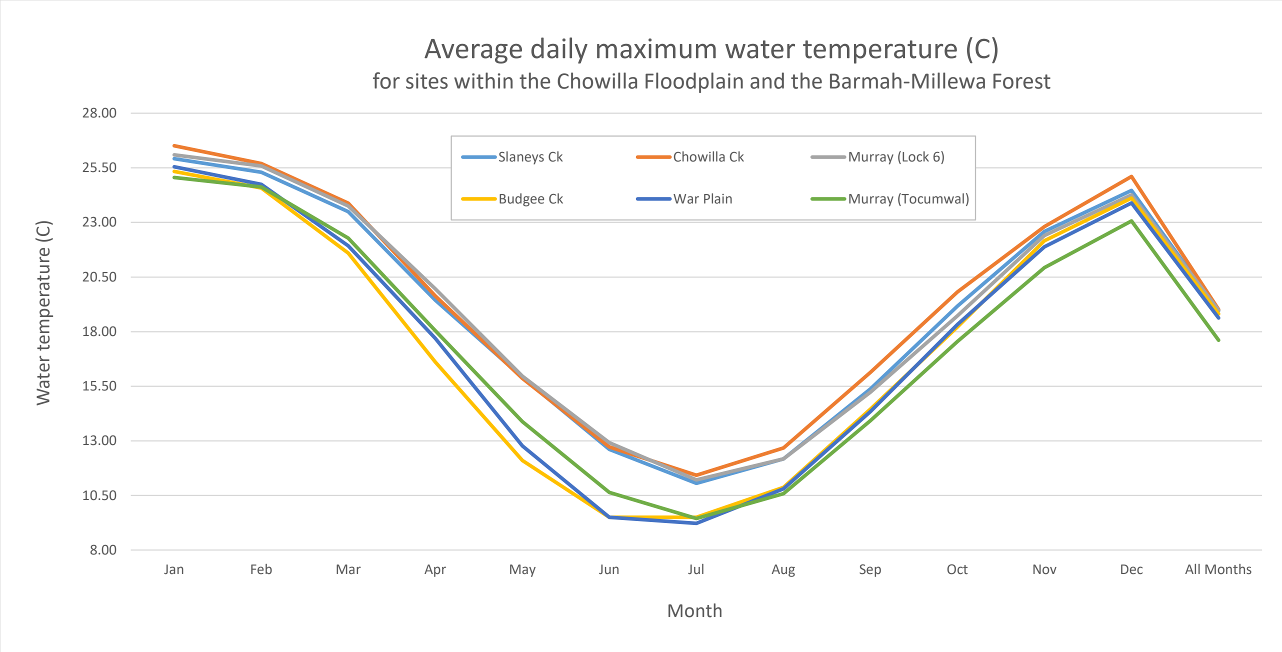

Figure 50 Water temperature within the Chowilla Floodplain and Barmah-Millewa Forest

Daily maximum water temperature (C) aggregated to a monthly average, for three sites within the Chowilla Floodplain and a local reach of the Murray River (upstream Lock 6) and three sites within the Barmah-Millewa Forest and a local reach of the Murray River (downstream of Tocumwal)

Source: raw data obtained from www.waterconnect.sa.gov.au and https: //riverdata.mdba.gov.au

12.2.6 Exposure pathways and risks for the Chowilla Floodplain

A generic assessment for the nine identified exposure pathways was undertaken in Section 11. The discussion below interprets the outcomes from Section 11 in the context of the Chowilla Floodplain ecosystem and its natural assets.

(a) Exposure of native aquatic species to low DO

Likelihood of exposure: under this pathway, hypoxic events are predicated on the accumulation of significant numbers of carp carcasses in a stationary or lentic waterbody. The scenario of highest risk in most ephemeral wetland settings is likely to be a lower-flow season, when carp are reasonably concentrated in available habitat. The Chowilla regulator, however, enables the inundation of a high proportion of wetlands and floodplains with relatively low channel flows. Additionally, there is a capacity to direct water to particular parts of the ecosystem and to maintain their connectivity. This level of regulation means that carp are less likely to be concentrated at a high biomass density in a lower-flow season, and that carcass accumulations are less likely to be substantial. With a large area within the floodplain potentially at risk, some exceptions to this are possible, but lower-flow seasons are nevertheless less likely to result in aggressive outbreaks and the precipitation of low DO events within the Chowilla Floodplain ecosystem than in other less-regulated ephemeral wetlands. The high water temperature within wetland and floodplain waters during summer months is also likely to constrain the aggressiveness of an outbreak. In high-flow seasons the risk is likely to be somewhat lower again, as carp will be largely dispersed throughout the maximally inundated floodplains. In this situation, outbreaks of CyHV-3 are likely to be localised and carcass accumulations minor and similarly dispersed. Pockets of low or zero DO are possible in relatively disconnected parts of the floodplains, although widespread hypoxia or anoxia is unlikely.

Likely consequences of exposure: low DO will primarily affect fish and crustaceans. Air-breathing aquatic animals will not be directly affected. Indirectly, low DO may also impact on ecological communities and on the integrity of wetlands. The Chowilla Floodplain anabranch and floodplain system provides habitats for at least 12 species of native fish, many of which are now poorly represented in the South Australian section of the Murray River. This includes the vulnerable Murray cod and the critically endangered silver perch. In addition to these 12 species, the Riverland Ramsar Site Ecological Character Description (Newall *et al.*, 2009) includes the endangered Murray hardyhead, the endangered trout cod and the southern pigmy perch.

Murray cod are sensitive to low DO and, although the sensitivity of trout cod is not known, they are closely related to Murray cod and are likely to be physiologically similar. Despite its mention within the Riverland Ramsar Site Ecological Character Description (Newall *et al.*, 2009), the Chowilla Floodplain is not known to have a sustainable population of trout cod. The population of silver perch is also somewhat doubtful, as this species is only rarely observed in South Australia. Silver perch are, however, likely to be more resilient to low DO than the two species of cod. Golden perch are physiologically adapted to lowland waterways with poorer water quality, and are able to sustain (and to some extent adapt to) low DO. The broader population of golden perch is also more distributed geographically than silver perch or cod, and thus less vulnerable at the population scale to local hypoxic events. Bony herring are a widespread, abundant and hardy fish, tolerating high temperatures (up to 38C), high turbidity and high salinity (up to at least 39 ppt). The Murray-Darling Basin Authority maintain that this species is relatively tolerant of low DO, although the Australian Museum states that the species is susceptible to low DO and likely to be the first to become stressed or die in the event of the drying of ephemeral waterbodies. It may be that the ubiquity and relatively high abundance of bony herring mean that deaths within a local population are visible earlier and are more obvious than some other species. Bony herring are a relatively short-lived fish (2 to 3 years is common), but are highly-fecund. This combination means that a failure to recruit in a given season may result in a noticeable drop in the population at that location – with rapid a rebound when conditions for breeding are more favourable. Freshwater catfish are a benthic fish and are considered the most resilient of the large-bodied native fish to low DO. Freshwater catfish and the other large-bodied native fish identified within the Chowilla Floodplain spawn in spring. Hypoxic events that occur during this time may thus result in a lower recruitment, although the impact of this on the sustainability of local populations may be lower for the long-lived species such as Murray cod than for silver perch and freshwater catfish. Notwithstanding this, the Murray cod are arguably the most exposed of the large-bodied native fish to hypoxic events within the Chowilla Floodplain ecosystem.

The small-bodied native fish (including both the wetland or floodplain specialists and the foraging generalists) are relatively more tolerant to low DO than are most of the larger fish – with the exception of the benthic freshwater catfish. Although there are differences between the species, most small-bodied fish will begin to use ASR at about 2.5 mg/L and may survive for some time while the oxygen concentration remains above about 1 mg/L. This notwithstanding, they are also generally short-lived and a disturbance to recruitment could have a significant impact on the sustainability of a local population. They may also be more exposed to precipitous drops in DO within shallow wetlands and may be less able to move substantial distances to evade poor water quality. The Chowilla Floodplain is likely to include some populations of the endangered Murray hardyhead, as well as the southern pigmy perch, and both may be exposed in the manner described above. The swamp yabby is likely to become stressed and emerge when DO falls below approximately 2 mg/L. The emergence of swamp yabbies predisposes them to predation from birds and other animals, as well as to desiccation. The river mussel favours moderate or fast-flowing channels within the Chowilla Floodplain ecosystem and is considered relatively intolerant to low DO. The floodplain mussel prefers slow-flowing and still-water habitats, and is relatively more tolerant (Sheldon and Walker, 1989).

The Chowilla Floodplain includes the largest remaining natural river red gum woodland and forests in the lower Murray River, as well as lignum shrublands, black box woodlands, grasslands and floodplain marshes. The impact of low DO events on these communities would be realised when they are inundated, and the high degree of regulation of flows within the Chowilla Floodplain means that this could include either high-flow or lower-flow seasons. Where this occurs, small communities – or smaller parts of a broader community – may experience the stress or death of fish and crustaceans. This affect is likely to be short-term (1 to several weeks) and is unlikely to affect the quality of the community when the floodplains are next inundated. The Chowilla Floodplain also supports a range of riparian and wetland communities, and many of these have become permanent or semi-permanent with altered flow regimes. The impact of low DO on these communities may be more marked in the sense that they are not transient and will not be regenerated with each inundation event.

Summary of risks: the Chowilla Floodplain includes a mixture of permanent or semi-permanent off-channel waterways and wetlands, and periodically-inundated floodplain forests and woodlands, shrublands, grasslands and floodplain marshes. The likelihood and likely consequences of a low DO event in these settings will follow in general terms the discussion in Section 11.1. Environmental assets particularly at-risk include the threatened Murray cod, silver perch and Murray hardyhead. Crustaceans may also be at risk, including the swamp yabby and a lotic species of freshwater mussel. The ephemeral floodplain forest, woodland and marshland communities would be affected if hypoxia was to follow from an outbreak of CyHV-3 at a time when the floodplains were substantially inundated. Permanent and semi-permanent riparian and wetland communities are likely to be more exposed, given that hypoxia could affect these during most seasons. Underpinning all of these concerns, however, is the relatively lower likelihood of an aggressive outbreak of CyHV-3 within the Chowilla Floodplain ecosystem that results in the accumulation of large numbers of carp carcasses. This lower likelihood reflects the enhanced regulation of the Chowilla Floodplain ecosystem, as enabled by the Chowilla regulator and associated structures, and the high summer temperature within wetland and floodplain waters.

Mitigation of risks: the mitigation of risks associated with low DO within the Chowilla Floodplain ecosystem would only focus on the removal of carp carcasses if these had accumulated in sufficient numbers to be likely to result in low DO (or the realisation of other carcass-related exposure pathways). Even where this occurs, it is likely to be relatively localised within the broader ecosystem. The overarching goal will be to prevent the DO within a given waterbody falling below about 3 to 4 mg/L or to enable it to return to that level. Consideration is also likely to be given in some outbreak situations to the manipulation of water flows to particular parts of the Chowilla Floodplain ecosystem to enable flushing of carcass components and the distribution of water with a relatively higher DO concentration. Options available through the Chowilla regulator and other structures include:

- Delivery of water to individual wetlands (pumping or gravity)
- Weir pool manipulation
- Pulse flows by way of Pipeclay and Slaney weirs
- In-channel rise
- Managed inundations, including
  – Low elevation
  – Moderate elevation
  – Maximum achievable elevation
- Managed hydrograph recession.

As explained elsewhere (Section 11), however, these options carry their own risks and complications, and would only be undertaken after expert consultation.

(b) Exposure of native species, livestock and humans to cyanobacterial blooms

Likelihood of exposure: as noted above, the level of regulation exercised within the Chowilla Floodplain means that carp are less likely to be concentrated at a high biomass density in a lower-flow season, and that carcass accumulations resulting from an outbreak of CyHV-3 are less likely to be substantial. With a large area within the floodplain potentially at risk, some exceptions to this are possible, but outbreaks of CyHV-3 are nevertheless less likely to result in the precipitation of widespread cyanobacterial blooms within the Chowilla Floodplain ecosystem than in other less-regulated ephemeral wetlands. The high temperature within wetland and floodplain waters during summer months is also likely to constrain the aggressiveness of an outbreak. Where a bloom occurs, however, it is likely to result in the direct exposure of most aquatic species and the indirect exposure of a wide range of terrestrial species – including amphibians and nesting waterbirds. In these situations, harm may be caused through either the direct effect of cyanotoxins (if the strain(s) of cyanobacteria involved are cyanotoxic) or indirectly when the bloom dies off and decomposes, raising the BOD and leading to a precipitous fall in DO.

Likely consequences of exposure: it was concluded within the discussion of this exposure pathway (Section 11.2) that the risks associated with widespread cyanobacterial blooms for large- and small-bodied native fish and crustaceans will largely mirror those associated with low DO. In addition to these, however, are risks to waterbirds, frogs and turtles. The risks posed by widespread cyanobacterial blooms to ecological communities and wetlands are also different to those posed by low DO.

Waterbird breeding events within the Chowilla Floodplain are in general smaller than in some other wetlands, although more regular and dependable. The Chowilla Floodplain ecosystem also provides a refuge during drought times for bird life from surrounding areas, and may help to support non-breeding waterbirds. The vulnerable regent parrot is a bush-bird endemic to the Chowilla Floodplain and listed as vulnerable under the EPBC Act. The risk drivers for this and other birds will be the same within the Chowilla Floodplain as discussed in a generic sense in the pathway description for cyanobacterial blooms (Section 11.2). In particular, the risks will be higher for those functional groups or species whose feeding, foraging, nesting and other behaviours place them in close and regular contact with water. Colonial nesters are also at higher risk, as stressors to these populations are likely to be applied to a large number of birds at a time when harm can fall to adults and the success of recruitment can be threatened. Diet is also important, in particular as cyanotoxins bioaccumulate in animal tissues and regular exposure to affected (living) biota can mean that a toxic threshold is eventually breached.

Eight species of frog have also been recorded in the area, including the southern bell frog, which is listed as vulnerable under the EPBC Act. If a widespread outbreak of cyanotoxic cyanobacteria was precipitated by the decomposition of carp carcasses, then significant numbers of frogs are likely to be exposed. The broad-shelled turtle is also endemic to the Chowilla Floodplain and this would be exposed to cyanotoxin if a widespread bloom was to occur.

The floodplain forest, woodland and marshland communities would be affected if a widespread bloom was precipitated by the accumulation of carp carcasses. Permanent and semi-permanent riparian and wetland communities are likely to be more exposed, given that a widespread bloom could be precipitated within these during most seasons. In any affected community, the impacts of a widespread cyanobacterial bloom may include the direct effect of cyanotoxin (if the strain involved is cyanotoxic) on all species in contact with water, consuming water or consuming biota that is itself affected with cyanotoxins; the effect of the precipitous drop in DO that will inevitably follow from collapse of the bloom; the decline in water quality that will follow from the death and decomposition of cyanobacteria and aquatic biota; and the ecosystem-wide impacts of these processes on food webs.

Livestock and humans are also susceptible to cyanotoxins, and the risk to both were considered in a generic sense within the description of this exposure pathway (Section 11.2). The Chowilla Floodplain includes a wide range of land tenures, and some stock may have access to its peripheral waters. Cyanobacterial blooms are not uncommon within the Chowilla Floodplain ecosystem, and it is understood that managers will be accustomed to monitoring water quality. Although recreational fishing is permitted under permit it is very unlikely that drinking water, fish or crustaceans would be taken for human consumption from an area where mass fish mortalities or destructive cyanobacterial blooms were occurring. This likelihood would be further ameliorated through signage in public places. Caution would extend to the use of contaminated waters for swimming and other recreation, although the malodorous nature of a fish kill is likely to be a sufficient deterrent. On balance there are no realistic scenarios under which humans or farmed (c.f. feral) livestock would be significantly exposed to harm as a result of an outbreak of CyHV-3 within the Chowilla Floodplain ecosystem.

Summary of risks: although cyanobacterial blooms are not uncommon in ephemeral wetlands, the high degree of regulation available within the Chowilla Floodplain means that the high-risk lower-flow scenario that characterises some other wetland ecosystems is less applicable. Regulation also means that the waterbird population within the Chowilla Floodplain ecosystem is more constant year-to-year than in some other ephemeral wetland settings. This in turn means that birds whose feeding, foraging, nesting and other behaviours place them in close and regular contact with water are at highest risk. Colonial nesters are also at high risk, as are the birds whose diet includes fish and other aquatic animals. Frogs and turtles will also be directly affected if the species of cyanobacteria is cyanotoxic.

Mitigation of risks: the mitigation of risks associated with widespread cyanobacterial blooms within the Chowilla Floodplain ecosystem would only focus on the removal of carp carcasses if these had accumulated in sufficient numbers and across a sufficiently broad area to be likely to result in the initiation of a bloom. As was noted for the low DO exposure pathway (above) consideration may also be given in some outbreak situations to the manipulation of water flows to particular parts of the Chowilla Floodplain ecosystem to enable flushing of carcass components and the distribution of uncontaminated water. Options available through the Chowilla regulator and other structures include:

- Delivery of water to individual wetlands (pumping or gravity)
- Weir pool manipulation
- Pulse flows by way of Pipeclay and Slaney weirs
- In-channel rise
- Managed inundations, including
  - Low elevation
  - Moderate elevation
  - Maximum achievable elevation
- Managed hydrograph recession.

As explained elsewhere (Section 11), however, these options carry their own risks and complications, and would only be undertaken after expert consultation.

(c) Exposure of native species, livestock and humans to the pathogens associated with decomposing fish carcasses

Likelihood of exposure: little information is available about the pathogens that may proliferate in the event of a fish kill, and their possible impacts on native species, livestock or humans. This deficit was discussed in the generic description of this pathway (Section 11.3) and in the underpinning review of the literature (Part II: Section 6.3.4). That aside, it is possible that carp gut flora, spoilage organisms, *E. coli*, *Pseudomonas spp*, *Aeromonas spp* and other opportunistic microorganisms might proliferate and, in doing so, may present a hazard to native aquatic and terrestrial species. The degree of water regulation that can be exercised within the Chowilla Floodplain ecosystem means that there is less likely to be a scenario where the accumulation of carcasses and decomposition products is sufficient to trigger the proliferation of hazardous waterborne microorganisms.

Likely consequences of exposure: although there is substantial uncertainty (and likely variation) around the pathogens that may be released following a substantial fish kill, and their ability to survive in an aquatic environment and cause harm to aquatic or terrestrial biota, it is reasonable to expect that the relative exposure of communities, functional groups and species will be similar to that obtained for widespread cyanobacterial blooms – albeit with a lower likelihood of realising the identified ecological endpoints. Balancing this, it was also noted within the generic evaluation of this exposure pathway (Section 11.3) that the experience obtained from field observations of Australian freshwater waterways is that while substantial fish kills are not uncommon, they have not to-date been linked to subsequent epidemics of waterborne pathogens. Some waterborne microorganisms may affect people and livestock, although people are likely to be protected to a large extent by the innate repulsiveness of a waterbody that contains the detritus of a significant outbreak. Livestock, however, may have less ability to avoid affected drinking water if there are limited or no alternatives. The risks to ecological communities are understandably highest for wetlands and marshlands, although an outbreak affecting a waterbody within or proximal to a forest and woodland, or grassland, ecological community, may also impact on species that utilise that water.

Summary of risks: the likelihood that microorganisms associated with the decomposition of carp carcasses would proliferate following a carp die-off in the Chowilla Floodplain ecosystem and become a threat to native species or communities, or to livestock or people, will be lowered by the extent of water regulation that can be exercised. Water regulation will also mean that there is less variance in risk between high-flow seasons, when carp are maximally dispersed, and lower-flow seasons. Accepting this, the profile of risks associated with waterborne microorganisms for individual species, functional groups, communities, livestock and humans will be reasonably similar to those associated with widespread cyanobacterial blooms – albeit with lower likelihoods.

Mitigation of risks: as for both low DO and widespread cyanobacterial blooms, mitigation of risks associated with waterborne microorganisms within the Chowilla Floodplain ecosystem is likely to focus primarily on the careful application of strategic environmental watering. If carp carcasses have accumulated in sufficient quantities in particular parts of the ecosystem to be considered a threat from the perspective of waterborne microorganisms (and extending to low DO or cyanobacterial risk) then these would be removed to the extent that this is practicable.

(d) Exposure of native piscivorous species to the removal of a dominant and stable food source

Likelihood of exposure: the breeding waterbird population within the Chowilla Floodplain is smaller than in many other wetlands. The extent of regulation, however, means that it is more constant from year-to-year, and is able to provide a refuge for bush-birds and non-breeding waterbirds during dry seasons. The relatively high temperature of wetland and floodplain waters during the summer months means that an outbreak of CyHV-3 is likely to be curtailed and that juvenile carp utilising the wetland and floodplain as nursery habitat during the summer months may be less exposed to the virus. Collectively, these considerations mean that there is likely to be less exposure of waterbirds (in particular, the colonial-nesting and strictly piscivorous species) within the Chowilla Floodplain ecosystem to the precipitous loss of a stable food source (that is, juvenile carp) during the summer months. Toward the end of summer, juveniles will drift back toward the channels and anabranches where the water temperature will be lower. In this setting, the outbreak may be revived amongst both juvenile and adult carp and this may result in a decline in the availability of food for nesting waterbirds.

Likely consequences of exposure: if colonial-nesting and strictly piscivorous waterbird species within the Chowilla Floodplain ecosystem are exposed to the sudden removal of stable and dominant food source (juvenile carp) then the absence of an alternative waterbody within the area means that they are likely to compensate by switching to alternative food sources – including frogs, crustaceans, small-bodied native fish and juvenile large-bodied native fish (prey-switching). If these alternatives cannot be achieved adequately, then there may be an impact on the survival rate of chicks and this may in turn impact on the resilience of some populations of piscivorous waterbird species. Although considered less likely within the Chowilla Floodplain ecosystem than in some other wetlands, the loss of a stable food source (juvenile carp) would nevertheless impact on ecological communities through the stress and possible death of chicks and the increased predation of other native aquatic species. Extrapolated across many colonies of nesting waterbird species, this could have a very marked effect on Chowilla Floodplain ecosystem.

Summary of risks: the food-web effects of freshwater fish kills in Australia and elsewhere are not well understood. The Chowilla Floodplain ecosystem is, however, likely to be protected to some extent by the fact that the population of breeding waterbirds within the Chowilla Floodplain ecosystem, while relatively constant year-to-year, is substantially smaller than in many other ephemeral wetlands. Combined with the suppressive effect that high water temperature during the summer months is likely to have on the exposure of juvenile carp to CyHV-3, it is relatively less likely that breeding waterbirds within the Chowilla Floodplain ecosystem would be exposed to a precipitous loss of a stable food source.

Mitigation of risks: the key treatment option identified for this pathway in the context of the Barmah-Millewa Forest case study (Section 12.1) was to consider the release the virus into the Barmah-Millewa Forest ecosystem a year ahead of releasing it into the Kow Swamp or Gunbower ecosystems. This would minimise the likelihood of concurrent outbreaks and enabling highly-mobile piscivorous species an opportunity to source food from other waterbodies. It was also suggested that it may be possible to release the virus into the Barmah-Millewa Forest during a lower-flow season, when the exposure of colonial-nesting piscivorous waterbirds is likely to be lower. These strategies, however, are less applicable to the Chowilla Floodplain as there are not alternative waterbodies in close proximity and the effect of season on the aggressiveness of the outbreak (high-flow as opposed to lower-flow) is likely to be less marked. The most effective strategy for the Chowilla Floodplain is likely to be the release of farmed juvenile large-bodied native fish species, as these will provide both a source of food for nesting waterbirds and protection for the endemic population of both large-bodied and small-bodied species within the ecosystem (below).

(e) Exposure of native species to increased predation as a result of prey-switching

Likelihood of exposure: the exposure of small-bodied and juvenile large-bodied native fish, crustaceans and turtle eggs and hatchlings to prey-switching is conditional on the exposure of piscivorous species to the sudden removal of a stable food source. It was explained above, however, that the Chowilla Floodplain ecosystem is likely to be protected by the facility for environmental watering offered by the Chowilla regulator and associated structures; and by the fact that the population of breeding waterbirds within the Chowilla Floodplain ecosystem, while relatively constant year-to-year, is substantially smaller than in many other ephemeral wetlands. Combined with the suppressive effect that high water temperature during the summer months is likely to have on the exposure of juvenile carp to CyHV-3, it is relatively less likely that breeding waterbirds within the Chowilla Floodplain ecosystem would be exposed to a precipitous loss of a stable food source.

Likely consequences of exposure: the native species potentially at risk as a result of prey-switching include the juveniles of large-bodied fish, small-bodied fish, crustaceans, frogs, and turtle eggs and hatchlings (Section 11.5). Murray cod juveniles could be at risk in some settings, although these will tend to remain within river channels and major anabranches. Silver perch prefer to spawn in faster-flowing parts of the river system, and over gravel or rock substrate, and their juveniles will also be relatively less exposed. Golden perch juveniles may be exposed, although the broader geographic distribution of this species renders it inherently more resilient. Freshwater catfish spawn within an elaborate nest, and juveniles could be exposed in some shallower parts of the Chowilla Floodplain ecosystem. All six of the small-bodied native fish identified within the Chowilla Floodplain ecosystem are likely to be exposed. Of these, the Murray hardyhead is listed as endangered under the EPBC Act and the impact on this and some other fragmented species could be substantial. The swamp yabby and all eight species of frog are also likely to be quite exposed, as these species are abundant within the ecosystem and pre-exist as components of the diet of many carnivorous (but not strictly piscivorous) waterbirds. The extent to which the eggs or hatchlings of the broad-shelled turtle might be targeted is unknown, although it is likely that many carnivorous waterbirds would opportunistically feed on either or both if a large proportion of carp juveniles was removed.

Summary of risks: the risks associated with this pathway are conditional on the realisation of the prior pathway – that is, stress to populations of (in particular) colonial-nesting piscivorous waterbirds as a result of the removal of juvenile carp. This event in itself is less likely in the Chowilla Floodplain ecosystem, given the facility for environmental watering offered by the Chowilla regulator and associated structures, the relatively smaller (albeit more consistent) population of breeding waterbirds and the suppressive effect of high water temperature during the summer months on an outbreak of CyHV-3 within wetland or floodplain waters. If a loss of juvenile carp was to occur, piscivorous waterbirds are likely to switch to other prey species, or increase their dependence on other prey species. Of these, juvenile Murray cod, the small-bodied native fish, frogs and swamp yabbies are likely to be most exposed.

Mitigation of risks: the key means by which to support local populations of alternative prey species will be through strategic restocking. This is not likely to be as effective in an open and dynamic ecosystem, such as the Chowilla Floodplain, as it might be in a more contained and predictable ecosystem, such as one of the irrigation reservoirs.

(f) Exposure of native species, livestock and humans to an outbreak of botulism

Likelihood of exposure: an outbreak of type C (or C/D mosaic) botulism can arise in two key ways: (a) through the death of animals carrying spores within their gastrointestinal tracts; and (b) through the germination of spores within the environment (Part II: Section 7.6.1). In both cases, the germination of spores is triggered by the development of anaerobic conditions. For carcasses, this occurs when aerobic organisms exhaust available oxygen. Spores within the environment can germinate following any oxygen-depleting process – including the decomposition of large numbers of fish or bird carcasses, or the decomposition of a cyanobacterial bloom. Both pathways for the development of type C (or C/D mosaic) botulism can be linked to an outbreak of CyHV-3. The first pathway relies on the establishment of the carcass-maggot cycle, and this is a highly probabilistic process – the larger the number of affected carcasses, and the population feeding on maggots, the higher the likelihood that the cycle will be sustained. The scenario of highest risk in most ephemeral wetland settings is likely to be a lower-flow season, when carp are reasonably concentrated in available spawning habitat. The Chowilla regulator, however, enables the inundation of a high proportion of wetlands and floodplains with relatively low channel flows. Additionally, there is a capacity to direct water to particular parts of the ecosystem and to maintain their connectivity. This level of regulation means that carp are less likely to be concentrated at a high biomass density in a lower-flow season, and that carcass accumulations are less likely to be substantial. With a large area within the floodplain potentially at risk, some exceptions to this are possible, but lower-flow seasons are nevertheless less likely to result in aggressive outbreaks and the precipitation of the carcass-maggot cycle within the Chowilla Floodplain ecosystem than in other less-regulated ephemeral wetlands. The carcass-maggot cycle also requires aggregations of waterbirds (in particular, insectivorous waterbirds), and most outbreaks of type C (or C/D mosaic) botulism in Australia have been associated with waterbird breeding and nesting events in wetlands or at the edges of waterholes, lagoons, billabongs or shallow lakes. Although the number of nesting waterbirds within the Chowilla Floodplain ecosystem is less than in some larger wetland systems, it is likely to be sufficient for the initiation of a carcass-maggot cycle. The population of breeding or nesting waterbirds within the Chowilla Floodplain is also quite consistent year-to-year, and less dependent on the extent of flow through the river system. This means that an outbreak of botulism could arise in most years.

Likely consequences of exposure: the impacts of an outbreak of type C (or C/D mosaic) botulism within the Chowilla Floodplain ecosystem would extend to most terrestrial and aquatic species, although the exposure of birds and other biota that feed on aquatic and other insects is likely to be higher. This would include, in particular, the waterfowl, large waders and Gruiformes. Insectivorous songbirds and bush-birds would also be at risk, as would frogs and turtles and any livestock with access to the Chowilla Floodplain. Humans are not susceptible to type C (or C/D mosaic) botulinum toxin.

Summary of risks: the reasons why outbreaks of type C (or C/D mosaic) botulism in wildlife occur in some settings, and not in others with an ostensibly similar profile of risk factors, are not well understood but are likely to reflect the strongly probabilistic nature of outbreak initiation and expansion. A range of pathways could be relevant to the Chowilla Floodplain ecosystem, including the carcass-maggot cycle and the germination of environmental spores following an hypoxic or anoxic event. Because carcass accumulation as a result of an aggressive outbreak of CyHV-3 is relatively less likely within the Chowilla Floodplain ecosystem than in some other ephemeral wetland settings, the initiation of an outbreak of botulism is also relatively less likely. The population of waterbirds, while relatively consistent, is also lower than some other wetlands, and this may mean that there is a lower likelihood that the carcass-maggot cycle would be propagated on a large scale.

Mitigation of risks: prevention of an outbreak of type C (or C/D mosaic) botulism will rest on the timely removal of carp and other carcasses. The practicality of this on a large scale within the Chowilla Floodplain ecosystem is doubtful, and priority should be given to parts of the ecosystem in which the number and diversity of colonial-nesting and other waterbirds is highest. This may help to minimise the impacts of an outbreak of botulism but may also help to constrain the probabilistic element of its initiation. In some situations, consideration might also be given to environmental watering as a means by which to disperse and dilute carcasses or help to remove a cyanobacterial bloom from a particularly high-value part of the ecosystem. The impacts of environmental watering are complex however, and this should only be contemplated following the advice of experts.

12.3 Case study 3: Mid-Murray River (Lake Mulwala to Tocumwal)

12.3.1 Physical description of the Lake Mulwala to Tocumwal case study site

This site within the mid-Murry River consists of a section of the main channel from Lake Mulwala (upstream of Yarrawonga Weir) and downstream to the Newell Highway Bridge at Tocumwal (Figure 51). The site is located within the Riverina region of New South Wales and the Hume region of Victoria, and is downstream of Hume Dam and the Ovens River confluence and upstream of Barmah-Millewa Forest. The site includes a relatively wide (60 to 100 metre) reach of river channel that is confined to a corridor of approximately 1.5 km width. Historical movement of the channel has created a diversity of additional off-channel habitats, including anabranches, billabongs, flood-runners, wetlands and floodplains, and forms part of the longest continuous river red gum forest in Australia.

Figure 51 Mid-murray from lake Mulwala to Tocumwal

Source: Google Maps[74]

Lake Mulwala is located above the Yarrawonga Weir, and is also included within the case study (Figure 52 and Figure 53). Yarrawonga Weir, constructed in 1939, is the largest of the 14 weirs on the Murray River downstream of Hume Dam.[75] The weir is located nearly 2,000 km upstream from the Murray River mouth and is the only weir in the Murray system below Hume Dam not to have a lock for the purpose of navigation. A fish lift was added in 1994 to provide passage for native fish. The weir has three principle purposes, namely to raise the head for irrigation, to provide regulation (including flood mitigation), and to provide recreational amenity. Although Lake Mulwala is a relatively shallow lake (14.2 metre at full supply), it forms 4,450 ha of inundation with a storage capacity of 130,000 ML.[76] The majority of this volume is termed 'dead storage' and is used solely to raise the head. The lake is popular for recreation and water sports and supports an

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[74] See: https: //www.google.com/maps/@-35.9217694,145.8422085,39146m/data=!3m1!1e3

[75] See: https: //www.mdba.gov.au/river-information/running-river-murray/yarrawonga-weir

[76] See: https: //www.g-mwater.com.au/downloads/gmw/Storages/Lake_Mulwala_Water_Quality_Brochure.pdf

important recreational fishery – including Murray cod.

Water for irrigation is supplied through two main irrigation channels, the Mulwala Channel and the Yarrawonga Main Channel. The Mulwala Channel (Figure 54), on the New South Wales side, has a discharge capacity up to about 10,000 ML/day. The Yarrawonga Main Channel (Figure 55), on the Victorian side, has a discharge capacity of about 3,200 ML/day. At 2,880 km length, the Yarrawonga Main Channel is the longest irrigation channel in the southern hemisphere. The two channels serve a total area of more than 8,000 km² of irrigated land across the two states.

Figure 52 Railway and road bridges over Yarrawonga Weir.

Source: Murray-Darling Basin Authority (https: //www.mdba.gov.au)

Figure 53 Yarrawonga Weir during low flow (approximately 5,000 ML/day)

Source: P. Caley (June 2018)

Figure 54 Mulwala channel after upgrades in 2018

Source: Murray Irrigation (https: //www.murrayirrigation.com.au)

Figure 55 Yarrawonga Main Channel, drained for maintenance

Source: P. Caley (June 2018)

12.3.2 Hydrology of the Lake Mulwala to Tocumwal site

For the past century, river regulation – including the development of storages, weirs and locks – has increasingly altered the flow regime in the Murray River. Hume Dam, located upstream of the mid-Murray site, has a storage capacity of over 3,000 GL (Figure 57). While this water is used for both human consumptive and environmental uses, the dam has an operational history of reducing the magnitude, frequency and duration of flood events, as well as altering the time at which high flow events occur. Altering the natural patterns of the flow regime in such a way has a detrimental impact on many ecological systems by means including a reduction in the periodic inundation of floodplains, limitation of the connectivity to wetlands and alteration of biological cues such as the initiation of native fish spawning.

Since its completion, Hume dam has provided important water supply for downstream irrigation purposes, with Yarrawonga Weir and its associated off-take channels (Figure 55) being a key distribution point. Water ordered from Hume dam for irrigation purposes has a four-day travel time along the Murray River before arriving at Yarrawonga Weir. During this period, if significant and un-forecast rainfall occurs, demand for irrigation water drops resulting in an unwanted 'rain rejection' flow. Downstream of Yarrawonga and Tocumwal is the Barmah choke (Case Study 1: Section 12.1), which is a section of river with a reduced flow capacity. When flows in the Murray River downstream of Yarrawonga exceed the capacity of the Barmah Choke (approximately 10,400 ML/day), the regulators are progressively opened to allow water to enter the forest. At flows between 10,400 and 16,000 ML/day, channels, swamps and other low-lying areas, including about 16 percent of the Barmah-Millewa Forest, are inundated. While Lake Mulwala serves as a buffer to incoming rain rejected flow, its active storage capacity is limited,[77] with excess flows spilling and flooding during the summer irrigation season rather than the natural winter-spring regime (MDBA, 2015). Yarrawonga Weir also provides environmental flows to downstream ecosystems at suitable times and has a limited role in flood mitigation for localised rainfall. Very substantial downstream flows from Yarrawonga (in excess of 100,000 ML/day) were recorded in 1964, 1970, 1973, 1974, 1975, 1981, 1990, 1992, 1993, 1996, 2010 and 2016 (Figure 56).

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[77] See: https: //www.g-mwater.com.au/downloads/gmw/Storages/Lake_Mulwala_Water_Quality_Brochure.pdf

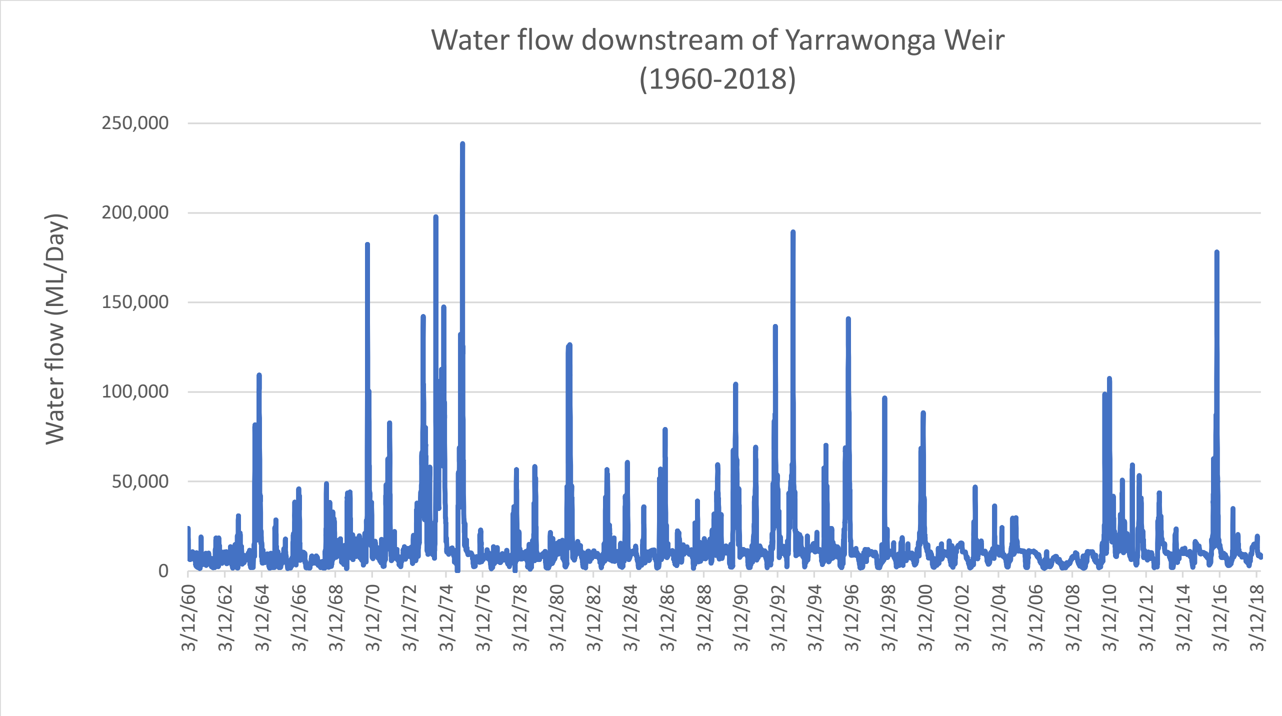

Figure 56 Average daily flow downstream of Yarrawonga from 1960 to 2018

Source: raw data obtained from https: //riverdata.mdba.gov.au/

Figure 57 Looking downstream from Hume Dam during low (environmental) flows

Source: P. Caley (June 2018)

Water is provided to irrigators through the period early August through to early May. The marked variance in annual flows downstream of Yarrawonga Weir through wet and dry seasons were highlighted in Figure 56 and noted above. Superimposed on this variance, however, is a reasonable level of consistency during the peak irrigation months of summer and autumn (Table

21). The variance in annual flows is accrued principally in winter and spring. Data for the period 1960 to 2018 (Figure 58) confirms that, on average, water flow peaks in winter and late spring. Flow decreases in late spring and early summer, and is then reasonably consistent until it begins to decline again in March. The lowest flows are recorded between April and June.

Table 21 Monthly mean stream flow downstream of Yarrawonga (ML/day)

Month	Very dry year (minimum on record)	Dry year (30th percentile)	Median year (50th percentile)	Wet year (70th percentile)
Jul	1,806	5,505	8,538	14,718
Aug	1,953	7,902	12,706	20,080
Sep	1,906	8,379	13,202	25,360
Oct	3,018	10,308	13,919	20,499
Nov	1,800	11,454	15,210	17,785
Dec	1,806	10,600	11,236	12,903
Jan	3,044	8,891	10,339	10,600
Feb	1,786	8,126	8,921	9,910
Mar	3,209	8,920	9,660	10,506
Apr	1,800	7,033	8,619	9,926
May	1,806	2,991	4,204	5,866
Jun	1,800	3,236	4,973	8,463

Source: Ecological Associates and SKM (2011)

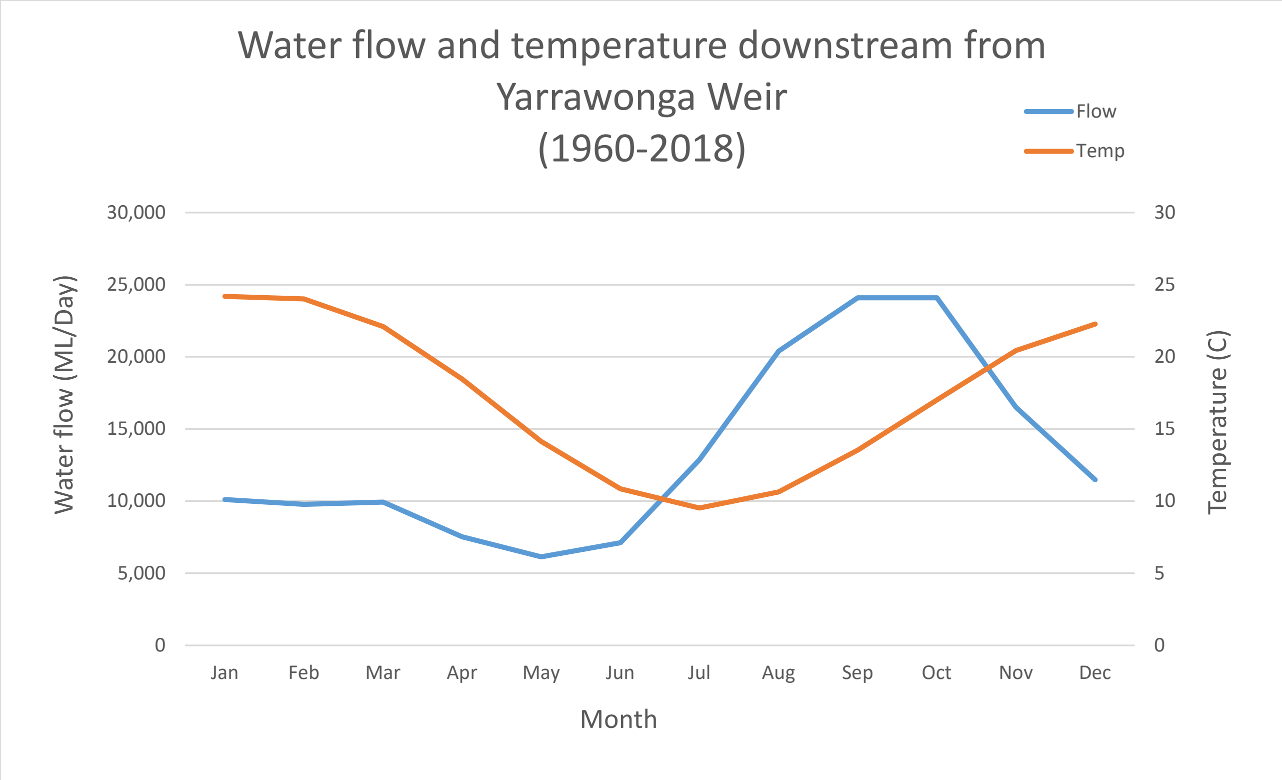

Figure 58 Flow downstream of Yarrawonga Weir (1960-2018)

Water flow (ML/day) and daily average water temperature, aggregated to a monthly average. Data observed downstream of the Yarrawonga Weir, 1960 to 2018.

Source: raw data obtained from https: //riverdata.mdba.gov.au/

12.3.3 Ecological values of the Lake Mulwala to Tocumwal site

The Lake Mulwala to Tocumwal section of the Murray River contains important ecosystems and habitats for a diversity of species. These are protected through a series of national parks and reserves, including Yarrawonga Regional Park, Cobram Regional Park, Big Reedy Lagoon Wildlife Reserve and Tocumwal regional Park in Victoria. The region encompasses part of a large continuous river red gum forest and includes a reserve system containing threatened animal and plant species (MDBA, 2015). Sections of the reach consist of a multi-branched channel with disconnected anabranches remaining from historical movement of the channel through the floodplain by erosion. This movement of the channel has created a diverse system with a number of billabongs, flood-runners and wetlands that connect with different flow volumes (MDBA, 2015) (Figure 59). The resulting diversity of aquatic systems provide important habitat for a range of species.

Lake Mulwala is a sink for a range of substances including nutrients, hydrocarbons, pesticides and heavy metals. During most periods, these remain at levels that are not of concern.[78] Nutrient levels, including phosphorus, in Lake Mulwala hover can be high. When nutrient levels are high,

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[78] See: https: //www.g-mwater.com.au/downloads/gmw/Storages/Lake_Mulwala_Water_Quality_Brochure.pdf

this can contribute to the prevalence of harmful algal blooms. There have been a number of sever algal blooms in the lake in recent years.

Figure 59 Downstream of Yarrawonga Weir during low flow (approximately 5,000 ML/Day)

Source: P. Caley (June 2018)

In a report for the Goulburn Broken Catchment Management Authority, Heard (2006) describes the ecological values of the Murray River and its southern (Victorian) environs. In combination with the broader Victorian riverine plain, this region was termed the Yarrawonga Landscape Zone, and included Tocumwal, Cobram and Yarrawonga Regional Parks and Big Reedy Lagoon Wildlife Reserve.

The native aquatic species known to occupy Yarrawonga Landscape Zone include the broad-shelled tortoise, giant bullfrog (*Limnodynastes interioris*), golden perch, growling grass frog, Murray cod, river blackfish, silver perch and trout cod (Heard, 2006). Important additions to the list provided by Heard (2006) include the Murray crayfish and the freshwater catfish, both of which are commonly found in the Murray River downstream of Yarrawonga Weir.[79] Of these aquatic species, the Murray cod and growling grass frog are listed under the EPBC Act as vulnerable, the trout cod is endangered and the silver perch is critically endangered. Both the trout cod and silver perch are believed to breed within the reach between Lake Mulwala and Tocumwal. The breeding population of non-migratory trout cod within this reach (extending to

[79] See: https: //vfa.vic.gov.au/

Barmah-Millewa Forest, and perhaps to Gunbower) is the only remaining breeding population considered to be sustainable. The mid-Murray River channel is wide and fast-flowing with a sandy-to-silty substrate, and contains a high number of snags. These habitat conditions are optimal for the trout cod and the key reason for the stability of the population in this reach of the Murray River. The breeding population of silver perch is also one of a few remaining sustainable populations, although this species is highly migratory. In February 2018 researchers sampled for both pygmy perch and flat headed galaxias at 12 sites along the Murray River from Lake Mulwala *upstream* to Tintaldra.[80] It is not known if these species exist within the lake or downstream of the Yarrawonga Weir. Historically, the Murray hardyhead is believed to have existed upstream from Mildura, as far as the Yarrawonga Weir, although it is doubtful that this small-bodied fish currently remains within the Lake Mulwala to Tocumwal reach.

The Lake Mulwala to Tocumwal reach (encompassing the Yarrawonga Landscape Zone) also contains a wide range of birdlife – including numerous waterbird species (Table 22). Of these species, the Australasian bittern is listed under the EPBC Act as endangered while the superb parrot is vulnerable. Waterbirds are substantially more diverse and numerous at Lake Mulwala than they are in habitats along the Murray River channel (MDBA, 2008). At both locations, piscivorous birds are the dominant functional group, although herbivorous species are relatively more abundant within habitats along the river channel than at Lake Mulwala (MDBA, 2008).

Table 22 Native birds inhabiting the Yarrawonga Landscape Zone

Common name	Scientific name	Category	Status
Royal spoonbill	*Platalea regia*	Seabird	Not listed
Greater painted snipe	*Rostratula benghalensis*	Shorebird	Not listed
Australasian shoveler	*Anas rhynchotis*	Waterfowl	Not listed
Blue-billed duck	*Oxyura australis*	Waterfowl	Not listed
Freckled duck	*Stictonetta naevosa*	Waterfowl	Not listed
Hardhead	*Aythya australis*	Waterfowl	Not listed
Musk duck	*Biziura lobate*	Waterfowl	Not listed
Australasian bittern	*Botaurus poiciloptilus*	Large wader	Endangered
Great egret	*Ardea alba*	Large wader	Not listed
Baillon's crake	*Porzana pusilla*	Gruiforme	Not listed
Brolga	*Grus rubicunda*	Gruiforme	Not listed
Intermediate egret	*Ardea intermedia*	Gruiforme	Not listed
Little egret	*Egretta garzetta*	Gruiforme	Not listed
Diamond firetail	*Stagonopleura guttata*	Songbird	Not listed
Grey-crowned babbler	*Pomatostomus temporalis*	Songbird	Not listed
Ground cuckoo-shrike	*Coracina maxima*	Songbird	Not listed
Bush stone-curlew	*Burhinus grallarius*	Bush-bird	Not listed

[80] See: https: //www.csu.edu.au/research/ilws/research/summaries/2018/murray-small-bodied-fish

Common name	Scientific name	Category	Status
Red-chested button-quail	*Turnix pyrrhothorax*	Bush-bird	Not listed
Superb parrot	*Polytelis swainsonii*	Bush-bird	Not listed
Swift parrot	*Lathamus discolour*	Bush-bird	Not listed
Barking owl	*Ninox connivens*	Raptor	Not listed
Black falcon	*Falco subniger*	Raptor	Not listed
Grey falcon	*Falco hypoleucos*	Raptor	Not listed
Powerful owl	*Ninox strenua*	Raptor	Not listed
White-bellied sea-eagle	*Haliaeetus leucogaster*	Raptor	Not listed

Source: Heard (2006)

The floodplain component of the Yarrawonga Landscape Zone includes a mixture of river red gum dominated ecological communities, including riverine grassy woodlands, sedgy riverine forest and wetlands, and is flanked in parts by patches of sand ridge woodland (Heard, 2006). Grassy woodland communities border ephemeral drainage lines and creeks. These typically support an over-storey of river red gums and an understorey of wattles, and are lined with tall sedges (Heard, 2006). River red gum wetlands are dominated by river red gum, sedges and rushes. Plains grassy wetlands are in shallow depressions further onto the riverine plain, where meanders of prior or ephemeral steams occurred. These shallow seasonal wetlands are typically treeless, with a grassland structure grading into sedgeland or herbland (Heard, 2006).

12.3.4 Carp biomass and population dynamics within the Lake Mulwala to Tocumwal site

The NCCP project team modelling carp biomass density across Australia found that the average density across the mid-Murray was approximately 115 kg/ha (Stuart *et al.*, 2019). Importantly, however, this estimate spanned both the reach between Yarrawonga Weir and Tocumwal, and the Barmah-Millewa Forest. The latter included very low estimates from the Barmah-Millewa Forest floodplain, which tended to skew the overall average value for the 'mid-Murray'. With that in mind, a higher estimate of 251 kg/ha was obtained for Lake Mulwala, while the carp biomass density within the river channel ranged from about 188 to about 200 kg/ha. The density within anabranches and billabongs was generally higher than in the river channel (toward 209 kg/ha) although in some places dropped down as low as 170 kg/ha. The reasons for this variance were not given, but are likely to reflect differences in the connectivity of individual waterbodies and the time of year and season (high-flow or lower-flow) when the data were obtained. Substantial resources are committed to electrofishing and carp population estimates for this reach of the Murray River (Figure 60), and the data used for biomass modelling is likely to be some of the most extensive and robust available.

Figure 60 Electrofishing in the Murray River, downstream of the Yarrawonga Weir

Source: P. Caley (June 2018)

One of the key concerns in respect of this assessment is the spawning behaviour of carp within the Lake Mulwala to Tocumwal case study site. Nicol *et al.* (2004) found very few juvenile fish within the reach of the Murray River between Yarrawonga Weir and Tocumwal, when compared with spawning on off-stream floodplain habitats in the Barmah-Millewa Forest. Citing the carp movement studies of Stuart and Jones (2002), Nicol *et al.* (2004) noted that adult carp from this reach had been observed to move downstream into the Barmah-Millewa Forest at times that corresponded with carp spawning activities. Individuals had also been observed to move from the Barmah-Millewa Forest upstream into the study reach. The apparent lack of spawning in the study reach, and the movement dynamics reported by Stuart and Jones (2002), suggested to Nicol *et al.* (2004) that carp recruitment into the reach of the Murray River between Yarrawonga Weir and Tocumwal may be driven primarily by spawning within the Barmah-Millewa Forest. These observations and inferences were broadly reciprocated through the later work of Stuart and Jones (2006b). These authors also noted that of the recaptured tagged carp that had moved longer distances after spawning in the Barmah-Millewa Forest, almost half (44 percent) had migrated upstream. These recaptured carp were located as far upstream as Cobrawonga.

On the basis of these observations and deductions, it is likely that a significant proportion of spawning and recruitment to the population of carp within the reach of the Murray River between Yarrawonga Weir and Tocumwal occurs within the Barmah-Millewa Forest. Although unverified within the literature, it also seems likely that this proportion would increase in high-flow seasons when the Barmah-Millewa floodplains are inundated and carp recruitment across the Murray-Darling system is most active. Beyond the Barmah-Millewa Forest, spawning is also likely to occur in the lentic off-channel anabranches and billabongs – some of which may become partially or completely disconnected during lower-flow seasons. Carp biomass density (see above) was estimated to be slightly higher at many of these locations than in adjacent segments of the main river channel (Stuart *et al.*, 2019). In places, these off-channel waters also include wetland or marshland ecosystems and the fringes of these would provide idea spawning habitat for carp.

Lake Mulwala (Figure 61) provides a separate ecosystem to the Murray River channel and its anabranches and billabongs, and supports a slightly higher biomass density of carp (above). Farmed juvenile native fish are also introduced into the lake for recreation and environmental purposes. The eastern end of the lake (including its confluence with the Ovens River) encompasses numerous small forested islands and semi-connected protrusions from the banks of the river channel, and the fringes of these are likely to provide suitable spawning habitat for carp. The western end of the lake is progressively more urbanised, and culminates at Yarrawonga Weir. Along the fringes of the western part of the lake, pockets of suitable spawning habitat are also likely. Carp within Lake Mulwala are less likely to migrate in significant numbers, and larger females in particular will tend to display a certain degree of site fidelity in respect of their year-to-year spawning grounds (Stuart and Jones, 2006b).

Figure 61 Lake Mulwala, including southern confuence with the Ovens River

Source: Google Maps[81]

12.3.5 CyHV-3 outbreak scenarios for the Lake Mulwala to Tocumwal reach

The character of an outbreak of CyHV-3 is likely to be different within: (a) the main Murray River channel; (b) its minor anabranches, billabongs and wetlands; and (c) Lake Mulwala. There is a range in carp biomass density amongst these environments (above); as well as a range in the connectivity of individual waterbodies and the likely temperature and quality of the water, and the diversity of species and communities that may be at risk. These factors are likely to influence both the behaviour of the disease and its impacts.

The **Murray River channel** is a lotic and relatively deep-water setting. Its flow varies most in winter and spring, as a result of rainfall within the upstream catchment, but is reasonably stable through the balance of the year. Even in exceptionally dry seasons, flow from Hume Dam and through this segment of the Murray River is effectively guaranteed by the importance of downstream irrigation and environmental watering. Carp within the Murray River channel are likely to spawn in the lentic

[81] See: https: //www.google.com/maps/@-36.0001123,146.1291171,11760m/data=!3m1!1e3

off-channel habitats, including the minor anabranches, billabongs and wetlands (as discussed below). In high-flow seasons, a certain proportion may also migrate downstream to the spawning grounds within the Barmah-Millewa Forest ecosystem. Relatively fewer carp are likely to spawn within the main channel, and these will tend to choose pockets of protected and less rapid flow around (for example) extruding banks or large snags. As for most other settings, an outbreak is likely to be initiated by an aggregation event if the water temperature exceeds the accepted minimum for effective transmission (approximately 18C: Section 5.3.4). Aggregations are frequently observed immediately below the Yarrawonga Weir. Further transmission may then occur between individual spawning fish. Although an outbreak that develops in this setting may be reasonably aggressive, the strong flow and water depth are likely to mean that carcass accumulations are minor and the outbreak itself is less visible. By way of illustration and comparison, while substantial fish deaths are known to have resulted from the blackwater event that affected approximately 1,800 km of the Murray River channel over a 4-month period in 2010-11 (Part II: Section 6.2), there were very few reports of observed massed fish kills.

The **minor anabranches, billabongs and wetlands** within the Yarrawonga to Tocumwal reach of the Murray River are lentic and relatively shallow waterbodies, and in parts may be ephemeral and dependent on the extent of flow through the main river channel. Although a diversity of habitats will be encompassed, an outbreak within these settings is in general likely to be more aggressive and more outwardly visible than an outbreak in the river channel. This is because the carp biomass density is in general slightly higher, and the water velocity, depth and quality generally lower. It is also likely that more spawning will occur in these habitats than in the main river channel and, thus, more opportunity for ongoing transmission following from an aggregation event. Carcass accumulation within these settings, and the accumulation of decomposition products, may also be more significant as there is less potential for flushing the system through substantial river flows. As these waterbodies are in general quite shallow, there is also less opportunity for carcasses to sink to benthic layers to complete their decomposition.

An outbreak in **Lake Mulwala** is the least predictable of the three settings. Although the lake includes some complex forested lentic sections (in particular, the eastern segment, and including the confluence awith the Ovens River) it is also reasonably deep in comparison with other outbreak settings (approximately 14 metres when full) and large (approximately 4,450 ha, with storage capacity of 130,000 ML) and may service downstream flows in excess of 100,000 ML/day. Collectively these characteristics mean that it would be relatively difficult for an outbreak of CyHV-3 to result in the accumulation of substantial numbers of carp carcasses or decomposition products. Some accumulation may occur in relatively more protected, shallow and lentic parts of the lake, although these will also be flushed to some extent by flows toward and through the Yarrawonga Weir. The above notwithstanding, an aggressive outbreak (albeit a less visible one) remains possible in this setting as the carp biomass density is relatively high and predictable flows from Hume Dam ensure that substantial carp spawning events within the lake are also likely in most seasons.

12.3.6 Exposure pathways and risks for the Lake Mulwala to Tocumwal reach

A generic assessment for the nine identified exposure pathways was undertaken in Section 11. The discussion below interprets the outcomes from Section 11 in the context of the ecosystems within the reach between Lake Mulwala and Tocumwal and its natural assets.

(a) Exposure of native aquatic species to low DO

Likelihood of exposure: the accumulation of carp carcasses in sufficient numbers to result in deoxygenation is unlikely in either Lake Mulwala or the Murray River main channel. The carp biomass density is not high in these locations, by comparison with other parts of the Murray-Darling Basin, and the depth and flow of the water are likely to be sufficient to ensure that carcass materials are redistributed, and dissolved oxygenation replenished. Carcass accumulation is, however, possible in some less connected minor anabranches, billabongs and wetlands, where water is able to warm and stagnate. Although there may be some variation in inundation and flow through these waterbodies in wet and dry years, it is unlikely to be as significant to the risk of a low DO event as in some other settings.

Likely consequences of exposure: low DO will primarily affect fish and crustaceans. Air-breathing aquatic animals will not be directly affected by low DO. Indirectly, low DO may also impact on ecological communities and on the integrity of wetlands. Although the endangered trout cod are present in the main river channel, they are primarily a lotic species and less likely to occupy the minor anabranches, billabongs and wetlands. Murray cod are less fixed to a home range during spawning season and may venture into these lentic waters. As a mobile species, however, most are equally able to leave waters in which DO is diminishing and return to the river channels. Silver perch prefer to spawn in faster-flowing parts of the river system, and over gravel or rock substrate, and are less likely to enter the lentic off-channel waters. Golden perch juveniles may be exposed, although the broader geographic distribution of this species renders it inherently more resilient. Freshwater catfish spawn within an elaborate nest, and juveniles could be exposed in some shallower parts of the Chowilla Floodplain ecosystem. Small-bodied native fish would be exposed, although the species and their population strength is this part of the river system was not established. Murray crayfish prefer permanent rivers and large streams where the water flows moderately fast, and are less likely to inhabit minor anabranches, billabongs and wetlands. Overall, Murray cod are arguably the most exposed of the large-bodied native fish to hypoxic events within the minor anabranches, billabongs and wetlands of this case study. Hypoxic events that occur during this time may result in a lower recruitment, although the impact of this on the sustainability of the local population may be lower for a long-lived species such as the Murray cod.

The minor anabranches, billabongs and wetlands support a range of ecological communities, including river red gum forests and woodlands and floodplain marshes. The impact of low DO on these communities is most likely to be realised during high-flow seasons, when these areas are inundated. Although widespread low DO is less probable under the high-flow scenario, pockets of affected water may develop. Where this occurs, small communities – or smaller parts of a broader community – may experience the stress or death of fish and crustaceans. This affect is likely to be short-term (1 to several weeks), and is unlikely to affect the quality of the community when the floodplains are next inundated. The minor anabranches and billabongs also support a range of riparian and wetland communities, which may be permanent or semi-permanent. The impact of low DO on these communities may be more marked in the sense that they are not transient, and will not be regenerated with each inundation event. A low DO event could also potentially occur in many of these communities during any season – that is, it would not be dependent on inundation of the floodplains.

Summary of risks: the Lake Mulwala to Tocumwal reach includes the river channel; minor anabranches, billabongs and wetlands; and Lake Mulwala. Of these habitats, the minor anabranches, billabongs and wetlands are the most likely to experience an accumulation of carp carcasses sufficient to result in deoxygenation. The Murray cod would arguably be the most exposed should a low DO event occur within this setting, although most cod would also be able to evade pockets of poor water quality by returning toward the river channel. The floodplain forest, woodland and marshland communities would be affected if hypoxia was to follow from an outbreak of CyHV-3 during a high-flow season, although this scenario is less likely given the spatial distribution (low density) of carp when the broader riparian areas are inundated. Permanent and semi-permanent riparian and wetland communities are likely to be more exposed, given that hypoxia could affect these during most seasons.

Mitigation of risks: the mitigation of risks associated with low DO will focus largely on the timely removal of carp carcasses. The extent to which this will be necessary, and practicable, will vary amongst settings although the overarching goal will be to prevent the DO within a given waterbody falling below about 3 to 4 mg/L or to enable it to return to that level. Access to affected areas might be difficult. Unlike regulated wetlands, there would be limited ability to use environmental watering to help flush out carcasses and carcass materials and to replenish areas with oxygenated water.

(b) Exposure of native species, livestock and humans to cyanobacterial blooms

Likelihood of exposure: as noted above, carcass accumulation is only likely to occur within the minor anabranches, billabongs and wetlands of this reach. Although cyanobacterial blooms are not uncommon in both the river channel and Lake Mulwala, they are unlikely to be precipitated in these settings by the accumulation of carp carcasses as a result of an outbreak of CyHV-3. Within the minor anabranches, billabongs and wetlands, however, a lower water velocity in combination with shallower and warmer water and a higher carp biomass density, mean that an aggressive local outbreak is possible. This possibility may be enhanced by the observation that carp favour these habitats for spawning. If a widespread cyanobacterial bloom was to be precipitated within this setting, it is likely to result in the direct exposure of most aquatic species and the indirect exposure of a wide range of terrestrial species – including amphibians and nesting waterbirds. Harm may then result from either the direct effect of cyanotoxins (if the strain(s) of cyanobacteria involved are cyanotoxic) or indirectly when the bloom dies off and decomposes, raising the BOD and leading to a precipitous fall in DO.

Likely consequences of exposure: it was concluded within the discussion of this exposure pathway (Section 11.2) that the risks associated with widespread cyanobacterial blooms for large- and small-bodied native fish and crustaceans will largely mirror those associated with low DO. In addition to these, however, are risks to waterbirds, frogs and turtles. The risks posed by widespread cyanobacterial blooms to ecological communities and wetlands are also different to those posed by low DO.

Waterbirds are substantially less numerous and diverse within the minor anabranches, billabongs and wetlands, and the river channel, than they are within the environs of Lake Mulwala. Although piscivorous birds are the dominant functional group, herbivorous species are more plentiful than they are within the environs of Lake Mulwala. The endangered Australasian bittern is likely to be present, as is the vulnerable superb parrot. The risk drivers for this and other birds will be the same within the minor anabranches, billabongs and wetlands of this reach of the Murray River as discussed in a generic sense in the pathway description for cyanobacterial blooms (Section 11.2). In particular, the risks will be higher for those functional groups or species whose feeding, foraging, nesting and other behaviours place them in close and regular contact with water. Colonial nesters are also at higher risk, as stressors to these populations are likely to be applied to a large number of birds at a time when harm can fall to adults and the success of recruitment can be threatened. Diet is also important, in particular as cyanotoxins bioaccumulate in animal tissues and regular exposure to affected (living) biota can mean that a toxic threshold is eventually breached.

The giant bullfrog and the vulnerable growling grass frog have also been recorded in the reach. If a widespread outbreak of cyanotoxic cyanobacteria was precipitated by the decomposition of carp carcasses, then significant numbers of frogs are likely to be exposed. The broad-shelled turtle is also endemic to the reach and this would be exposed to cyanotoxin if a widespread bloom was to occur.

The floodplain forest, woodland and marshland communities would be affected if a widespread bloom was precipitated by the accumulation of carp carcasses. Permanent and semi-permanent riparian and wetland communities are likely to be more exposed, given that a widespread bloom could be precipitated within these during most seasons. In any affected community, the impacts of a widespread cyanobacterial bloom may include the direct effect of cyanotoxin (if the strain involved is cyanotoxic) on all species in contact with water, consuming water or consuming biota that is itself affected with cyanotoxins; the effect of the precipitous drop in DO that will inevitably follow from collapse of the bloom; the decline in water quality that will follow from the death and decomposition of cyanobacteria and aquatic biota; and the ecosystem-wide impacts of these processes on food webs.

Livestock and humans are also susceptible to cyanotoxins, and the risk to both were considered in a generic sense within the description of this exposure pathway (Section 11.2). This reach of the Murray River includes a wide range of land tenures, and some stock may have access to its waters. Cyanobacterial blooms are not uncommon, and it is understood that managers will be accustomed to monitoring water quality. Although recreational fishing is permitted under permit it is very unlikely that drinking water, fish or crustaceans would be taken for human consumption from an area where mass fish mortalities or destructive cyanobacterial blooms were occurring. This likelihood would be further ameliorated through signage in public places. Caution would extend to the use of contaminated waters for swimming and other recreation, although the malodorous nature of a fish kill is likely to be a sufficient deterrent. On balance there are no realistic scenarios under which humans or farmed (c.f. feral) livestock would be significantly exposed to harm as a result of an outbreak of CyHV-3 within the minor anabranches, billabongs and wetlands of this reach of the Murray River.

Summary of risks: although cyanobacterial blooms are not uncommon in the Murray River and Lake Mulwala, carcass accumulation is only likely to occur within the minor anabranches, billabongs and wetlands. Significant waterbirds occupy these habitats, albeit in lower numbers and with less diversity in species than within the environs of Lake Mulwala. As for other settings, birds whose feeding, foraging, nesting and other behaviours place them in close and regular contact with water are at highest risk. Colonial nesters are also at high risk, as are the birds whose diet includes fish and other aquatic animals. Frogs and turtles will also be directly affected if the species of cyanobacteria is cyanotoxic.

Mitigation of risks: the mitigation of risks associated with widespread cyanobacterial blooms within the minor anabranches, billabongs and wetlands of this reach of the Murray River would only focus on the removal of carp carcasses if these had accumulated in sufficient numbers and across a sufficiently broad area to be likely to result in the initiation of a bloom. As was noted for the low DO exposure pathway (above) the extent to which this will be practicable, will vary amongst settings. Unlike regulated wetlands, there would be limited ability to use environmental watering to help flush out carcasses and carcass materials and to replenish areas with oxygenated water.

(c) Exposure of native species, livestock and humans to the pathogens associated with decomposing fish carcasses

Likelihood of exposure: little information is available about the pathogens that may proliferate in the event of a fish kill, and their possible impacts on native species, livestock or humans. This deficit was discussed in the generic description of this pathway (Section 11.3) and in the underpinning review of the literature (Part II: Section 6.3.4). That aside, it is possible that carp gut flora, spoilage organisms, *E. coli*, *Pseudomonas spp*, *Aeromonas spp* and other opportunistic microorganisms might proliferate and, in doing so, may present a hazard to native aquatic and terrestrial species. This would be as likely within the minor anabranches, billabongs and wetlands of this reach of the Murray River as in any other freshwater setting.

Likely consequences of exposure: although there is substantial uncertainty (and likely variation) around the pathogens that may be released following a substantial fish kill, and their ability to survive in an aquatic environment and cause harm to aquatic or terrestrial biota, it is reasonable to expect that the relative exposure of communities, functional groups and species will be similar to that obtained for widespread cyanobacterial blooms – albeit with a lower likelihood of realising the identified ecological endpoints. Balancing this, it was also noted within the generic evaluation of this exposure pathway (Section 11.3) that the experience obtained from field observations of Australian freshwater waterways is that while substantial fish kills are not uncommon, they have not to-date been linked to subsequent epidemics of waterborne pathogens. Some waterborne microorganisms may affect people and livestock, although people are likely to be protected to a large extent by the innate repulsiveness of a waterbody that contains the detritus of a significant outbreak. Livestock, however, may have less ability to avoid affected drinking water if there are limited or no alternatives. The risks to ecological communities are understandably highest for wetlands and marshlands, although an outbreak affecting a waterbody within or proximal to a forest and woodland, or grassland, ecological community, may also impact on species that utilise that water.

Summary of risks: the likelihood that microorganisms associated with the decomposition of carp carcasses would proliferate following a carp die-off and become a threat to native species or communities, or to livestock or people, is as significant in the minor anabranches, billabongs and wetlands of this reach of the Murray River as it is in any other freshwater setting. Accepting this, the profile of risks associated with waterborne microorganisms for individual species, functional groups, communities, livestock and humans will be reasonably similar to those associated with widespread cyanobacterial blooms – albeit with lower likelihoods.

Mitigation of risks: as for both low DO and widespread cyanobacterial blooms, mitigation of risks associated with waterborne microorganisms within the minor anabranches, billabongs and wetlands of this reach of the Murray River is likely to focus primarily on the timely removal of carp carcasses. As was noted for the low DO exposure pathway (above) the extent to which this will be practicable, will vary amongst affected anabranches, billabongs and wetlands.

(d) Exposure of native piscivorous species to the removal of a dominant and stable food source

Likelihood of exposure: although the accumulation of large numbers of carp carcasses is less likely within Lake Mulwala than it is in the minor anabranches, billabongs and wetlands of the downstream reach of the Murray River, the relatively higher carp biomass density and consistent spawning means that there is nevertheless an opportunity for an aggressive outbreak of CyHV-3 to occur. As the water temperature within the lake is also unlikely to exceed the upper threshold for the transmission of CyHV-3 during the grow-out season for highly-susceptible carp juveniles, there is also opportunity for these to be affected significantly and for a sudden drop in the availability of a stable food source for piscivorous waterbirds. Lake Mulwala hosts a very substantial population of breeding waterbirds, including the piscivorous colonial-nesting waterbirds.

Likely consequences of exposure: if colonial-nesting and strictly piscivorous waterbird species within the environs of Lake Mulwala are exposed to the sudden removal of stable and dominant food source (juvenile carp) then they may be able to compensate for this by feeding on alternative waterbodies in the vicinity (including the river channel, or its anabranches, billabong and wetlands) or by switching to alternative food sources – including frogs, crustaceans, small-bodied native fish and juvenile large-bodied native fish (prey-switching). If these alternatives cannot be achieved adequately, then there may be an impact on the survival rate of chicks and this may in turn impact on the resilience of some populations of piscivorous waterbird species. An impact on breeding waterbirds would in turn have a very marked effect on ecological communities within and in the environs of Lake Mulwala.

Summary of risks: the food-web effects of freshwater fish kills in Australia and elsewhere are not well understood. Lake Mulwala is, however, protected by the proximity of alternative waterbodies, and while an aggressive outbreak of CyHV-3 is possible in this setting it is relatively less likely to result in the stress of the substantial community of colonial-nesting piscivorous waterbirds.

Mitigation of risks: the key treatment option identified for this pathway in the context of the Barmah-Millewa Forest case study (Section 12.1) was to consider the release the virus into the Barmah-Millewa Forest ecosystem a year ahead of releasing it into the Kow Swamp or Gunbower ecosystems. This would minimise the likelihood of concurrent outbreaks and enabling highly-mobile piscivorous species an opportunity to source food from other waterbodies. This strategy may also be possible within the Lake Mulwala to Tocumwal reach, in the sense that the virus could be released within Lake Mulwala ahead of its release downstream of Yarrawonga Weir. Further mitigation might also be possible through the release of farmed juvenile large-bodied native fish species, as these will provide both a source of food for nesting waterbirds and protection for the endemic population of both large-bodied and small-bodied species within the ecosystem (below).

(e) Exposure of native species to increased predation as a result of prey-switching

Likelihood of exposure: the exposure of small-bodied and juvenile large-bodied native fish, crustaceans and turtle eggs and hatchlings to prey-switching is conditional on the exposure of piscivorous species to the sudden removal of a stable food source. It was explained above, however, that despite its very high diversity and number of breeding waterbirds Lake Mulwala (the most exposed to the food-web effects of CyHV-3 of the three parts to this case study) is likely to be protected by its proximity to alternative waterbodies.

Likely consequences of exposure: the native species potentially at risk as a result of prey-switching include the juveniles of large-bodied fish, small-bodied fish, crustaceans, frogs, and turtle eggs and hatchlings (Section 11.5). Murray cod and silver perch juveniles would be amongst the large-bodied native species at risk within Lake Mulwala. Golden perch juveniles may be exposed, although the broader geographic distribution of this species renders it inherently more resilient. Freshwater catfish spawn within an elaborate nest, and juveniles could be exposed in some shallower parts of the lake. Any of the small-bodied native fish would also be exposed, although the extent of their populations within this setting is not known. Both species of frog are also likely to be quite exposed, as these species pre-exist as components of the diet of many carnivorous (but not strictly piscivorous) waterbirds. The extent to which the eggs or hatchlings of the broad-shelled turtle might be targeted is unknown, although it is likely that many carnivorous waterbirds would opportunistically feed on either or both if a large proportion of carp juveniles was removed.

Summary of risks: the risks associated with this pathway are conditional on the realisation of the prior pathway – that is, stress to populations of (in particular) colonial-nesting piscivorous waterbirds as a result of the removal of juvenile carp. This event in itself is less likely within the Lake Mulwala ecosystem, given the proximity of alternative waterbodies. If a loss of juvenile carp was to occur, piscivorous waterbirds are likely to switch to other prey species, or increase their dependence on other prey species. Of these, juvenile Murray cod and silver perch, any small-bodied native fish, the two species of frogs and broad-shelled turtles are likely to be most exposed.

Mitigation of risks: the key means by which to support local populations of alternative prey species will be through strategic restocking. This is ongoing within Lake Mulwala, as a component of the receational fishing industry.

(f) Exposure of native species, livestock and humans to an outbreak of botulism

Likelihood of exposure: an outbreak of type C (or C/D mosaic) botulism can arise in two key ways: (a) through the death of animals carrying spores within their gastrointestinal tracts; and (b) through the germination of spores within the environment (Part II: Section 7.6.1). In both cases, the germination of spores is triggered by the development of anaerobic conditions. For carcasses, this occurs when aerobic organisms exhaust available oxygen. Spores within the environment can germinate following any oxygen-depleting process – including the decomposition of large numbers of fish or bird carcasses, or the decomposition of a cyanobacterial bloom. Both pathways for the development of type C (or C/D mosaic) botulism can be linked to an outbreak of CyHV-3. The first pathway relies on the establishment of the carcass-maggot cycle, and this is a highly probabilistic process – the larger the number of affected carcasses, and the population feeding on maggots, the higher the likelihood that the cycle will be sustained. Aggregations of waterbirds (in particular, insectivorous waterbirds) are ideal for this scenario, and most outbreaks of type C (or C/D mosaic) botulism in Australia have been associated with waterbird breeding and nesting events in wetlands or at the edges of waterholes, lagoons, billabongs or shallow lakes. Of the three environments that make up this case study, the minor anabranches, billabongs and wetlands are the most likely to experience conditions suited to the development of an outbreak of type C (or C/D mosaic) botulism. Although the number of nesting waterbirds within this setting is lower than on Lake Mulwala, it is likely to be sufficient for the initiation of a carcass-maggot cycle. The population of breeding or nesting waterbirds within the minor anabranches, billabongs and wetlands is also quite consistent year-to-year, given the predictability of flow within this reach of the Murray River. This means that an outbreak of botulism could arise in most years.

Likely consequences of exposure: the impacts of an outbreak of type C (or C/D mosaic) botulism within the minor anabranches, billabongs and wetlands of this reach of the Murray River would extend to most terrestrial and aquatic species, although the exposure of birds and other biota that feed on aquatic and other insects is likely to be higher. This would include, in particular, the waterfowl, large waders and Gruiformes. Insectivorous songbirds and bush-birds would also be at risk, as would frogs and turtles and any livestock with access to the affected waterbodies. Humans are not susceptible to type C (or C/D mosaic) botulinum toxin.

Summary of risks: the reasons why outbreaks of type C (or C/D mosaic) botulism in wildlife occur in some settings, and not in others with an ostensibly similar profile of risk factors, are not well understood but are likely to reflect the strongly probabilistic nature of outbreak initiation and expansion. A range of pathways could be relevant to the minor anabranches, billabongs and wetlands of this reach of the Murray River, including the carcass-maggot cycle and the germination of environmental spores following an hypoxic or anoxic event. The population of waterbirds, while relatively consistent, is lower than some other wetlands, and this may mean that there is a lower likelihood that the carcass-maggot cycle would be propagated on a large scale.

Mitigation of risks: prevention of an outbreak of type C (or C/D mosaic) botulism will rest on the timely removal of carp and other carcasses. The practicality of this on a large scale within the minor anabranches, billabongs and wetlands of this reach of the Murray River is doubtful, and priority should be given to parts of the ecosystem in which the number and diversity of colonial-nesting and other waterbirds is highest. This may help to minimise the impacts of an outbreak of botulism, although may also help to constrain the probabilistic element of its initiation.

12.4 Case study 4: Moonie River Catchment

12.4.1 Physical description of the Moonie River Catchment case study site

This case study differed from others in that it was undertaken at a catchment scale (Figure 65). The reasons for this were the importance of capturing the catchment-wide ecosystem characteristics of the Moonie River and a lack of published information about particular reaches.

An overview of water resources and environmental issues within the Moonie River Catchment was undertaken 1999 by the Queensland Department of Natural Resources (DNR, 1999), who explained that the catchment extends in a southwest direction from the Wambo Shire in the north east to the junction of the Moonie and Barwon Rivers, approximately 30 km south of the

Queensland-New South Wales border. In total, the catchment covers an area of approximately 14,870 square kilometres, with the majority of this (approximately 97 percent) within Queensland. The Moonie River Catchment drains into the Barwon River, and is part of the Murray-Darling River system. The principal stream within the catchment is the Moonie River, which terminates at its junction with the Barwon River. The river's source is approximately 475 km upstream of the border in the hills west of Dalby. The Thomby Range provides a catchment divide to the north of the river.

The Moonie River Catchment is essentially flat with low relief hills scattered throughout its floodplains (Davis, 2017). The Moonie River is a simple channel system (Figure 62) with few tributaries compared with other rivers in the Basin.[82] The river exists for most of the time as a series of disconnected waterholes (Figure 63 and Figure 64).[83] The catchment is heavily cleared and impacted by agricultural development, with highly-eroded banks and riparian zones. The region features remnant areas of brigalow scrub, mixed eucalypt woodland areas and open grasslands.

Figure 62 Moonie River channel

Source: Jon Marshall (2019)

[82] See: https: //www.mdba.gov.au/discover-basin/catchments/moonie

[83] Note that here and throughout this case study, the term 'waterhole' is used to encompass true waterholes as well as lagoons and billabongs

Figure 63 Diminishment of flow in the Moonie River channel

Source: Jon Marshall (2019)

Figure 64 Disconnected terminal waterhole within the Moonie River channel

Source: Jon Marshall (2019)

The Moonie catchment has a hot to warm semi-arid climate, with an average annual rainfall of 500 to 600 mm. There is considerable annual variation in rainfall and temperature, resulting in irregular and infrequent river flows. There are major shallow and deep groundwater aquifers in the Moonie catchment in the St George alluvium, and in sedimentary aquifers above the Great Artesian Basin. The sedimentary rock aquifers of the Great Artesian Basin, deep beneath the catchment, are also an important water source for the region. More than 70% of the catchment is used for grazing beef cattle on native and improved pasture. About 10% of the land is used for dryland cropping and about 15% is native vegetation. There is a very small area of irrigated cropping in the west of the catchment, mainly cotton, and this accounts for about 90% of water use in the region. Almost all irrigation in the region depends on surface water. However, these diversions are small, accounting for only 0.2% of surface water diverted for irrigation in the Basin. Groundwater use is less than 0.1% of the Basin total, excluding water from the confined aquifers of the Great Artesian Basin, which provides around 70% of the groundwater used in the region.

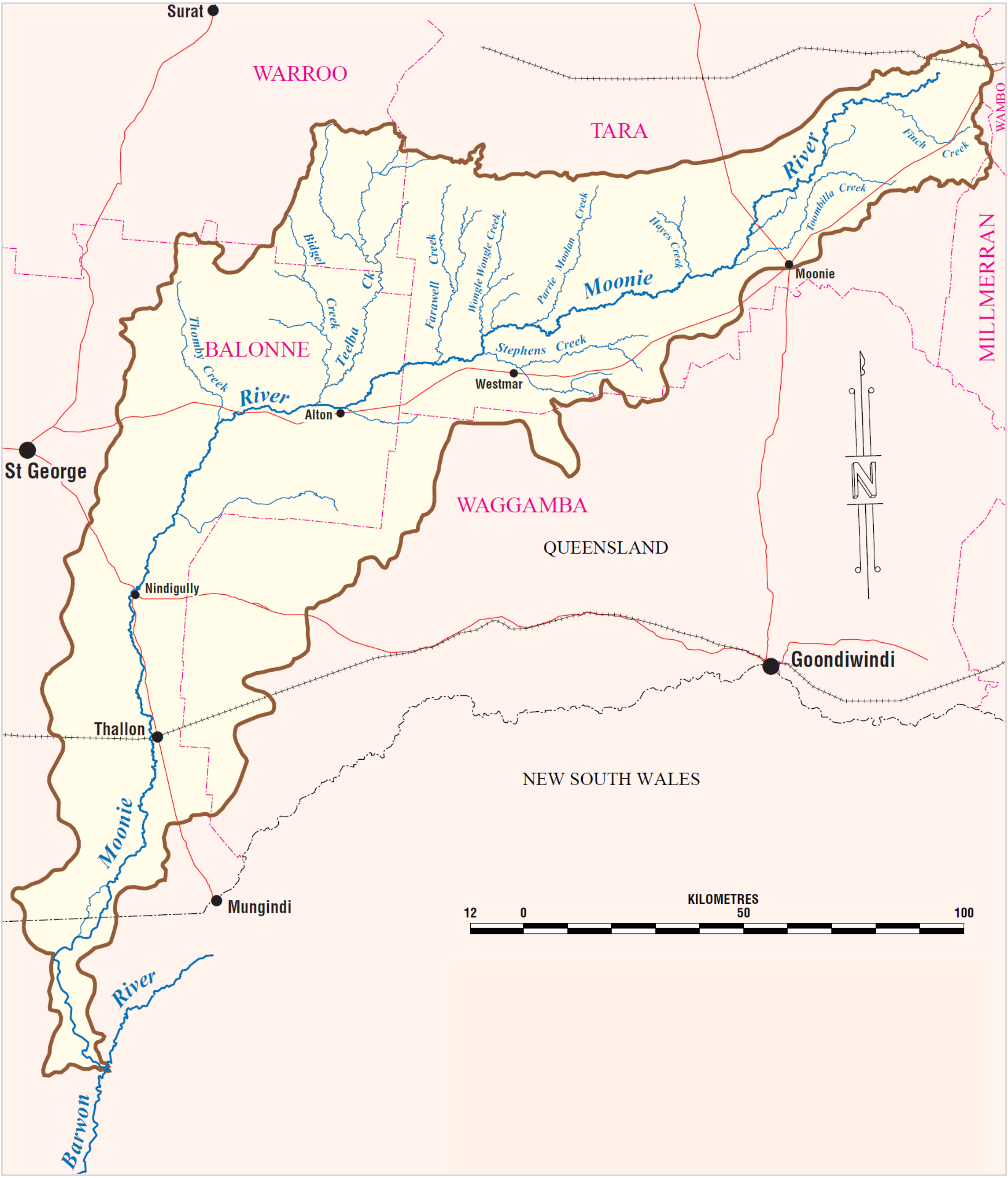

Figure 65 Moonie River Catchment

Source: DNR (1999)

12.4.2 Hydrology of the Moonie River Catchment

The Moonie River is a predominantly unregulated system and has no water major storages. A weir was built over the river at Thallon in 1959 to supply town water. Small-to-medium weirs are dispersed along the river for stock and domestic purposes, with some larger structures for irrigation (predominantly cotton). The majority of water stored is harvested through capture of overland flows and the diversion of floodwater during episodic flow events. Water is stored in large shallow floodplain storages known as ‘ring tanks’ or ‘turkey nest dams’.

Hydrology is rainfall-runoff dominated, with very little base-flow contribution (Davis, 2017). Due to the shallow gradient of the river channel, flow velocities during flow events are low and the river's moderately expansive floodplains of cracking clay throughout the middle and lower reaches are inundated for several days to 2 to 3 weeks at a time, with an average flood event frequency of 1.5 to 2 years (Marshall *et al.*, 2016). During periods without flow, which are common and can last up to 700 days, a series of isolated waterholes along the main river channel provide refugia for aquatic biota, including native and introduced fish (Marshall *et al.*, 2016). Waterholes connect only during flow events, which tend to be brief and associated with large rainfall events, typically during the summer months (Marshall *et al.*, 2016). The highest flood on record occurred in March 2010 when the river rose to a height of 4.65 metres at Nindigully and to 5.50 metres at Thallon (Davis, 2017).

Marshall (*pers. comm.*, 2019)[84] undertook an analysis of a range of ephemeral rivers within the northern (Queensland) Murray-Darling Basin to determine the times of the years when disconnection of regional waterholes was most likely to occur. The following six gauges were chosen for the analysis:

- Paroo at Caiwarra (424201A)
- Warrego at Wallen (423206A)
- Nebine at Reseleigh Crossing (422502A)
- Balonne at St George (422201F)
- Narran at Dirranbandi-Hebel Road (422206A)
- Moonie at Nindigully (417201B).

Modelled daily flows representing current management settings over 116 years (1895 to 2011) were analysed to identify the proportion of total days in each month when there was no flow (that is, <5 ML/day). This was then averaged for each month across the six locations. The analysis showed that the probability that a watercourse in this region will be flowing exceeds 50 percent from approximately late-December to mid-March (Figure 66). Conversely, waterholes are most likely to be isolated from approximately April through to November. No flow is the normal state for many of these rivers and individual flow events are rainfall-runoff generated, discrete and last only days to weeks.

[84] Dr Jon Marshall, Principal Scientist (Aquatic Ecosystems) | Water Planning Ecology, Science Division, Queensland Department of Environment and Science

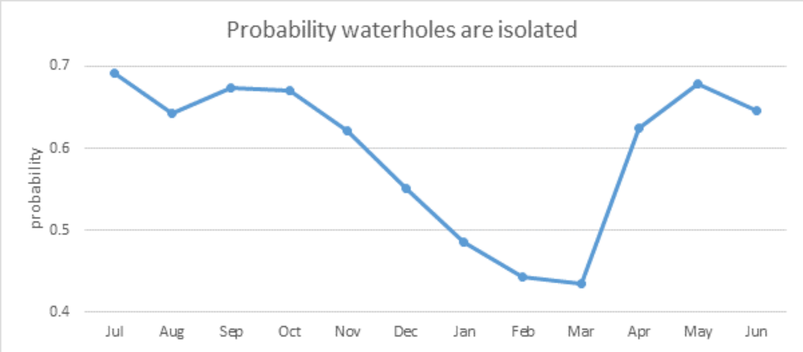

Figure 66 Monthly probability of flow cessation in the northern Murray-Darling Basin

Source: Marshall *pers. comm.* (2019)

In a review of the Moonie River watering plan (2003), the Queensland Department of Environment and Sciences (DES, 2018) noted that waterholes diminish to the point of providing poor habitat quality (termed, waterhole failure) when their average depth falls below approximately 0.5 metre. While refuge waterholes often experience changes in water depth, and waterhole biota are adapted to cope with these conditions, prolonged periods of very shallow water represent high risk. At low water levels, there are a range of threats to aquatic biota, including extreme water temperature fluctuations, the concentration of toxicants, increased predation, a limitation in the availability of food and increased potential for disease transmission. Waterholes that remain deeper than approximately 0.5 m throughout the dry season, particularly those with higher turbidity, provide suitable refuge conditions for fish and avoid the risk of exposure to increased water temperatures.

12.4.3 Ecological values of the Moonie River Catchment

The Moonie catchment is ecologically significant as it flows through the endangered southern brigalow (*Acacia harpophylla*) belt, which contains remnants of brigalow forests, poplar box (*Eucalyptus populnea*), wilga (*Geijera parviflora*) and white cypress pine (*Callitris glaucophylla*).[85] Remnant vegetation only makes up a small percentage of the catchment and is concentrated around the headwaters around Teelba Creek, and along many of the streams (DNR, 1999). The major vegetation types throughout the catchment include brigalow and belah open forest to woodland, with associations of poplar box, molly box (*Eucalyptus pilligaensis*) and black tea-tree (*Melaleuca bracteata*) communities sometimes present. The hillsides and ridges along the northern boundary of the catchment are populated with narrow-leaved ironbark (*Eucalyptus crebra*), silver-leaved ironbark (*Eucalyptus melanophloia*) and bendee (*Acacia catenulata*). Cypress pine forests and bull oak (*Allocasuarina luehmannii*) occur in areas on the gently undulating country running along the south eastern boundary of the top half of the catchment. Poplar box, brigalow and belah open forest to woodland are the major vegetation communities in the area south of the Moonie Highway crossing of the Moonie River at Mt Driven.

The Moonie River floodplain contains more than 100 floodplain wetlands larger than one hectare in size (CSIRO, 2008), with all wetlands in the catchment covering a total of 9,611 ha. Most of

[85] See: https: //www.mdba.gov.au/discover-basin/catchments/moonie

these are found in the southern lowland section of the catchment, downstream of Nindigully. Throughout the catchment there are 158 palustrine wetlands and 87 riverine wetlands. A study by Kingsford *et al.* (1997a; as cited in DNR, 1999) using aerial survey data has identified a wetland area that supports up to 19,000 waterbirds. This wetland is located approximately 5.5 km to the northeast of Thallon (Figure 65). The wetland is comprised of two lakes of approximately 12 ha and 21 ha and is a relatively permanent source of water. There is little published information about bird species inhabiting this site, although evidence of the breeding of black swans (*Cygnus atratus*), grey teal (*Anas gibberifrons*) and little black cormorants (*Phalacrocorax sulcirostris*) has been observed (DNR, 1999). The wetlands are filled by overbank flows of the Moonie River during significant flow events, as well as being supplemented by water from a water diversion scheme consisting of an in-stream structure and a gravity diversion channel. A further study by Kingsford *et al.* (1997b; as cited in DNR, 1999) has used GIS interpretation of satellite imagery to identify wetlands greater than 5 ha within the Murray-Darling Basin. Using this methodology, they have identified a total of 23 wetlands covering 942 ha within the Moonie River Catchment. Six of these were categorised as 'vegetated wetland', fifteen as 'dam', and two were unable to be categorised on the basis of their spectral signature or with the aid of ancillary information (DNR, 1999). There are also believed to be other wetland areas that support waterbirds in the Moonie River Catchment. A billabong that fills mainly from overland flows and is next to the Carnarvon Highway, about 15 km south of Nindigully, and a swamp about 10 km downstream of Thallon, are two examples of wetlands known locally to support a variety of birds (DNR, 1999).

The Queensland Department of Natural Resources also provided a list of fauna and flora that 'may be found' in the Moonie River Catchment (DRN, 1999). The 48 species of bird on this list are shown in Table 23. Of these, a large proportion are bush-birds, songbirds and raptors – most of which are only loosely associated with waterbodies. Of the true waterbirds, most are waterfowl with only two large waders, one Gruiforme and two shorebirds. The plains wanderer is listed as critically endangered under the EPBC Act, while the Australian painted snipe is endangered, and the painted honeyeater and red goshawk are vulnerable.

Table 23 Native birds inhabiting the Moonie River Catchment

Common name	Scientific name	Category	Status
Australian painted snipe	*Rostratula australis*	Shorebird	Endangered
Masked lapwing	*Vanellus miles*	Shorebird	Not listed
Black swan	*Cygnus atratus*	Waterfowl	Not listed
Chestnut teal	*Anas castanea*	Waterfowl	Not listed
Freckled duck	*Stictonetta naevosa*	Waterfowl	Not listed
Great crested grebe	*Podiceps cristatus*	Waterfowl	Not listed
Grey teal	*Anas gibberifrons*	Waterfowl	Not listed
Maned duck	*Chenonetta jubata*	Waterfowl	Not listed
Pacific black duck	*Anas superciliosa*	Waterfowl	Not listed
Black-necked stork	*Ephippiorhynchus asiaticus*	Large wader	Not listed
Great egret	*Egretta alba*	Large wader	Not listed
Lewin's rail	*Dryolimnas pectoralis*	Gruiforme	Not listed

Common name	Scientific name	Category	Status
Black-throated finch	*Poephila cincta cincta*	Songbird	Not listed
Eastern yellow robin	*Eopsaltria australis*	Songbird	Not listed
Golden whistler	*Pachycephala pectoralis*	Songbird	Not listed
Grey fantail	*Rhipidura fuliginosa*	Songbird	Not listed
Grey shrike-thrush	*Colluricincla harmonica*	Songbird	Not listed
Jacky winter	*Microeca leucophaea*	Songbird	Not listed
Rufous whistler	*Pachycephala rufiventris*	Songbird	Not listed
South-west crested shriketit	*Falcunculus frontatus leucogaster*	Songbird	Not listed
Willie wagtail	*Rhipidura leucophrys*	Songbird	Not listed
Black-chinned honeyeater	*Melithreptus gularis*	Bush-bird	Not listed
Fork-tailed swift	*Apus pacificus*	Bush-bird	Migratory
Glossy-black cockatoo	*Calyptorhychus lathami*	Bush-bird	Not listed
Ground cuckoo-shrike	*Coracina maxima*	Bush-bird	Not listed
Helmeted honeyeater	*Lichenostomus melanops cassidix*	Bush-bird	Not listed
Leaden flycatcher	*Myiagra rubecula*	Bush-bird	Not listed
Melville cicadabird	*Coracina tenuirostris melvillensis*	Bush-bird	Not listed
Painted honeyeater	*Grantiella picta*	Bush-bird	Vulnerable
Paradise parrot	*Psephotus pulcherrimus*	Bush-bird	Extinct
Pink cockatoo (Major Mitchell)	*Cacatua leadbeateri*	Bush-bird	Not listed
Plains wanderer	*Pedionomus torquatus*	Bush-bird	Critically endangered
Regent honeyeater	*Xanthomyza phrygia*	Bush-bird	Not listed
South-eastern red-tailed black cockatoo	*Calyptorhynchus banksia graptohyne*	Bush-bird	Not listed
Squatter pigeon	*Geophaps scripta scripta (southern subspecies)*	Bush-bird	Not listed
Turquoise parrot	*Neophema pulchella*	Bush-bird	Not listed
Western long-billed corella	*Cacatua pastinator pastinator*	Bush-bird	Not listed
Yellow-tufted honeyeater	*Lichenostomus melanops*	Bush-bird	Not listed
Australian kestrel	*Falco cenchroides*	Raptor	Not listed
Black falcon	*Falco subniger*	Raptor	Not listed
Brown goshawk	*Accipiter fasciatus*	Raptor	Not listed
Grey falcon	*Falco hypoleucos*	Raptor	Not listed
Grey goshawk	*Accipiter novaehollandiae*	Raptor	Not listed
Masked owl (northern subspecies)	*Tyto novaehollandiae kimberli*	Raptor	Not listed
Powerful owl	*Ninox strenua*	Raptor	Not listed
Red goshawk	*Erythrotriorchis radiates*	Raptor	Vulnerable

Common name	Scientific name	Category	Status
Square-tailed kite	*Lophoictinia isura*	Raptor	Not listed
Wedge-tailed eagle	*Aquila audax*	Raptor	Not listed

Source: DNR (1999)

Davis (2017) captured and identified a total of 1,438 individual fish within the Moonie catchment. These represented nine native species and three invasive species. Six of the native fish species were large-bodied (Murray cod, golden perch, spangled perch [*Leiopotherapon unicolor*], bony herring, freshwater catfish and Hyrtl's catfish [*Neosilurus hyrtlii*]); while a further three were small-bodied (olive perchlet, carp gudgeons and Australian smelt). This author observed that although the fish community within the Moonie catchment was dominated by bony herring, golden perch, spangled perch and eastern gambusia, its composition differed significantly between dry and wet periods. During dry periods, there was a higher abundance of invasive fish (in particular, carp and goldfish) relative to native species (principally freshwater catfish). This reflected these species' physiological adaptations to deteriorating water quality – in particular, low DO and high turbidity and salinity. During high-flow periods there was a higher abundance and diversity of juvenile and small-bodied native fish, while carp numbers were lower. The author surmised that the low carp numbers observed within waterholes during the high-flow period suggested that they had moved to the inundated floodplains. Alternatively, the low biomass density of carp observed in this study may simply reflect the effect of dilution that arises when the in-channel habitat becomes inundated (Marshall *pers. comm.*, 2019). It is relevant that the period during which the field surveys were undertaken was shortly following the highest flood on record for the Moonie catchment, with the river system completely connected and the floodplains inundated.

In a study of water availability in the Moonie catchment, CSIRO (2008) noted that golden perch, spangled perch, freshwater catfish, Australian smelt, olive perchlet, bony herring and carp gudgeons all relied upon persistent waterholes for their long-term viability. In addition to native fish, the broad-shelled turtle, long-necked (or snake-necked) turtle (*Chelodina longicolis*) and Macquarie turtle are found in the Moonie catchment (CSIRO, 2008), as is the warty water-holding or rough frog (*Cyclorana verrucosa*) (DNR, 1999). Parts of the results of CSIRO (2008) appeared to be at odds with Davis (2017) who observed a greater diversity of species within ephemeral waterholes and stressed their importance for species continuity. This author only identified Murray cod, olive perchlet, carp gudgeons and Hyrtl's catfish in semi-permanent or ephemeral waterholes. The identification of Murray cod was itself also at odds with CSIRO (2018), which had stated that although Murray cod were anecdotally present in the system they not been identified in scientific surveys. As a final point of difference, CSIRO (2018) included silver perch as a species reliant upon persistent waterholes, although silver perch were not identified in the field surveys undertaken by Davis (2017). Murray cod are listed as vulnerable under the EPBC Act, while silver perch are critically endangered. Both species will be included for the purpose of this case study.

Considering these divergent views, Marshall (*pers. comm.*, 2019) noted that during extended periods, the longest interval without flow is likely to be the limiting factor and that all fish populations (and other aquatic biota) depend completely on permanent refuge waterholes. Over shorter periods the smaller and less-permanent waterholes are occupied and are important for some species where they survive and recruit in the short term. This may due in part to a lowered predation release or a higher availability of food. From an evolutionary standpoint, there is fitness to be gained from using small waterholes often although in prolonged dry seasons the strategy is lethal, and the evolutionary fitness is instead gained from use of permanent waterholes. Overall, the fluctuating hydrology allows both strategies to persist within the catchment although permanent waterholes remain at the core of long-term population viability.

Marshall (*pers. comm.*, 2019) also provided a summary of the outcomes of Queensland Government catches within the Moonie River during the period 2006 to 2010. This work is summarised in Table 24. It can be seen of the 10 native species caught in the Moonie River, bony herring were significantly the most numerous, followed by golden perch, freshwater catfish and spangled perch. The small-bodied native fish were less commonly caught, as were silver perch. Only three Murray cod were caught in the Moonie River during this 4-year period. Interestingly, the number of (introduced) mosquitofish caught in this survey was over four times higher than the number of carp. Likewise, the number of carp was only approximately twice that of goldfish.

Table 24 Species caught by Queensland Government in the Moonie River 2006-2010

Species	Native or introduced	Number caught	Percentage of catch
Bony herring	Native	6,956	48.9%
Eastern mosquitofish	Introduced	3,064	21.5%
Golden perch	Native	1,848	13.0%
Carp	Introduced	708	5.0%
Freshwater catfish	Native	413	2.9%
Spangled grunter (spangled perch)	Native	404	2.8%
Goldfish	Introduced	363	2.6%
Carp gudgeons	Native	235	1.7%
Agassiz's glass fish (perchlet)	Native	180	1.3%
Australian smelt	Native	28	0.2%
Silver perch	Native	16	0.1%
Hyrtl's catfish	Native	13	0.1%
Murray cod	Native	3	0.0%

Source: Marshall *pers. comm.* (2019)

12.4.4 Carp biomass and population dynamics within the Moonie River Catchment

The mark-recapture estimates obtained for two waterhole sites in the Moonie River from surveys undertaken for the NCCP by the Queensland Government were 165 and 109 fish per ha, and 142 and 118 kg/ha (Marshall *pers. comm.*, 2019). It is important to note, however, that these results were obtained shortly after a river flow event when the river channel was inundated, and available habitat area was maximised. When flow has ceased for a year or more, the available habitat diminishes to approximately 10 percent of this maximal area resulting in a 10-fold concentration of aquatic biota.

From these and other estimates, the NCCP project team modelling carp biomass density across Australia found that the average density for the Moonie River was approximately 132 kg/ha (Stuart *et al.*, 2019). This represented a broad range in estimates from as low as approximately 45 kg/ha for wetlands, dams and some other off-channel habitats, to approximately 185 kg/ha in the upper reaches of the catchment (close to Dalby). As a general trend, carp biomass density appeared to decrease as the river descended southwest toward Thallon, where it fluctuated around a value of approximately 115 kg/ha.

The above notwithstanding, single-point estimates of cap biomass density within the Moonie River are likely to be fraught for two key reasons.

- First, the Moonie River itself contracts during dry periods to a sequence of disconnected pools. Some of these are ephemeral and will eventually dry without flooding rainfall. Others are considered to be semi-permanent or permanent. Implicit to the process of contraction from a flowing river to disconnected waterholes (equating to as little as 10 percent of the original habitat) is the concentration of aquatic life within these waterholes. Toward the end of such dry periods, the concentrated carp biomass density within some waterholes is likely to be very high. While undertaking a comparison of carp removal methodologies, Norris *et al.* (2014) noted that during periods of low water, where migration is not possible, carp may be concentrated into smaller pools. Illustrating this, they found, for example, that 747 carp/ha could be removed from the Moonie River near to Thallon. A further 604 carp/ha were removed from a control site within the river channel, close to this point; while in a lagoon disconnected from the channel the carp biomass density was found to be almost five times as high again (3,144 carp/ha). This part of the Moonie River is located toward the southern end of the catchment, within a reach with an estimated average carp biomass density as modelled by the NCCP project team of approximately 115 kg/ha (Stuart *et al.*, 2019).
- The second (and likely less substantive) reason for a marked variance in estimates of carp biomass density is the movement of carp between waterholes and the river channel, and possibly to the floodplains, when water flows are high and the river is fully connected. It should be noted that this full effect will only be realised as a result of flooding rains and is a separate consideration to the seasonal reconnection of disconnected waterholes (above). This behavioural characteristic of carp is one of the dominant ecological attributes of the species and is linked to its invasive success in a continent characterised by periodic flooding (Bajer and Sorensen, 2010). Davis (2017) observed very few residual carp in waterholes following substantial flooding of the Moonie River Catchment and surmised that most had migrated to the floodplains. A similar result was reported by Brown *et al.* (2005) from field observations of the Barmah-Millewa Forest ecosystem. The effect of this migration will be to lower the apparent carp biomass density even more dramatically, as the carp are dispersed over a very broad geographical area.

On balance it is likely to be difficult to be prescriptive about carp biomass density within the Moonie River Catchment and, when assessing the epidemiology and ecological impacts of CyHV-3, it may be more useful to consider that: (a) in dry periods, when the river has diminished to a sequence of disconnected waterholes, the carp biomass density may be in the range of moderate to very high (possibly higher than 1,000 kg/ha in some severely contracted waterholes); (b) when waterholes are reconnected, the carp biomass density is likely to be in the range estimated by Stuart *et al.* (2019) (approximately 132 kg/ha across the catchment); and (c) in times of flood, the carp biomass density may be very low (less than 50 kg/ha).

The spawning and recruitment of carp within the Moonie River Catchment is also likely to vary from year to year. The spawning season commences in spring, when the water temperature reaches approximately 16 to 17C (Part II: Section 4.2.2), but substantial spawning is very unlikely to occur in the Moonie River Catchment during dry years when adult carp are concentrated within dwindling waterholes (Marshall *et al.*, 2019). Substantive rains within the Moonie River Catchment generally fall in the summer months. If these are moderate and the river is reconnected and flowing, then some spawning may occur within wetlands and at the fringes of channels. If summer rains are sufficient to cause inundation of the floodplains, then spawning may also occur in these habitats. Where spawning on the floodplains does occur, juveniles will drift back toward the river channel with receding floodwaters. If flooding rains occurred later in summer, then adult carp may migrate to the floodplains without this leading to substantial spawning and recruitment (Davis, 2017).

The above follows from a generally-established view of carp ecology in Australia and other countries typified by occasional inundation of floodplain environments. In the context of the Moonie River Catchment, however, some caveats are important. The period of floodplain inundation within the Moonie River Catchment is less than the case for many southern ephemeral wetland complexes (for example, the Barmah-Millewa Forest) and the floodplain itself is principally grazing country and likely to be less desirable to carp as spawning habitat (Marshall *pers. comm.*, 2019). Juvenile carp are also rarely observed within the Moonie River Catchment, and the existence or relative importance of local recruitment has been questioned (Marshall *pers. comm.*, 2019). In support of this and citing catch data from 15 sites within the Moonie River Catchment, each sampled nine times during the period January 2007 to November 2009, Marshall (*pers. comm.*, 2019) noted that approximately 20% of fish were less than 150mm in length (Table 25).

Table 25 Standard length of carp in the Moonie River 2007 to 2009

Standard length in mm	Number	Cumulative percent
0	0	0.00%
50	2	0.39%
100	27	5.59%
150	70	19.08%
200	93	36.99%
250	105	57.23%
300	145	85.16%
350	58	96.34%
400	14	99.04%
450	3	99.61%
500	1	99.81%
550	1	100.00%

Source: Marshall *pers. comm.* (2019)

Recruitment of carp to the Moonie River Catchment was also examined by Bennett (2008), who used otolith chemistry to determine that adult carp within the Moonie River Catchment may form a sink population that declined between recruitment years. The study concluded that the likely source of juvenile carp was external to the Moonies River Catchment. Further work undertaken by the Queensland Department of Environment and Science (Woods *pers. comm.*, 2019[86]) suggested that the Moonie River Catchment may be both a source and sink for regional carp populations, in the sense that flooding within the Moonie River Catchment that is timed to coincide with spawning may result in recruitment to both the Moonie River itself and to other regional connected rivers. Conversely, flooding within these connected river systems may result in recruitment to the Moonie River Catchment. In the period between flood events in either of these systems, the population of adult carp within the Moonie River Catchment is likely to decline. Ongoing work using acoustic tagging in lower Balonne River has shown that some adult carp undertake upstream and downstream movements of more than 200 km during single-flow events, while others remain sedentary (Woods *pers. comm.*, 2019).

12.4.5 CyHV-3 outbreak scenarios for the Moonie River Catchment

The characteristics of an outbreak of CyHV-3 within the Moonie River Catchment are likely to be driven to a large extent by: (a) carp biomass density; (b) the presence and extent of flow through the river system; and (c) the temperature of the water. While the delineation between high-flow and lower-low seasons has been used to describe the characteristics of outbreaks in most other case studies in this ecological risk assessment, it is more useful to view the Moonie River Catchment (and other seasonally-disconnected dryland river systems within the northern and western Murray-Darling Basin) within the context of the flow and no-flow parts of a typical year.

The hydrology of the Moonie River (including the flow / no-flow cycle) was summarised in Section 12.4.2. Superimposed on this is the seasonal variance in water temperature. This was evaluated by Marshall and communicated to the NCCP in 2018 (Marshall *pers. comm.*, 2019). The evaluation is summarised in Figure 67, which shows the following:

- Potency of the virus is limited by temperature to two optimal water temperature periods (a) per year in the Queensland portion of the Murray-Darling Basin, although it can survive for slightly longer permissive water temperature periods;
- During periods without flow river waterholes stratify (b) at a depth of 0.5-1.0 m for much of the year;
- During periods without flow, dissolved oxygen saturation at the surface (d) reduces with increasing water temperature (d), and based on respiration exceeding primary production in these systems, we assume DO saturation is lower below the thermocline during stratification periods (c); and
- The likelihood that the river will cease to flow and revert to disconnected waterholes (e) is lowest during summer – and conversely, that the likelihood that the river will be characterised by seasonally-disconnected waterholes is highest from autumn through to spring.

The evaluation presented in Figure 67 shows that there are two periods in a typical calendar year when the water temperature is likely to be permissive for the transmission of CyHV-3. Both these

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[86] Dr Ryan Woods, Senior Scientist | Water Planning Ecology, Science Division, Queensland Department of Environment and Science

periods occur when there is also a high probability that the river itself will have contracted to a series of disconnected waterholes.

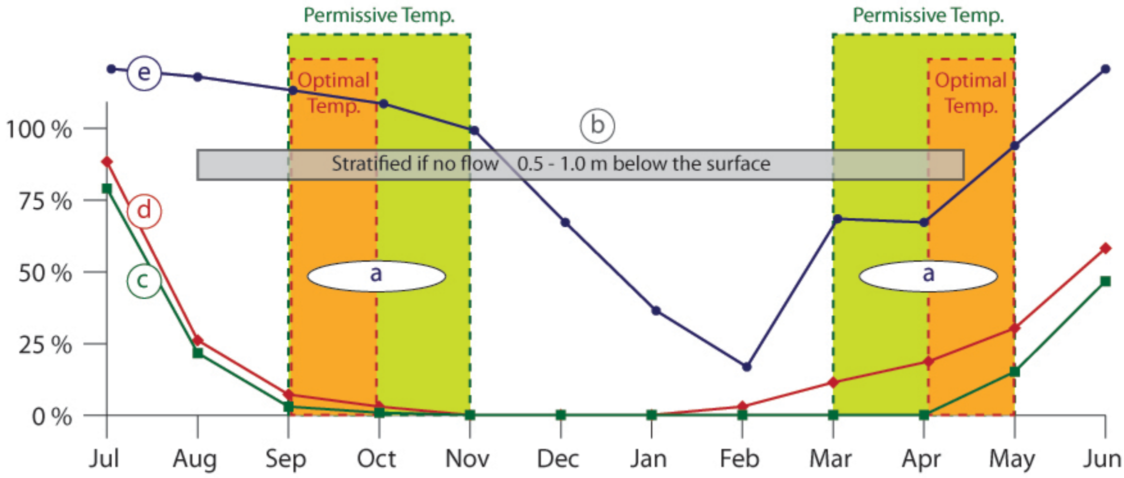

Figure 67 Water temperature and flow in the Moonie River through a calendar year

Source: Marshall *pers. comm.* (2019)

If the virus (CYHV-3) was seeded into the Moonie River in early spring (start of September), when the water temperature is permissive, then substantial transmission is likely to occur during pre-spawning aggregations. At this time, the river is likely to have contracted to disconnected waterholes with relatively poor baseline water quality. An aggressive outbreak is likely in this setting, and carcass accumulation may be sufficient to result in an impact on water quality.

Carp exposed toward the end of spring (end of November), when the water temperature is likely to approach or exceed the threshold for effective transmission, may develop a latent infection (Part II: Section 5.3.6). With rains and temporary reconnection of the river likely from late December, these latently-infected carp will then be distributed throughout the population within the catchment. As noted above, acoustic tagging has shown that some adult carp undertake upstream and downstream movements of more than 200 km during single-flow events. Reconnection of the river is in general only transient (days to weeks), and it then contracts again to a series of disconnected waterholes. The volume and quality of the water within these waterholes declines through the remaining summer period until the river is reconnected in the following season. Importantly, there would be little or no transmission of CyHV-3 as the summer progresses and the temperature of the water remains above the upper threshold (Figure 67).

In early autumn (March), water within the contracting waterholes is likely to have cooled sufficiently to become permissive once again for the transmission of CyHV-3 (Figure 67). At this point, latently-infected fish will become actively infected and infectious (termed recrudescence, see Part II: Section 5.3.6). In combination with the increasing carp biomass density (as a result of the contraction of waterholes and the concentration of biota) this is likely to result in aggressive outbreaks within individual waterholes. If the seasonal rains were sufficient to result in inundation of the floodplains, the reconnection of isolated wetlands and the reconnection with other regional river systems, then these autumn outbreaks in disconnected waterholes may also be fuelled by the presence of highly-susceptible juvenile carp. With reconnection of the river system and the distribution of latently-infected carp throughout, it is also likely that autumn outbreaks would occur concurrently in many (potentially all) waterholes within the catchment.

Autumn outbreaks within disconnected waterholes may be terminated either by their direct impact on the population of susceptible carp, or by an indirect impact on the ecosystem within each waterhole and its ability to sustain a population of aquatic biota. Autumn outbreaks may also be terminated by the cooling of water to below the lower threshold for the transmission of CyHV-3 (Figure 67). In this scenario, a proportion of infected carp would again move into a latent state. The cycle would then resume the following spring, as the temperature of the water within the affected waterholes increased and latency transitioned to active infection and transmission to susceptible carp.

The scenario within the Moonie River Catchment is therefore likely to be one of outbreaks in disconnected waterholes in both spring and autumn. Waterholes will be maximally contracted in spring, resulting in the highest carp biomass density and the lowest baseline water quality. The latter includes stratification, as well as low DO (even above the thermocline) and an increased risk of cyanobacterial blooms. There may also be an increased risk of botulism at this time. Collectively, these considerations point toward a higher aggressiveness of spring outbreaks. Although the aquatic and terrestrial species that are able to sustain their populations through the cyclic disconnection of the Moonie River are in general very resilient, and relatively tolerant to poor water quality, survival thresholds may be breached in the event of the accumulation of large numbers of carp carcasses within contracted waterholes and the impact that this may have on DO, cyanobacterial risk and the risk of botulism. This is considered further in Section 12.4.6 below.

12.4.6 Exposure pathways and risks for the Moonie River Catchment

A generic assessment for the nine identified exposure pathways was undertaken in Section 11. The discussion below interprets the outcomes from Section 11 in the context of the Moonie River Catchment and its natural assets.

(a) Exposure of native aquatic species to low DO

Likelihood of exposure: under this pathway, hypoxic events are predicated on the accumulation of significant numbers of carp carcasses in a stationary or lentic waterbody. This scenario is plausible within the Moonie River Catchment during the spring and autumn when the river has contracted to a series of disconnected waterholes and the water temperature is within the limits for the transmission of CyHV-3 (approximately 18 to 28C). Waterholes will be maximally contracted in spring, resulting in the highest carp biomass density and the lowest baseline water quality. Norris *et al.* (2014) observed between 604 and 747 carp/ha in waterholes and as high as 3,144 carp/ha in a lagoon. Although these figures are substantially in excess of those reported from the biomass modelling work of Stuart *et al.* (2019), the progressive concentration of carp likely within diminishing waterholes means that they are not unrealistic. Walsh *et al.* (2018) examined the effect of carcass decomposition on DO, and although their experiments were complicated by a range of issues (Part II: Section 6.3) these authors nevertheless observed that a carp carcass biomass density in the range of approximately 1,200 to 2,500 kg/ha resulted in hypoxic or anoxic conditions. The situation within waterholes in the Moonie River Catchment is likely to be exacerbated by an already-low baseline DO (in particular, in spring) and by the warmth of the water – noting that oxygen becomes progressively less soluble in water as its temperature increases. Stratification is also likely (in particular, during spring), with low DO or anoxia below the thermocline. This will mean that the volume of water with a sufficient DO to support aquatic biota is limited to the top 50 cm to 1 m of an affected waterhole (Marshall *pers. comm.*, 2019). As this is also the warmest water, the depletion of oxygen through an elevated BOD presents a risk to the waterhole.

On balance, it is likely that an outbreak of CyHV-3 within a waterhole in the Moonie River Catchment during spring or autumn would be aggressive and would result in sufficient carcass accumulation to lead to hypoxic or anoxic conditions. Whether these conditions extend to the entire waterhole will depend on its size and depth, and opportunities for re-oxygenation through wind and other means. As noted below, there is also the possibility that the distribution of latently-infected carp during a flow event would mean that outbreaks occur concurrently in a series of disconnected waterholes (potentially, all waterholes).

Likely consequences of exposure: low DO primarily affects fish and crustaceans. Air-breathing aquatic animals will not be directly affected. Indirectly, low DO may also impact on ecological communities and on the integrity of wetlands. The Moonie River Catchment provides habitats for at least ten large-bodied native fish species as well as at least three small-bodied species (Table 24). Of these, the Murray cod is listed as vulnerable under the EPBC Act while the silver perch is critically endangered. Murray cod are sensitive to low DO. Silver perch are slightly less sensitive, although not considered to be a tolerant species. Golden perch are physiologically adapted to lowland waterways with poorer water quality and are able to sustain (and to some extent adapt to) low DO. The broader population of golden perch is also more distributed geographically than silver perch or cod, and thus less vulnerable to local hypoxic events. Bony herring are a widespread, abundant and hardy fish, tolerating high temperatures (up to 38C), high turbidity and high salinity (up to at least 39 ppt). The Murray-Darling Basin Authority maintain that this species is relatively tolerant of low DO, although the Australian Museum states that the species is susceptible to low DO and likely to be the first to become stressed or die in the event of the drying of ephemeral waterbodies. It may be that the ubiquity and relatively high abundance of bony herring mean that deaths within a local population are visible earlier and are more obvious than some other species. Bony herring are a relatively short-lived fish (2 to 3 years is common), although are highly-fecund. This combination means that a failure to recruit in a given season may result in a noticeable drop in the population at that location – with rapid a rebound when conditions for breeding are more favourable. Freshwater catfish are a benthic fish and are considered the most tolerant of the large-bodied native fish to low DO. Freshwater catfish are also the only native species to be found in a high abundance in dwindling waterholes within the Moonie River Catchment (Davis, 2017). The tolerance of Hyrtl's catfish to low DO is not known although, as another benthic species, is likely to be relatively high. Most of the large-bodied native fish identified within the catchment spawn in spring. Hypoxic events that occurred during this time may result in a lower recruitment, although the impact of this on the sustainability of local populations is likely to be lower for the long-lived species such as Murray cod than for silver perch and freshwater catfish. Ostensibly, Murray cod and silver perch will be the two large-bodied species of native fish most exposed if hypoxic or anoxic conditions were to follow from an outbreak of CyHV-3. Although the presence of Murray cod has been demonstrated through field surveys (Davis, 2017; and the data from Marshall [*pers. comm.*, 2019] as depicted in Table 24) its prevalence and the sustainability of its population within the Moonie River catchment is not known. Silver perch were not identified by Davis (2017) although were caught by the Queensland

Government in low numbers (Table 24) and were included by CSIRO as a species endemic to the catchment. Again, their prevalence and sustainability within the catchment are not known.

The small-bodied native fish (including olive perchlet, carp gudgeons and Australian smelt) are in general relatively more tolerant to low DO than are most of the larger fish – with the exception of the benthic freshwater catfish. Although there are differences between the species, most small-bodied fish will begin to use ASR at about 2.5 mg/L and may survive for some time while the oxygen concentration remains above about 1 mg/L. This notwithstanding, they are also generally short-lived and a disturbance to recruitment could have a significant impact on the sustainability of a local population.

The Moonie River Catchment includes brigalow and belah open forest and woodland, with associations of poplar box, molly box and black tea-tree. The terrestrial ecological communities associated with these assemblages would not be threatened during a dry season, when the floodplains are not inundated. The impacts of a low DO event within dry season waterholes may, however, extend to any affiliated wetlands or marshlands. These impacts would include the food-web effects of a die-off of native and invasive species as a result of hypoxia or anoxia, as well as the impact of carcasses on water quality.

The summary above focuses on the possible impacts of CyHV-3 on a single waterhole. The more significant impact however is at a catchment scale. This is because the sustainability of most populations of native fish and other aquatic biota would be threatened with the simultaneous failure of multiple refugia (waterholes). This situation does not occur naturally in the seasonal cycle of the Moonie River, where refugia provide a base from which individual species can recruit and repopulate the catchment.

Summary of risks: the accumulation of carp carcasses in numbers sufficient to result in hypoxic or anoxic conditions may occur in either spring or summer, when the river has contracted to disconnected waterholes and the water temperature is within the upper and lower thresholds for the transmission of CyHV-3. Waterholes will be maximally contracted in spring, resulting in the highest carp biomass density and the lowest baseline water quality. Ostensibly, this would result in the most aggressive outbreaks and the highest likelihood of harm to species or communities. If DO concentration falls sufficiently, then all water-breathing aquatic animals will be affected and are likely to die. Under hypoxic (c.f. anoxic) conditions, the small-bodied and benthic native species will be more tolerant and likely to survive, as will the hardy invasive carp and goldfish. Murray cod and silver perch are two of the most exposed species, although the importance of the Moonie River Catchment to either was not established. Small wetlands associated with waterholes may also be exposed through the food web and water quality impacts of a low DO event.

There is also the probability that the distribution of latently-infected carp during a flow event will mean that outbreaks occur concurrently in a series of disconnected waterholes – potentially, all waterholes. As noted above, this scenario would have a significant impact on the sustainability of native fish and other aquatic biota within the Moonie River Catchment.

Mitigation of risks: the mitigation of risks associated with low DO within the Moonie River Catchment will focus on the removal of carp carcasses from waterholes with a high carp biomass density, and within which an aggressive outbreak had occurred. The overarching goal will be to prevent the DO within a given waterbody falling below about 3 to 4 mg/L or to enable it to return to that level. Achievement of this goal in the context of the Moonie River Catchment is likely to be challenging, however, for several reasons. The high turbidity means that carcasses will need to be identified while floating, as they won't otherwise be visible. This greatly narrows the period (2 to 3 days) in which clean-up can be undertaken. The river channel itself is also significantly obstructed with fallen timber and other debris (snags), making navigation by vessel difficult even during period of flow (Figure 68). The Moonie River channel traverses very sparsely populated agricultural land and this is likely to compromise both the monitoring of at-risk waterholes and clean-up activities. The difficulties noted above are likely to be particularly relevant in the situation where a number of waterholes are affected concurrently – and this in itself, is a realistic possibility.

Figure 68 Fallen timber compromises navigation of the Moonie River channel

Source: Jon Marshall (2019)

(b) Exposure of native species, livestock and humans to cyanobacterial blooms

Likelihood of exposure: the scenario most likely to result in the accumulation of carp carcasses in numbers sufficient to initiate a widespread cyanobacterial bloom will be the same as the scenario most likely to result in a low DO event – that it, an aggressive spring outbreak of CyHV-3 during the spring or autumn when the river has contracted to a series of disconnected waterholes and the water temperature is within the thresholds for the transmission of CyHV-3. Waterholes will be maximally contracted in spring, resulting in the highest carp biomass density and the lowest baseline water quality. Stratification is also likely with very low DO or anoxia below the thermocline. Cyanobacteria may already be proliferating, noting that the perpetually high turbidity limits photosynthesis to shallow surface waters with rapid light attenuation below that. Under this scenario, it is likely that carp carcasses would accumulate in numbers sufficient to result in a cyanobacterial bloom. Whether this extends to encompass the entire waterhole will depend on the aggressiveness of the outbreak, the alignment of risk factors for cyanobacterial blooms and the size of the waterhole. Where a cyanobacterial bloom occurs, it is likely to result in the direct exposure of most aquatic species and the indirect exposure of a wide range of terrestrial species – including amphibians and nesting waterbirds. In these situations, harm may be caused through either the direct effect of cyanotoxins (if the strain(s) of cyanobacteria involved are cyanotoxic) or indirectly when the bloom dies off and decomposes, raising the BOD and leading to a precipitous fall in DO. It is important to note that cyanotoxins can bioaccumulate, meaning that prolonged low exposure can result in breaching a toxic threshold. Livestock and humans are also susceptible to cyanotoxins.

Likely consequences of exposure: it was concluded within the discussion of this exposure pathway (Section 11.2) that the risks associated with widespread cyanobacterial blooms for large- and small-bodied native fish and crustaceans will largely mirror those associated with low DO. In addition to these, however, are risks to waterbirds, frogs and turtles. The risks posed by widespread cyanobacterial blooms to ecological communities and wetlands are also different to those posed by low DO.

Although waterbird breeding and nesting events within the Moonie Catchment are not large by comparison with major wetlands, as many as 19,000 birds have been identified by an aerial survey of the wetland to the northeast of Thallon (Kingsford *et al.*, 1997a; as cited in DNR, 1999). These birds are mostly waterfowl and are not (in the main) colonial nesters. The plains wanderer is listed as critically endangered under the EPBC Act, while the Australian painted snipe is endangered, and the painted honeyeater and red goshawk are vulnerable. A widespread cyanobacterial bloom would be likely to impact on the quality of the wetland habitat, as well as the availability of most forms of aquatic food. Most wetlands and waterholes within the Moonie River Catchment, however, are reasonably close to other waterholes. If the outbreak of CyHV-3 has not resulted in a similar deterioration of water quality within these other waterholes, then many waterbirds will be able to relocate – or to source food from beyond their immediate nesting places. The wetlands and waterholes of the Moonie River Catchment also provide drought refuge and a source of drinking water for many species of songbird, bush-bird and raptor (Table 23). Again, the extent to which these will be exposed to either cyanotoxin (if the species of cyanobacteria is cyanotoxic) or to water stress will depend on whether the outbreak of CyHV-3 affects only one or a few waterholes and wetlands, or most within the catchment. The risk drivers for birds exposed to a cyanobacterial bloom will be the same within the Moonie River Catchment as discussed in a generic sense in the pathway description for cyanobacterial blooms (Section 11.2). In particular, the risks will be higher for those functional groups or species whose feeding, foraging, nesting and other behaviours place them in close and regular contact with water. Colonial nesters are also at higher risk, as stressors to these populations are likely to be applied to a large number of birds at a time when harm can fall to adults and the success of recruitment can be threatened. Diet is also important, in particular as cyanotoxins bioaccumulate in animal tissues and regular exposure to affected (living) biota can mean that a toxic threshold is eventually breached.

The warty water-holding frog has also been recorded in the catchment and, if a widespread outbreak of cyanotoxic cyanobacteria was precipitated by the decomposition of carp carcasses, then significant numbers of these and other frogs are likely to be exposed. The broad-shelled turtle, long-necked turtle and Macquarie turtle are also endemic to the Moonie River Catchment and would be exposed to cyanotoxin if a widespread bloom was to occur.

The Moonie River Catchment includes brigalow and belah open forest and woodland, with associations of poplar box, molly box and black tea-tree. The terrestrial communities associated with these assemblages would not be threatened during a dry season, when the floodplains are not inundated. The impacts of a cyanobacterial bloom within a dry season waterhole may, however, extend to any affiliated wetland or marshland. These impacts would include the food-web effects of a die-off of native and invasive species as a result of cyanotoxins (if the cyanobacterial species involved was cyanotoxic) and the precipitous fall in DO that accompanies the collapse of the bloom.

Livestock and humans are also susceptible to cyanotoxins, and the risk to both were considered in a generic sense within the description of this exposure pathway (Section 11.2). The Moonie River Catchment includes a range of land tenures, and many livestock have access to its channels and waterholes. This is a riparian right under the Queensland Water Act 2000. Although cyanobacterial blooms are not uncommon within the Moonie River Catchment, and managers will be accustomed to monitoring water quality, it is nevertheless possible that cyanotoxins within drinking water would present a risk to livestock – in particular, as stock in some areas may not have an alternative source of drinking water. Recreational fishing is permitted within the Moonie River Catchment, although it is very unlikely that drinking water or fish would be taken for human consumption from an area where mass fish mortalities or destructive cyanobacterial blooms were occurring. This likelihood would be further ameliorated through signage in public places. Caution would extend to the use of contaminated waters for swimming and other recreation, although the malodorous nature of a fish kill is likely to be a sufficient deterrent.

Summary of risks: although cyanobacterial blooms are not uncommon within the Moonie River Catchment, this pathway considers the special case of a widespread bloom initiated by the decomposition of a large number of carp carcasses within a disconnected waterhole during an aggressive outbreak of CyHV-3 in spring or autumn. There is also the possibility that the distribution of latently-infected carp during a flow event would mean that outbreaks occur concurrently in a series of disconnected waterholes – potentially, all waterholes – a proportion of which may then experience cyanobacterial blooms. A widespread cyanobacterial bloom has the potential to impact on both aquatic and terrestrial biota, as well as on the quality and sustainability of riparian or wetland ecological communities. In the Moonie River Catchment, the relatively lower profile of breeding or nesting waterbirds means that the more significant environmental effects of a widespread cyanobacterial bloom are likely to be on native fish, livestock and domestic water users. All fish species will be exposed, with a profile of relative exposure and tolerance that follows broadly the profiles for low DO events (above). Waterbirds most at risk will be those most closely associated with waterbodies. In the Moonie River Catchment, these are principally waterfowl and a small number of large waders, shorebirds and Gruiformes.

Mitigation of risks: the mitigation of risks associated with widespread cyanobacterial blooms within the Moonie River Catchment would focus on the removal of carp carcasses from places where they had accumulated in sufficient numbers, and across a sufficiently broad area, to be likely to result in the initiation of a bloom. Achievement of this goal in the context of the Moonie River Catchment is likely to be challenging, however, for several reasons. The high turbidity means that carcasses will need to be identified while floating, as they won't otherwise be visible. This greatly narrows the period (2 to 3 days) in which clean-up can be undertaken. The river channel itself is also significantly obstructed with fallen timber and other debris (snags), making navigation by vessel difficult even during period of flow. The Moonie River channel traverses very sparsely populated agricultural land and this is likely to compromise both the monitoring of at-risk waterholes and clean-up activities. The difficulties noted above are likely to be particularly relevant in the situation where a number of waterholes are affected concurrently – and this in itself, is a realistic possibility.

(c) Exposure of native species, livestock and humans to the pathogens associated with decomposing fish carcasses

Likelihood of exposure: little information is available about the pathogens that may proliferate in the event of a fish kill, and their possible impacts on native species, livestock or humans. This deficit was discussed in the generic description of this pathway (Section 11.3) and in the underpinning review of the literature (Part II: Section 6.3.4). That aside, it is possible that carp gut flora, spoilage organisms, *E. coli*, *Pseudomonas spp*, *Aeromonas spp* and other opportunistic microorganisms might proliferate and, in doing so, may present a hazard to native aquatic and terrestrial species. This may be more probable for disconnected waterholes within the Moonie River Catchment than for most other settings considered within this assessment. This is because: (a) the waterholes are not connected to a source of clean water; (b) the waterholes generally contain warm water of a poorer quality; (c) the carp biomass density may be high; and (d) an outbreak of CyHV-3 may be aggressive.

Likely consequences of exposure: although there is substantial uncertainty (and likely variation) around the pathogens that may be released following a substantial fish kill, and their ability to survive in an aquatic environment and cause harm to aquatic or terrestrial biota, it is reasonable to expect that the relative exposure of communities, functional groups and species will be similar to that obtained for widespread cyanobacterial blooms – albeit with a lower likelihood of realising the identified ecological endpoints. Some waterborne microorganisms may affect people and livestock, although people are likely to be protected to a large extent by the innate repulsiveness of a waterbody that contains the detritus of a significant outbreak. There may some exceptions to this – in particular, properties on the Moonie River with no alternative water source. Livestock may also have less ability to avoid affected drinking water if there are limited or no alternatives. The risks to ecological communities are understandably highest for wetlands and marshlands, although an outbreak affecting a waterbody within or proximal to a forest and woodland, or grassland, ecological community, may also impact on species that utilise that water. Balancing this, it was also noted within the generic evaluation of this exposure pathway (Section 11.3) that the experience obtained from field observations of Australian freshwater waterways is that while substantial fish kills are not uncommon, they have not to-date been linked to subsequent epidemics of waterborne pathogens.

Summary of risks: the disconnected waterholes of the Moonie River Catchment may be at risk from contamination with a range of waterborne microorganisms in the event of an aggressive outbreak and the accumulation of significant numbers of carp carcasses. Although dependent on the pathogen(s) concerned, potentially all aquatic and terrestrial species may be at risk. This would include livestock, and in some settings may include people.

Mitigation of risks: the mitigation of risks associated with proliferating waterborne microorganisms within the disconnected pools and lagoons of the Moonie River Catchment will focus on the removal of carp carcasses from waterholes with a high carp biomass density, and within which an aggressive outbreak had occurred. Achievement of this goal in the context of the Moonie River Catchment is likely to be challenging, however, for several reasons. The high turbidity means that carcasses will need to be identified while floating, as they won't otherwise be visible. This greatly narrows the period (2 to 3 days) in which clean-up can be undertaken. The river channel itself is also significantly obstructed with fallen timber and other debris (snags), making navigation by vessel difficult even during period of flow. The Moonie River channel traverses very sparsely populated agricultural land and this is likely to compromise both the monitoring of at-risk waterholes and clean-up activities. The difficulties noted above are likely to be particularly relevant in the situation where a number of waterholes are affected concurrently – and this in itself, is a realistic possibility.

(d) Exposure of native piscivorous species to the removal of a dominant and stable food source

Likelihood of exposure: the limited breeding waterbird population within wetlands and riparian vegetation adjacent disconnected waterholes within the Moonie River Catchment is composed principally of waterfowl. This group can be divided loosely into herbivores (including swans and geese) and ducks (which includes the dabbling and diving ducks and grebes). Dabbling ducks feed in shallow water and are more likely to have a diet with more aquatic plants and insects, while diving ducks feed deeper in the water and typically eat more fish or crustaceans. Although some waterfowl may include juvenile carp in their diet, none will rely on carp to the extent that their survival or recruitment would be threatened by the removal of juvenile carp from their feeding grounds. Other birds commonly observed along the Moonie River waterholes include nankeen night herons, whistling kites and occasionally egrets and some cormorants (Marshall *pers. comm.*, 2019). There will also be songbirds and bush-birds, and some raptors, none of which will be exposed through the loss of juvenile carp. Across these, the large waders, Gruiformes and shorebirds may be relatively more exposed, although their diet in this location will be diverse and their numbers quite low. The observed population of juvenile carp is also very low (Marshall *pers. comm.*, 2019). On balance, the likelihood of exposure by this pathway was considered to be negligible and the pathway was not taken forward.

(e) Exposure of native species to increased predation as a result of prey-switching

Likelihood of exposure: the exposure of small-bodied and juvenile large-bodied native fish, crustaceans and turtle eggs and hatchlings to prey-switching is conditional on the exposure of piscivorous species to the sudden removal of a stable food source. It was explained above, however, that the absence of large numbers of piscivorous waterbirds within the Moonie River Catchment means that the sudden removal of juvenile carp would not be associated with a significant food-web effect. On this basis, the pathway was not taken forward.

(f) Exposure of native species, livestock and humans to an outbreak of botulism

Likelihood of exposure: an outbreak of type C (or C/D mosaic) botulism can arise in two key ways: (a) through the death of animals carrying spores within their gastrointestinal tracts; or (b) through the germination of spores within the environment (Part II: Section 7.6.1). In both cases, the germination of spores is triggered by anaerobic conditions and the presence of a suitable substrate. Within carcasses, this occurs when aerobic organisms exhaust available oxygen. Spores within the environment can germinate following any oxygen-depleting process – including the decomposition of large numbers of fish or bird carcasses, or the decomposition of a cyanobacterial bloom. Both pathways for the development of type C (or C/D mosaic) botulism can be linked to an outbreak of CyHV-3. The first pathway relies on the establishment of the carcass-maggot cycle, and this is a highly probabilistic process – the larger the number of affected carcasses, and the population of waterbirds feeding on maggots and other insects, the higher the likelihood that the cycle will be sustained. Large aggregations of breeding or nesting waterbirds (in particular, insectivorous waterbirds) are ideal for this scenario, and most outbreaks of type C (or C/D mosaic) botulism in Australia have been associated with waterbird breeding and nesting events in wetlands or at the edges of waterholes, lagoons, billabongs or shallow lakes. Although the number of nesting waterbirds within the Moonie River Catchment is less than in some larger wetland systems, it is relatively consistent and likely to be sufficient for the initiation of a carcass-maggot cycle. The highest risk scenario will be linked to the development of an aggressive outbreak within a disconnected waterhole, where the biomass density of carp can be high. In this setting, an outbreak of type C (or C/D mosaic) botulism might follow from the germination of spores within the carcasses of decomposing carp. Alternatively, and perhaps more likely, an outbreak could follow from the development of anoxic conditions within all or part of the waterbody. This might be the result of the BOD created by the decomposition of carcasses or may follow from the death and decomposition of a cyanobacterial bloom – noting that the DO within water below the thermocline in a stratified waterhole is likely to be very low or zero (anoxia) (Marshall *pers. comm.*, 2019). In either event, anoxic conditions within the waterhole or lagoon may trigger the germination of *C. botulinum* spores within the aquatic environment and this may then initiate an outbreak of botulism in susceptible animals (in particular, waterbirds) consuming contaminated water, insects or carcass materials. As a variant on this pathway, a proportion of the carcasses that sink and subsequently accumulate in the sediment will inevitably become exposed as the waterhole dries and contracts. Spores within these carcasses may then germinate if the detritus around them creates an anaerobic environment. Exposure of probing waterbirds to this material may result in intoxication, and that may then lead to the initiation of an outbreak.

Likely consequences of exposure: although botulism may follow from an aggressive outbreak of CyHV-3 within a disconnected waterhole or lagoon, it is a strongly probabilistic process and is not likely to extend across many waterbodies at a single point in time. This means that exposure is likely to be restricted to susceptible waterbirds, terrestrial animals and livestock with access to that waterbody. The worst case for the ecological communities within the Moonie River Catchment may be an outbreak of botulism that occurred in the wetlands north of Thallon, where as many as 19,000 waterbirds have been observed. Insectivorous waterfowl, songbirds and bush-birds are amongst the waterbirds most exposed to botulism, and these are likely to make up a large proportion of the wetland bird population. The less numerous large waders, Gruiformes and shorebirds would also be exposed. Raptors may be relatively protected from botulism, although this has not been confirmed beyond a small number of carrion-eating species. Frogs, turtles and any livestock with access to the affected wetland, waterhole or lagoon would also be exposed. Humans are not susceptible to type C (or C/D mosaic) botulinum toxin.

Summary of risks: the reasons why outbreaks of type C (or C/D mosaic) botulism in wildlife occur in some settings, and not in others with an ostensibly similar profile of risk factors, are not well understood but are likely to reflect the strongly probabilistic nature of outbreak initiation and expansion. That notwithstanding, most outbreaks of botulism in Australian wildlife occur on shallow, disconnected waterbodies where the water quality is poor and the water is relatively warm. These conditions are likely to prevail within most of the disconnected waterholes within the Moonie River Catchment during spring or autumn. The likelihood of an outbreak will be higher in places where the number of waterbirds is higher, such as the wetlands north of Thallon, and this will also result in the greatest losses. Most aquatic and terrestrial species are susceptible to botulism although the risk is generally highest for those that feed on insects.

Mitigation of risks: prevention of an outbreak of type C (or C/D mosaic) botulism will rest on the timely removal of carp and other carcasses from places where they have accumulated in high numbers. Achievement of this goal in the context of the Moonie River Catchment is likely to be challenging, however, for several reasons. The high turbidity means that carcasses will need to be identified while floating, as they won't otherwise be visible. This greatly narrows the period (2 to 3 days) in which clean-up can be undertaken. The river channel itself is also significantly obstructed with fallen timber and other debris (snags), making navigation by vessel difficult even during period of flow. The Moonie River channel traverses very sparsely populated agricultural land and this is likely to compromise both the monitoring of at-risk waterholes and clean-up activities. The difficulties noted above are likely to be particularly relevant in the situation where a number of waterholes are affected concurrently – and this in itself, is a realistic possibility. Consideration may also be given to the removal of waterbirds from waterbodies in which carp carcasses have led to an hypoxic or anoxic event or to a widespread cyanobacterial bloom. These waterbodies will present the highest risk of a subsequent outbreak of botulism. The removal of waterbirds may not be practical in all settings, and consideration of this course of action should include ecological advice.

12.5 Case study 5: Lower lakes and Coorong

12.5.1 Physical description of the Lower Lakes and Coorong case study site

The Coorong, Lower Lakes and Murray River mouth wetland is a freshwater lake and coastal lagoon system covering an area of approximately 142,530 ha in south-eastern South Australia (DEWNR, 2015). The case study site encompasses the Lower Lakes (Lakes Alexandrina and Albert), the lower reaches of Currency Creek and the Finniss River, the Murray River estuary, the Coorong and a number of ephemeral lakes (Figure 69).

The Coorong itself is a long, shallow saline lagoon more than 140 km in length and separated from the Southern Ocean by a narrow sand dune peninsula (DOE, 2010). Lakes Alexandrina and Albert are separated from the Coorong by a series of five barrages, Goolwa, Mundoo, Boundary Creek, Ewe Island and Tauwitchere (CSIRO, 2015). These were completed in 1940 to maintain lake levels for water supply and protect water quality in the Lakes and Murray channel from sea water incursions. The Coorong marks the termination of the Murray River.

The Coorong and Lakes Alexandrina and Albert wetland is one of Australia's most important wetland areas. Australia designated the site as a Wetland of International Importance under the Ramsar Convention on Wetlands in 1985 and is included as an Icon Site under the Living Murray program.[87] Parts of the Coorong also form the Coorong National Park and Game Reserve. The following individual wetlands are included (CSIRO, 2015):

- Lake Alexandrina, encompassing Tolderol, Mud Islands and Currency Creek Game Reserves
- Lake Albert
- Tributaries, including the Finniss River and Currency Creek
- The Coorong, encompassing principally the Coorong National Park and Game Reserve.

The Coorong supports a wide range of threatened species and ecological communities. It is also an important agricultural, commercial and recreational area.

[87] See: https: //www.mdba.gov.au/publications/brochure/Living-murray-program

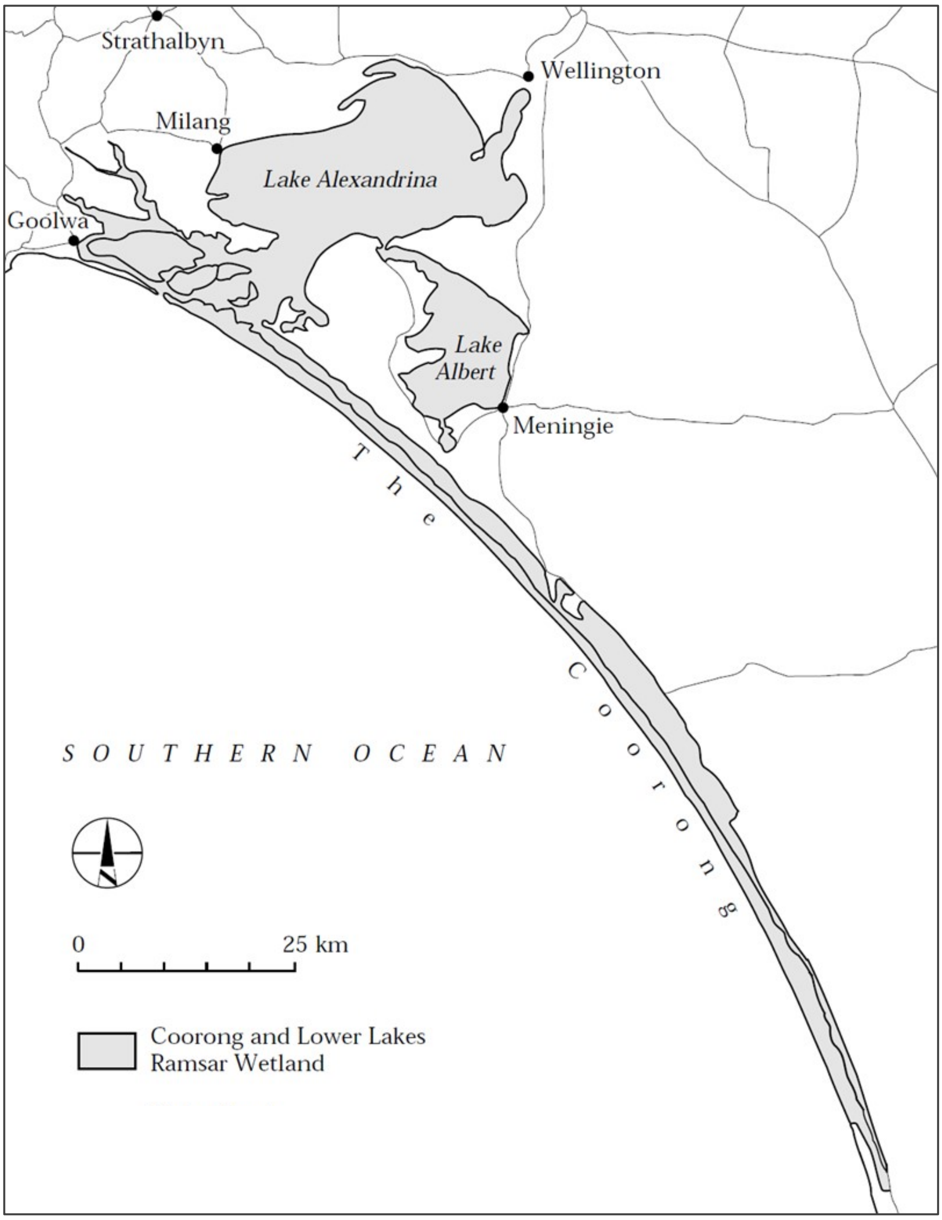

Figure 69 the Coorong Ramsar-listed wetland

Source: SADEH (2000)

12.5.2 Hydrology of the Lower Lakes and Coorong

Flows from the Murray River pass into Lake Alexandrina approximately 5 km south of Wellington, and out to the Southern Ocean through the mouth of the Murray River (DEWNR, 2015). Because the Coorong is included as an Icon Site under the Living Murray program, inflow may include up to 500 GL of environmental water (DEWNR, 2015). Lake Alexandrina also receives inflows from the tributary streams draining the eastern Mount Lofty ranges along the south-western edge of Lake Alexandrina (Phillips and Muller, 2006), and local rainfall and groundwater discharge provide additional inputs.

Lake Alexandrina connects to the terminal Lake Albert through a small channel on its eastern shore. Both lakes are then separated from the mouth of the Murray River and the Coorong by a complex of islands and channels, and by five barrages. The barrages are gated structures that typically maintain the Lower Lakes at near-full capacity at approximately +0.75 m AHD (Australian

Height Datum, corresponding approximately to mean sea level) (SAEPA, 2016). The barrages prevent ingress of saline water to the Lower Lakes and to regulate lake water levels. The mouth of the Murray River is the only site where sand and silt can exit the Murray-Darling Basin, and through-flow is now dependent on coordinated barrage releases and dredging. Since 2002, five fishways have also been incorporated into three of the barrages, allowing fish movements between freshwater and saline environments, and another seven were under construction in 2015 (DEWNR, 2015).

The Coorong itself is a shallow coastal lagoon complex that stretches over 140 km from the mouth of the Murray River (DEWNR, 2015). It receives inflows from Lake Alexandrina, the Southern Ocean and Salt Creek, and is separated from the sea by a narrow sand dune. The Coorong has a strong salinity gradient, from fresh to brackish in parts of the mouth of the Murray River to hypersaline in areas of its southern lagoon. This gradient varies temporally, depending on the respective inflows. The mouth of the Murray River is the only site where sand and silt can exit the Murray-Darling Basin, and through-flow is now dependent on coordinated barrage releases and dredging.

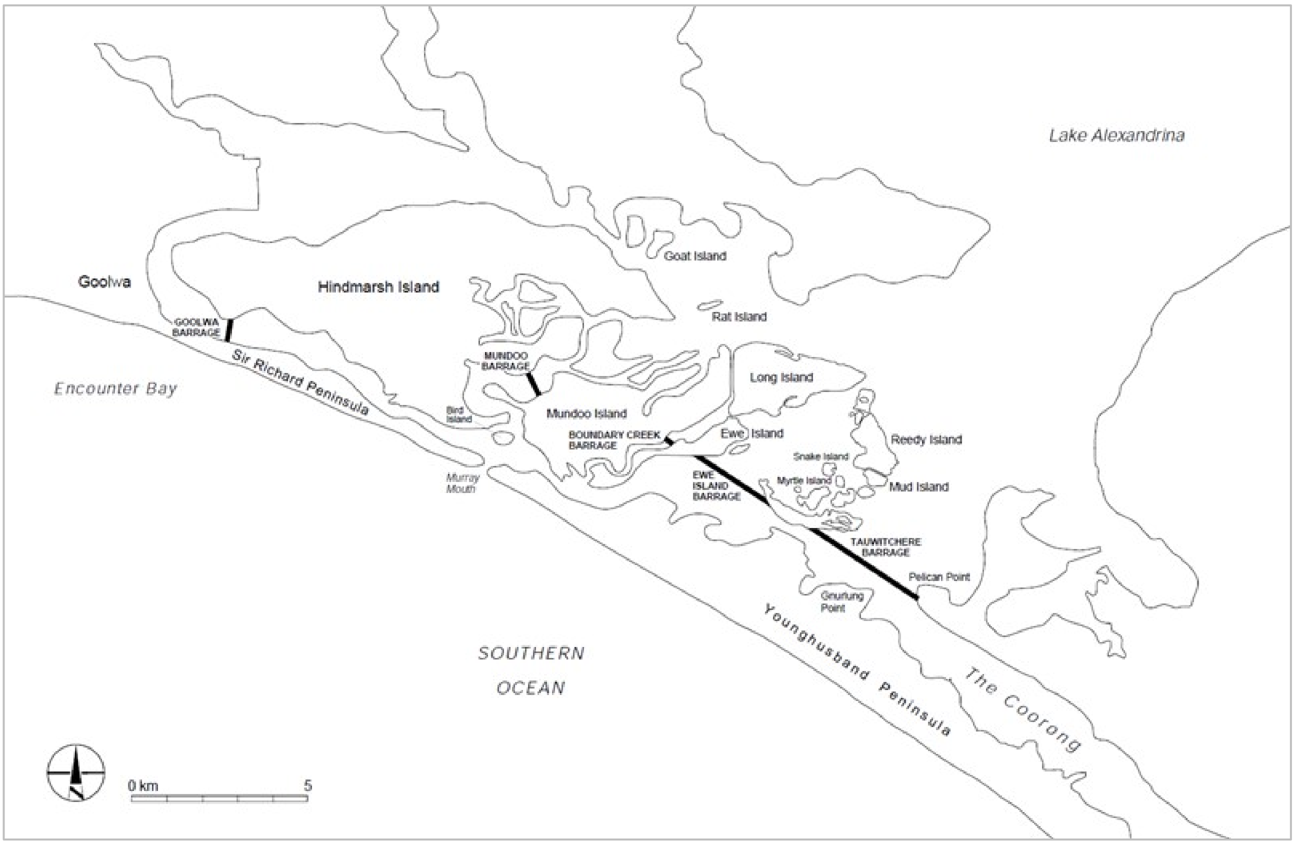

Figure 70 Barrages supporting the Coorong

Source: SADEH (2000)

Water quality has been a longstanding concern for Lakes Alexandrina and Albert. Documented reports of algal scums and discoloured water go back to at least 1853, with the first detailed scientific account of toxic cyanobacteria (SAEPA, 2016). This incident resulted in hundreds of stock deaths at Milang, on the shores of Lake Alexandrina, and through careful analysis was attributed to the cyanobacterium *Nodularia spumigena*. The phytoplankton balance within the Lower Lakes has shifted in recent years from green algae to cyanobacteria (SAEPA, 2016), although the concentration of chlorophyll-α in the Coorong shows a pronounced spatial variation – increasing from the northern to the southern end, where concentrations may exceed 100 μg/L (SAEPA, 2016). Diatoms (micro-algae) have been found to dominate the phytoplankton community in the South Lagoon of the Coorong. In the North Lagoon chlorophytes (green algae) have been found to be dominant up to a salinity of 20 g/L, but over this diatoms dominated the community (SAEPA, 2016).

The lakes have a high algal, total nitrogen and phosphorus levels (SAEPA, 2016). Dissolved nutrients however, are generally at very low levels and this has been attributed to rapid uptake by algae (SAEPA, 2016). A large proportion of the nutrient inputs from the Murray River are retained within the lake system in organic forms, and are not exported (SAEPA, 2016). Although determined to some extent by the source of inflow from the Murray River (that is, water from the Darling River as opposed to water from the mid-Murray River), the Lower Lakes are also in general quite turbid and this results in rapid light absorption and attenuation and influences the phytoplankton community structure (SAEPA, 2016). The Coorong itself is less turbid, due principally to its marine nature (SAEPA, 2016).

12.5.3 Ecological values of the Lower Lakes and Coorong

In the Ramsar Management Plan for the Coorong, and Lakes Alexandrina and Albert, the South Australian Department of Environment and Heritage describe essential characteristics of ecological character and communities (SADEH, 2000). Unless otherwise referenced, the outline that follows was adapted principally from that report.

Fresh water impounded by the barrages in Lakes Albert and Alexandrina maintains a variety of permanent and ephemeral wetlands. Currency Creek and the Finniss River form sheltered reedy freshwater estuaries on Lake Alexandrina. Tolderol and Mosquito points and the Narrows also support extensive sheltered reed beds. The Bremer River winds through red gum flats to the lake, while the Angas River flows seasonally through semi-arid woodlands into the lake. Both rivers have ephemeral wetlands near their mouths on Lake Alexandrina.

Together the lakes cover approximately 648 square kilometres which makes them the largest freshwater body in South Australia. Lake Albert is more saline than Lake Alexandrina and exhibits more of the original character of the lakes before their impoundment in 1940. Around the lakes are salt marshes and saline lagoons, many of which are occasionally inundated by floods. All retain some water as a result of local runoff from winter rains. In late winter and spring, many of these salt lakes display a bright pink colour due to the presence of the microorganism, *Dunaliella sp*. The lakes are fringed with tall reeds (*Phragmites sp*.) and bulrush or cumbungi (*Typha sp*.) and there are sheltered flats and lagoons in places. This lakeshore vegetation forms an almost unbroken habitat corridor around the lakes, which has a critical role in allowing birds and other animals to move between habitats with relative safety from predators and disturbance. Emergent macrophyte communities have thrived whilst communities dependent on variable water regimes have become restricted in their distribution (MDBA, 2014d).

Habitats around the edge of the lakes are influenced by, and change in response to, water regulation procedures at the barrages. As noted above, the barrages maintain the lakes at a nominal level of 0.75 metres AHD. However there is a cyclical change in levels from about 0.85

metres AHD in late spring, to a low of about 0.6 metres AHD in autumn and lower in drought years. This variation results in the seasonal exposure of mud flats around the lake edges. The slight seasonal rise and fall in lake levels also results in variations to habitats. Prolonged strong winds can elevate water on one side of the lakes and lower it on the other, resulting in daily and weekly variations of almost a metre.

Hindmarsh, Mundoo, Ewe and Tauwitchere islands lie within a transitional zone between Lake Alexandrina and the Coorong. These island areas comprise unique vegetation communities. The freshwater habitats on, and immediately surrounding the islands are critical habitats for fish, particularly EPBC-listed small-bodied native fish such as Murray hardyhead and Yarra pygmy perch (MDBA, 2014d). These transitional zones provide important ecological connectivity for migration of diadromous fish species such as congolli and common galaxias (MDBA, 2014d). The area around Hindmarsh, Mundoo, Ewe and Tauwitchere islands are also where mudflats would have occurred before river regulation stabilised water levels. Mudflats in this area are now exposed over short time scales by wind and act as habitat for wading birds.

The lagoon environment of the estuary (from the Goolwa Barrage to Pelican Point) includes habitats such as exposed mudflats and shallow waters, which provide important foraging grounds for many wader bird species (MDBA, 2014d). Habitats within the Coorong itself range from seasonally fresh near the barrages, when large quantities of water are being released, to brackish in the mouth of the Murray River and grading to hypersaline in the southern lagoon. Increased salinity and unfavourable water levels in the South Lagoon over recent years, brought about by low freshwater inflows, have led to the severe decline of keystone species such as *Ruppia tuberosa* (MDBA, 2014d). The Coorong experiences seasonal changes of as much as a metre in water level in the southern lagoon, from a high in late spring to a low in late autumn. As water levels fall from early summer, extensive tidal mud flats are exposed along the southern shores of the Coorong. These provide habitat for a number of species of wading birds, many of which are seasonal migrants to Australia and breed in Alaska, northern China and Siberia. On the peninsula side, there are freshwater soaks which provide further variety of habitats. Wind and tide also cause short term variations in water levels locally, while storm tide events can force seawater back through open barrage gates into the lakes and across causeways on Ewe and Tauwitchere islands. The seaward side of the coastal dune barrier is a high energy coast with a continuous sand beach broken only by the mouth of the Murray River, and stretching from Lacepede Bay to Encounter Bay – a distance of nearly 200 kilometres. About 150 kilometres of this beach is within the Ramsar area. The beach is habitat for a number of waders, gulls and terns.

River regulation, water abstraction and agriculture replacing the low open woodlands in the region have altered the lakes and the Coorong, contracting some habitats and creating others. The Lower Lakes and Coorong together still retain a mosaic of habitats which support a diversity of bird life and are an important drought refuge.

Waterbirds: Paton *et al.* (2016) undertook a ‘condition monitoring’ study of the Coorong, Lower Lakes and mouth of the Murray River Icon Site that focussed on the waterbird assemblage. This is the most recent and comprehensive of the numerous studies of the Coorong’s waterbirds. The following text was adapted directly from this report.

Over 185,000 waterbirds (57 species) were using the Coorong in January 2016. More than half (over 93,000) of these waterbirds were using the South Lagoon, with red-necked stints, grey teal, hoary-headed grebes and banded stilts all exceeding 10,000 birds. Australian shelduck, whiskered terns, silver gulls, red-necked avocets, red-capped plovers, sharp-tailed sandpipers, chestnut teal, and Australian pelicans were also prominent in the South Lagoon, with more than a thousand individuals of each. A little over 45,000 waterbirds were using the north lagoon, with grey teal, silver gulls, Australian shelducks, whiskered terns, sharp-tailed sandpipers, chestnut teal and black swans prominent in this region of the Coorong.

Over 85,000 waterbirds were counted in the Lower Lakes – notably, this is less than half the numbers of birds that were using the Coorong. Prominent species in the Lower Lakes included great cormorants, pied cormorants, Australian pelicans and whiskered terns – all fish-eating species; and Australian shelducks, grey teal, pacific black ducks, black swans and Eurasian coots – all largely herbivorous species. These ten species accounted for over 85% of the waterbirds using the Lower Lakes. As has been the case in recent years, only small numbers (approximately 700) of migratory shorebirds (stints, sandpipers) used the Lower Lakes in January 2016. Abundances of several endemic shorebirds (stilts, avocets, plovers, lapwings) were also low (approximately 1,000) but a little higher than the migratory species and comparable to abundances in previous years.

Around 46,000 waterbirds were using the Murray Estuary in January 2016. Sharp-tailed sandpipers, grey teal and red-necked stints were the most abundant species in this region, exceeding 5,000 individuals and collectively they accounted for more than half the birds counted in this region of the Coorong. A thousand or more silver gulls, whiskered terns, great cormorants, banded stilts, black swans and red-necked avocets were also present.

A list of the waterbird species observed by Paton *et al.* (2016) within the Coorong, Lower Lakes and Murray Estuary is given in Table 26. This was appended with species cited by MDBA (2014d). In addition to these true waterbirds, the case study site also provides habitat for a range of bush-birds and songbirds, including the critically endangered orange bellied parrot (*Neophema chrysogaster*) and the endangered southern Mount Lofty Ranges emu wren (*Stipiturus malachurus intermedius*).

Table 26 Waterbirds observed within the Lower Lakes and Coorong

Common name	Scientific name	Category	Status
Australasian darter	*Anhinga novaehollandiae*	Seabird	Unlisted
Australian pelican	*Pelecanus conspicillatus*	Seabird	Unlisted
Black-faced cormorant	*Phalacrocorax fuscescens*	Seabird	Unlisted
Great cormorant	*Phalacrocorax carbo*	Seabird	Unlisted
Little black cormorant	*Phalacrocorax sulcirostris*	Seabird	Unlisted
Little pied cormorant	*Microcarbo melanoleucos*	Seabird	Unlisted
Pied cormorant	*Phalacrocorax varius*	Seabird	Unlisted
Royal spoonbill	*Platalea regia*	Seabird	Unlisted
Yellow-billed spoonbill	*Platalea flavipes*	Seabird	Unlisted
Australian painted snipe	*Rostratula australis*	Shorebird	Endangered
Australian pied oystercatcher	*Haematopus longirostris*	Shorebird	Unlisted
Australian reed-warbler	*Acrocephalus australis*	Songbird	Unlisted
Banded lapwing	*Vanellus tricolor*	Shorebird	Unlisted
Banded stilt	*Cladorhynchus leucocephalus*	Shorebird	Unlisted

Common name	Scientific name	Category	Status
Bar-tailed godwit	*Limosa lapponica*	Shorebird	Vulnerable
Black-tailed godwit	*Limosa limosa*	Shorebird	Unlisted
Black-winged stilt	*Himantopus himantopus*	Shorebird	Unlisted
Caspian tern	*Hydroprogne caspia*	Shorebird	Unlisted
Common greenshank	*Tringa nebularia*	Shorebird	Unlisted
Common sandpiper	*Actitis hypoleucos*	Shorebird	Unlisted
Common tern	*Sterna hirundo*	Shorebird	Unlisted
Crested tern	*Thalasseus bergii*	Shorebird	Unlisted
Curlew sandpiper	*Calidris ferruginea*	Shorebird	Unlisted
Double-banded plover	*Charadrius bicinctus*	Shorebird	Unlisted
Eastern curlew	*Numenius madagascariensis*	Shorebird	Critically endangered
Fairy tern	*Sternula nereis*	Shorebird	Vulnerable
Flesh-footed shearwater	*Ardenna carneipes*	Shorebird	Unlisted
Great knot	*Calidris tenuirostris*	Shorebird	Critically endangered
Greater sand plover	*Charadrius leschenaultia*	Shorebird	Unlisted
Grey plover	*Pluvialis squatarola*	Shorebird	Unlisted
Grey-tailed tattler	*Tringa brevipes*	Shorebird	Unlisted
Gull-billed tern	*Gelochelidon nilotica*	Shorebird	Unlisted
Hooded plover	*Thinornis rubricollis*	Shorebird	Unlisted
Latham's snipe	*Gallinago hardwickii*	Shorebird	Unlisted
Lesser sand plover	*Charadrius mongolus*	Shorebird	Endangered
Little tern	*Sternula albifrons*	Shorebird	Unlisted
Long-toed stint	*Calidris subminuta*	Shorebird	Unlisted
Masked lapwing	*Vanellus miles*	Shorebird	Unlisted
Pacific golden plover	*Pluvialis fulva*	Shorebird	Unlisted
Pacific gull	*Larus pacificus*	Shorebird	Unlisted
Pectoral sandpiper	*Calidris melanotos*	Shorebird	Unlisted
Pied oystercatcher	*Haematopus longirostris*	Shorebird	Unlisted
Red knot	*Calidris canutus*	Shorebird	Endangered
Red-capped plover	*Charadrius ruficapillus*	Shorebird	Unlisted
Red-kneed dotterel	*Erythrogonys cinctus*	Shorebird	Unlisted
Red-necked avocet	*Recurvirostra novaehollandiae*	Shorebird	Unlisted
Red-necked phalarope	*Phalaropus lobatus*	Shorebird	Unlisted
Red-necked stint	*Calidris ruficollis*	Shorebird	Unlisted
Ruddy turnstone	*Arenaria interpres*	Shorebird	Unlisted
Ruff	*Philomachus pugnax*	Shorebird	Unlisted
Sanderling	*Calidris alba*	Shorebird	Unlisted
Sharp-tailed sandpiper	*Calidris acuminate*	Shorebird	Unlisted
Silver gull	*Chroicocephalus novaehollandiae*	Shorebird	Unlisted
Sooty oystercatcher	*Haematopus fuliginosus*	Shorebird	Unlisted

Common name	Scientific name	Category	Status
Terek sandpiper	*Xenus cinereus*	Shorebird	Unlisted
Wandering tattler	*Tringa incana*	Shorebird	Unlisted
Whimbrel	*Numenius phaeopus*	Shorebird	Unlisted
Whiskered tern	*Chlidonias hybrida*	Shorebird	Unlisted
Australasian shoveler	*Anas rhynchotis*	Waterfowl	Unlisted
Australian shelduck	*Tadorna tadornoides*	Waterfowl	Unlisted
Australian wood duck	*Chenonetta jubata*	Waterfowl	Unlisted
Black swan	*Cygnus atratus*	Waterfowl	Unlisted
Blue-billed duck	*Oxyura australis*	Waterfowl	Unlisted
Cape barren goose	*Cereopsis novaehollandiae*	Waterfowl	Unlisted
Chestnut teal	*Anas castanea*	Waterfowl	Unlisted
Eurasian coot	*Fulica atra*	Waterfowl	Unlisted
Freckled duck	*Stictonetta naevosa*	Waterfowl	Unlisted
Great crested grebe	*Podiceps cristatus*	Waterfowl	Unlisted
Grey teal	*Anas gracilis*	Waterfowl	Unlisted
Hardhead duck	*Aythya australis*	Waterfowl	Unlisted
Hoary-headed grebe	*Poliocephalus poliocephalus*	Waterfowl	Unlisted
Musk duck	*Biziura lobate*	Waterfowl	Unlisted
Pacific black duck	*Anas superciliosa*	Waterfowl	Unlisted
Pink-eared duck	*Scientific name:*	Waterfowl	Unlisted
Australasian bittern	*Botaurus poiciloptilus*	Large wader	Endangered
Australian white ibis	*Threskiornis moluccus*	Large wader	Unlisted
Cattle egret	*Ardea ibis*	Large wader	Unlisted
Eastern great egret	*Ardea modesta*	Large wader	Unlisted
Great egret	*Ardea alba*	Large wader	Unlisted
Intermediate egret	*Ardea intermedia*	Large wader	Unlisted
Little egret	*Egretta garzetta*	Large wader	Unlisted
Nankeen night-heron	*Nycticorax caledonicus*	Large wader	Unlisted
Straw-necked ibis	*Threskiornis spinicollis*	Large wader	Unlisted
White-faced heron	*Egretta novaehollandiae*	Large wader	Unlisted
Australian spotted crake	*Porzana fluminea*	Gruiforme	Unlisted
Black-tailed native-hen	*Tribonyx ventralis*	Gruiforme	Unlisted
Dusky moorhen	*Gallinula tenebrosa*	Gruiforme	Unlisted
Lewin's rail	*Lewinia pectoralis*	Gruiforme	Unlisted
Purple swamphen	*Porphyrio porphyrio*	Gruiforme	Unlisted
Spotless crake	*Porzana tabuensis*	Gruiforme	Unlisted
Eastern osprey	*Pandion cristatus*	Raptor	Unlisted
Grey falcon	*Falco hypoleucos*	Raptor	Unlisted
Peregrine falcon	*Falco peregrinus*	Raptor	Unlisted
White-bellied sea-eagle	*Haliaeetus leucogaster*	Raptor	Unlisted

Source: Paton *et al.* (2016) and MDBA (2014d)

Native fish: the Lower Lakes and Coorong provide critical fish habitats including important nursery and feeding areas for commercial and non-commercial fish species (MDBA, 2014d). The site is utilised by a number of fish groups including obligate freshwater, diadromous, euryhaline, estuarine and marine species. Over 75 species of fish have been recorded within the icon site, although 34 of these are of marine origin and are only irregular visitors to the Coorong (MDBA, 2014d). The ecological character description for the Coorong Ramsar site (Phillips and Muller, 2006) states that the native fish community includes 48 species. Declines in formerly common estuary species (including the estuary perch, *Macquaria colonorum* and the jumping mullet, *Liza argentea*) have been observed by local fishers (MDBA, 2014d).

Amongst the native species known from the icon site are three species that are listed under the EPBC Act: Murray cod, Murray hardyhead and Yarra pygmy perch. Freshwater outflows from the Lower Lakes, and an open mouth of the Murray River, promote connectivity, improves estuarine habitats and promotes the flux of nutrients in the Coorong. This is highly favourable for productivity and enhances the growth and survival of juvenile and small-bodied fish and helps to sustain population strength (MDBA, 2014d). Little is known about how Lakes Alexandrina and Albert are used by large-bodied native fish, including the Murray cod (MDBA, 2014d).

The ecological character description for the Coorong Ramsar site (Phillips and Muller, 2006) (Table 27) and the Environmental Water Management Plan for the Lower Lakes, the Coorong and the mouth of the Murray River (MDBA, 2014d) include a range of native species that have not appeared in surveys of the fish assemblage (Bice *et al.*, 2016). This includes Murray cod and silver perch. In the most recent survey (2015-16) the catch across all locations revealed just 26 species, and was dominated by bony herring and the catadromous congolli (*Pseudaphritis urvillii*). The freshwater Australian smelt, semi-catadromous common galaxias (*Galaxias maculatus*) and marine sandy sprat (*Hyperlophus vittatus*) were the next most abundant species.

Table 27 Native fishes of the Lower Lakes and Coorong

Common name	Scientific name	Status
Agassiz glassfish	*Ambassis agassizii*	Unlisted
Australian smelt	*Retropinna semoni*	Unlisted
Big-bellied seahorse	*Hippocampus abdominalis*	Unlisted
Black bream	*Acanthopagrus butcheri*	Unlisted
Blue sprat	*Spratelloides robustus*	Unlisted
Bony bream	*Nematalosa erebi*	Unlisted
Bridled goby	*Acentrogobius bifrenatus*	Unlisted
Climbing galaxias	*Galaxias brevipinnis*	Unlisted
Common galaxias	*Galaxias maculatus*	Unlisted
Congolli	*Pseudaphritis urvillii*	Unlisted
Dwarf flathead gudgeon	*Philypnodon sp.*	Unlisted
Estuary perch	*Macquaria colonorum*	Unlisted
Flathead gudgeon	*Philypnodon grandiceps*	Unlisted
Fly-specked hardyhead	*Craterocephalus stercusmuscarum fulvus*	Unlisted
Freshwater catfish	*Tandanus tandanus*	Unlisted

Common name	Scientific name	Status
Goblin shark	*Mitsukurina owstoni*	Unlisted
Golden perch	*Macquaria ambigua ambigua*	Unlisted
Greenback flounder	*Rhombosolea tapirina*	Unlisted
Hybrid carp gudgeon (e.g. Lakes carp gudgeon)	*Hypseleotris spp.*	Unlisted
Jumping mullet	*Liza argentea*	Unlisted
Lagoon goby	*Tasmanogobius lasti*	Unlisted
Midgley's carp gudgeon	*Hypseleotris sp.*	Unlisted
Mountain galaxias	*Galaxias olidus*	Unlisted
Mulloway	*Argyrosomus japonicus*	Unlisted
Murray cod	*Maccullochella peelii peelii*	Vulnerable
Murray hardyhead	*Craterocephalus fluviatilis*	Endangered
Murray-Darling carp gudgeon	*Hypseleotris sp.*	Unlisted
Pouched lamprey	*Geotria australis*	Unlisted
Prickly toadfish	*Contusus brevicaudus*	Unlisted
Purple-spotted gudgeon	*Mogurnda adspersa*	Unlisted
Ricardson's toadfish	*Tetractenos hamiltoni*	Unlisted
River blackfish	*Gadopsis marmoratus*	Unlisted
River garfish	*Hyporhamphus regularis*	Unlisted
Sand fish	*Crapatalus arenarius lasti*	Unlisted
Sandy sprat	*Hyperlophus vittatus*	Unlisted
Short-finned eel	*Anguilla australis*	Unlisted
Shortheaded lamprey	*Mordacia mordax*	Unlisted
Silver perch	*Bidyanus bidyanus*	Critically endangered
Small-mouthed hardyhead	*Atherinosoma microstoma*	Unlisted
Smooth toadfish	*Tetractenos glaber*	Unlisted
South Australian cobbler	*Gymnapistes marmoratus*	Unlisted
Southern pygmy perch	*Nannoperca australis*	Unlisted
Striped perch	*Helotes sexlineatus*	Unlisted
Tamar goby	*Afurcagobius tamarensis*	Unlisted
Western blue spot (swan river) goby	*Pseudogobius olorum*	Unlisted
Western carp gudgeon	*Hypseleotris klunzingeri*	Unlisted
Yarra pygmy perch	*Nannoperca obscura*	Vulnerable
Yellow-eye mullet	*Aldrichetta forsteri*	Unlisted

Source: Phillips and Muller (2006)

Several commercially important native species spawn and recruit within or adjacent to the Murray

River estuary during the spring and summer (A/Prof Qifeng Ye, *pers. comm.*, 2019).[88] These include mulloway (*Argyrosomus japonicus*), greenback flounder (*Rhombosolea tapirina*) and black bream (*Acanthopagrus butcheri*). Adult mulloway spawn in the surf zone during spring and summer, and epibenthic larvae enter protected juvenile habitat in the Murray River estuary via the Murray Mouth during late summer (Ferguson *et al.*, 2008; Ferguson *et al.*, 2014). Greenback flounder spawn within the Murray River estuary and Coorong, and likely in the adjacent marine environment from March to October (Earl, 2014). The Murray River estuary and the Coorong provide important nursery habitat for greenback flounder through their first 2 to 3 years of life, after which individuals migrate to the Southern Ocean via the Murray Mouth (Earl, 2014). Abundance of adult greenback flounder in the Murray River Estuary and the Coorong is extremely variable among years. This variation relates, in part, to the movement of individuals between the estuary and the Southern Ocean via the Murray Mouth (Earl *et al.*, 2017).

Yelloweye mullet (*Aldrichetta forsteri*) is also a commercially important species. It spawns at sea and enters the Murray River Estuary and the lagoons of the Coorong in large numbers via the Murray Mouth (Earl and Ferguson, 2013). Pipi (*Donax deltoides*) spawn during October and November (although multiple, later spawning events may occur), have a short larval phase (approximately 14 days) (Gluis and Li, 2014), and recruit to the beaches on Younghusband and Sir Richard Peninsulas from November onwards. Abundance of *D. deltoides* is likely to be driven by highly variable annual recruitment. Longshore currents are driven by prevailing winds which tend to be south-westerly in winter and moving to south-easterly in spring and summer. Change in the direction of longshore currents tends to occur in spring and summer. Mass mortalities of *D. deltoides* have occurred in the past (for example, along Goolwa beach in October and November 1984) likely due to reduced salinities (Wiltshire *et al.*, 2009), associated with the Murray River plume (Clarke, 1985; King, 1985).

The South Australian Seafood Quality Assurance Program (SASQAP) and the pipi fishery conduct regular water quality sampling along Younghusband Peninsula from the Murray Mouth. Increased levels of *Escherichia coli*, or shellfish biotoxins, result in extensions of the harvesting exclusion zone which impacts the fishery. For fish species with high ecological and conservation values, the Murray Mouth and barrage flows are highly important for the migration (fish passage) and recruitment of diadromous species such as the lamprey and congolli (Bice *et al.*, 2019). Barrage flows (quantity and quality) are also important in maintaining estuarine habitat, productivity, and the ecosystem health in the Coorong (Ye *et al.*, 2015).

Frogs: approximately 10 species of frog occupy the Lower Lakes and Coorong, although both the diversity of species and their population sizes vary between seasons (SADEH, 2000). Generally, frog distribution is limited by salinity and aridity, with the greatest species richness occurring in the estuaries of the Finniss River, Currency Creek and the western shore of Lake Alexandrina near Milang. Frogs in these parts of the Lower Lakes are an important prey species for herons, egrets and bitterns. The most widespread species in the region is the banjo frog (*Neobatrachus centralis*), although the common eastern froglet (*Crinia signifera*) and the spotted grass frog (*Limnodynastes

[88] The material in this section (the impact of flows and plumes on the native species in the Coorong) was adapted from written correspondence with Associate Professor Qifeng Ye, Principal Scientist and Science Leader | Inland Waters & Catchment Ecology, SARDI Aquatic Sciences, Primary Industries and Regions South Australia (05-09-19)

tasmaniensis) may also be present in large numbers at times (SADEH, 2000). The ecological character description for the Coorong Ramsar site (Phillips and Muller, 2006) also includes the southern bell frog as an endemic species; while the Environmental Water Management Plan for the Lower Lakes, the Coorong and the mouth of the Murray River (MDBA, 2014d) includes the Bibron's toadlet (*Pseudophryne bibroni*). The southern bell frog is listed under the EPBC Act as vulnerable, and inhabits fringing wetlands of Lake Alexandrina with known populations in Pelican Lagoon, Clayton Bay and Hindmarsh Island channels (MDBA, 2014d).

Turtles: the long-necked turtle is locally common in the area, and the short-necked or Murray turtle (*Emydura macquarii*) has also been reported (SADEH, 2000). The Environmental Water Management Plan for the Lower Lakes, the Coorong and the mouth of the Murray River (MDBA, 2014d) also includes the broad-shelled turtle (*Chelodina expansa*).

Vegetation communities: the Lower Lakes and Coorong include a number of ecologically-important terrestrial plant communities, as well as a number of submerged and emergent aquatic plant populations (MDBA, 2014d). These plants provide several ecosystem services including habitat structure, and direct and indirect food resources for aquatic fauna and birds. Plant diversity in the icon site is greatest near areas of confluence such as the lower reaches of the eastern Mount Lofty Ranges tributaries. Sections of the near shore environment around the Lower Lakes have extensive stands of *Phragmites australis* and *Typha domingensis* (MDBA, 2014d). These macrophytes provide excellent shelter and habitat for a range of fish and other vertebrate species. Key plant assemblages include those that contain macrophytes, *Ruppia spp*., *Gahnia filum*, *Myriophyllum spp*., *Melaleuca halmaturorum* and samphire (*Sarcocornia spp*. and *Suaeda australis*) (MDBA, 2014d). The site also contains a section of the critically endangered 'Swamps of the Fleurieu Peninsula', as well as the threatened *Gahnia filum* sedgeland ecosystems and a number of nationally listed plant species (MDBA, 2014d). The Swamps of the Fleurieu Peninsula are localised wetlands occurring in high rainfall areas in the local catchment areas of Tookayerta, Hindmarsh, Parawa, Myponga, Yankalilla, Onkparinga, Currency Creek and Finniss.[89] They are densely vegetated and occur adjacent to waterlogged soils around low-lying creeks and flats. The Swamps are typified by reedy or heathy vegetation growing on peat, silt, peat silt, or black clay soils.

12.5.4 Carp biomass density and population dynamics within the Lower Lakes and Coorong

In their analysis of the fish assemblage structure, movement and recruitment in the Lower Lakes and Coorong in 2015-16, Bice *et al.* (2016) caught just four (4) carp from fishway structures at Tauwitchere (large and small vertical slots, and the rock ramp), Goolwa (vertical slot and the adjacent Goolwa Barrage) and Hunters Creek (vertical slot). In the same study, these authors caught 86,613 bony herring and 76,729 congolli. The six traps were on structures at the edge of the more brackish waters of the mouth of the Murray River and the Coorong and (for reasons

[89] See: http: //www.environment.gov.au/biodiversity/threatened/publications/fleurieu-swamp

unknown) it seems very unlikely that such a small number of carp (four individual fish) is representative of the biomass density across the Lower Lakes and Coorong.

The NCCP project team modelling carp biomass density across Australia found that the density within Lake Alexandrina and the Goolwa Channel was approximately 325 kg/ha and approximately 385 kg/ha in Lake Albert (Stuart *et al.*, 2019). The carp biomass density within the Coorong and the mouth of the Murray River was approximately 250 kg/ha. This estimate was also given, however, to the hypersaline waterbodies at the southern end of the Coorong, and seems unlikely in that setting. The Stuart *et al.* (2019) estimates were based on a total carp population within the Lower Lakes of approximately 28,000 tonnes. This was at odds with Koehn *et al.* (2016), who estimated that the two lakes contained approximately 846,000 adult carp. The latter correlates to approximately 1,700 tonnes, or approximately 22 kg/ha. Stuart *et al.* (2019) questioned the very small capture/recapture study upon which Koehn *et al.* (2016) estimate was based, and considered their own estimates in the range of 325 to 385 kg/ha (for the Lower Lakes) to be more realistic.

The estimates of Stuart *et al.* (2019) were adopted for the purpose of this case study, although the uncertainty inherent in these estimates was also kept in mind.

Koehn *et al.* (2016) also noted that the carp biomass density within the Lower Lakes is strongly linked to the extent of flow through to the mouth of the Murray River. Population estimates fall as the flow diminishes through drought and dry years, and experience a marked resurgence with flood rains in the catchment (Koehn *et al.*, 2016). This pattern is typical of the ecology of carp in most waterbodies (Part II: Section 4.2). Koehn *et al.* (2016) also explained, however, that the while the population of carp falls with the extent of flow, the biomass density may *increase* in some parts of the Lower Lakes and Coorong ecosystem. The reason for this is the concurrently falling water level, and the subsequent concentration of carp. Conversely, immediately following the arrival of floodwaters, and before recruitment has occurred, carp biomass density is likely to be very low as the existing carp are dispersed within the floodwaters. These effects are illustrated in Figure 71, which shows carp biomass density increasing steadily through the years of the Millennium Drought (2000 to 2010), then falling with flooding rains at the end of 2010 (Koehn *et al.*, 2016).

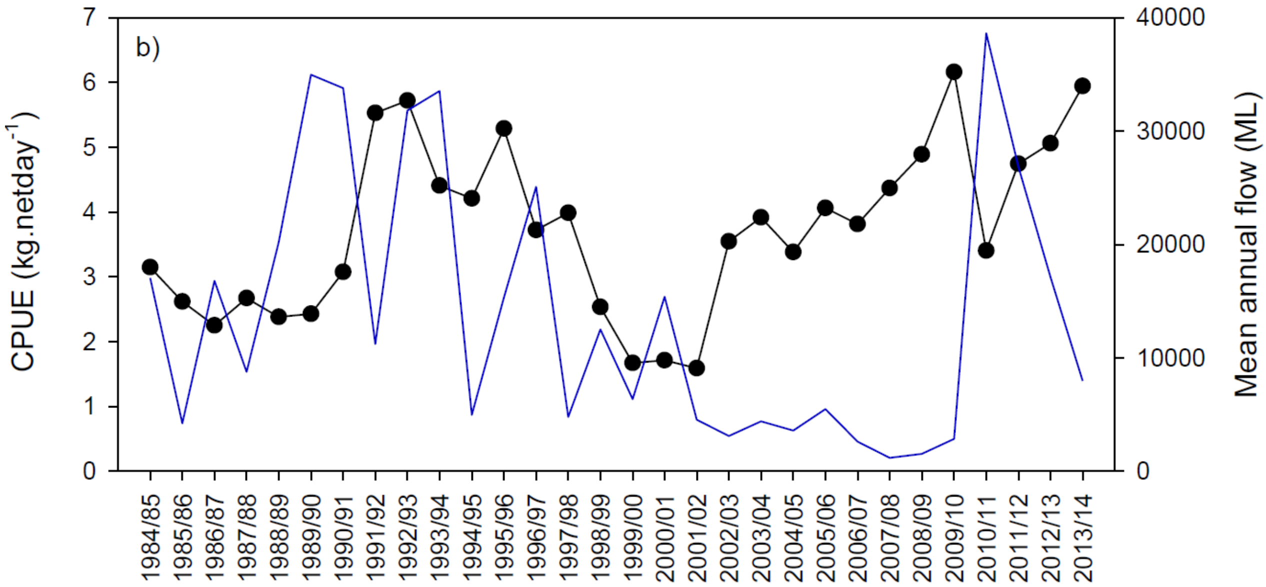

Figure 71 Carp biomass within the Lower Lakes 1984 to 2014

Source: Koehn *et al.*, (2016)

Carp within the Lower Lakes spawn in spring when the water temperature exceeds approximately 16 to 17C (Part II: Section 4.2). Spawning is likely to be concentrated in shallow areas protected by aquatic vegetation, such as the sheltered reedy freshwater estuaries adjacent the ingress of Currency Creek and the Finniss River. Tolderol and Mosquito points and the Narrows also support extensive sheltered reed beds. The Bremer River winds through red gum flats to the lake, while the Angas River flows seasonally through semi-arid woodlands into the lake, and both rivers have ephemeral wetlands near their mouths on Lake Alexandrina. Both lakes are fringed with tall reeds, and bulrush or cumbungi, forming occasional salt marshes and saline lagoons. These areas retain some water as a result of local runoff from winter rains, and may be inundated by floods. Juvenile carp will remain in these shallow waters, drifting toward the main body of the lake in late summer and autumn.

Carp resident in the freshwater habitats on (and surrounding) Hindmarsh, Mundoo, Ewe and Tauwitchere islands are likely to spawn amongst macrophytes and other submerged vegetation. These habitats are critical habitats for small-bodied fish, such as the Murray hardyhead and Yarra pygmy perch (MDBA, 2014d). Carp within the Coorong are likely to be drawn principally to the seasonally freshened waters near to the barrages, rather than the brackish mouth of the Murray River or the hypersaline southern lagoon. Spawning will again take place on submerged vegetation, including the aquatic herb *Ruppia tuberosa*.

12.5.5 CyHV-3 outbreak scenarios for the Lower Lakes and Coorong

As noted above, carp will begin to spawn when the water temperature exceeds approximately 16 to 17C. In the Lower Lakes, this will occur in spring – in general, approximately mid-September (Figure 72). The commencement of spawning will correlate approximately with the lower end of the permissive range for the transmission of CyHV-3 (approximately 18C, Part II: Section 5.3.4). An outbreak of CyHV-3 will typically be initiated by an aggregation event, as commonly occurs prior to the commencement of spawning. A number of spring aggregation events within Lake Alexandrina were reported in CarpMap.[90] Further transmission is then likely with the interaction between individual carp during spawning itself.

Unlike some warmer settings (for example, the Chowilla Floodplain – see Case Study 2: Section 12.2) transmission is unlikely to be curtailed by high water temperatures during the peak of summer. This is evident in Figure 72, which compares the mean daily temperature of the water in Lake Alexandrina with the Murray River adjacent to the Chowilla Floodplain. When it is considered that these data show the mean daily temperature averaged a second time across each month, it can be seen that whereas daily maximums for the Murray River are likely to exceed the upper threshold for the transmission of CyHV-3 (approximately 28C) this is not the case for Lake Alexandrina. One of the key contributors to the relatively low temperature of Lake Alexandrina is the strong and persistent wind. This is frequently sufficient to raise the water level on the downwind side of the lake by almost a metre, when compared with the upwind side (SADEH, 2000). The persistent wind also acts to maintain oxygenation of the lake – as discussed further in Section 12.5.6.

In view of the lower water temperature, the outbreak of CyHV-3 is likely to continue through to the hatching of larvae and their growth to juveniles. The juveniles will be highly-susceptible to CyHV-3, and their presence in large numbers may amplify the outbreak in some settings. The outbreak would otherwise continue until the decline in susceptible fish means that the probably of contact is not sufficient for it to be sustained. At this point, or with the fall in water temperature in autumn to below the minimum threshold for transmission, the outbreak would decline and eventually cease.

The scenario above references data from Lake Alexandrina, but the situation is likely to be broadly similar in Lake Albert and the Coorong. At each site within the ecosystem, aggregations of carp are likely to trigger an outbreak if the water temperature is permissive. The outbreak would then be maintained through the spawning season and the growth of juveniles, before declining in autumn – or before that if the population of susceptible fish has been sufficiently depleted. The environment within the area of the mouth of the Murray River is brackish, and southern parts of the Coorong are saline or hypersaline, and it is unknown if this may diminish the viability of the virus. Estimates for the carp biomass density (Section 12.5.4) for these parts of the ecosystem may also be higher than their reality and, if so, this is likely to diminish the aggressiveness of the outbreak at a local level.

A final factor to consider is the impact of the extent of water flowing through the ecosystem to the mouth of the Murray River. Water flow may alter the inundation of spawning habitat and may also alter the apparent carp biomass density – that is, when flows are low the volume of water in key spawning grounds is less and carp are effectively concentrated. This effect notwithstanding, ephemeral wetlands within the Lower Lakes and Coorong receive rainfall in winter, and the height of the water is generally at its highest in spring (SADEH, 2000). The water level then falls through to a low in autumn. This cycle is likely to mean that carp spawning grounds are maximally inundated at the time when spawning occurs, and that an outbreak of CyHV-3 may be aided in its

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[90] See: https: //carpmap.org.au/carp/content

later stages by the concentration of carp (including highly-susceptible juveniles) in diminishing waterbodies.

Significantly dry or drought years are likely to create a different outbreak scenario as the failure of winter rain may mean that ephemeral wetlands are not inundated in spring, and that the (lessened) spawning of carp will be restricted to shallow water at the fringe of the lakes. An outbreak in these circumstances may be less aggressive as the carp will remain in larger waterbodies and there are likely to be less highly-susceptible juveniles.

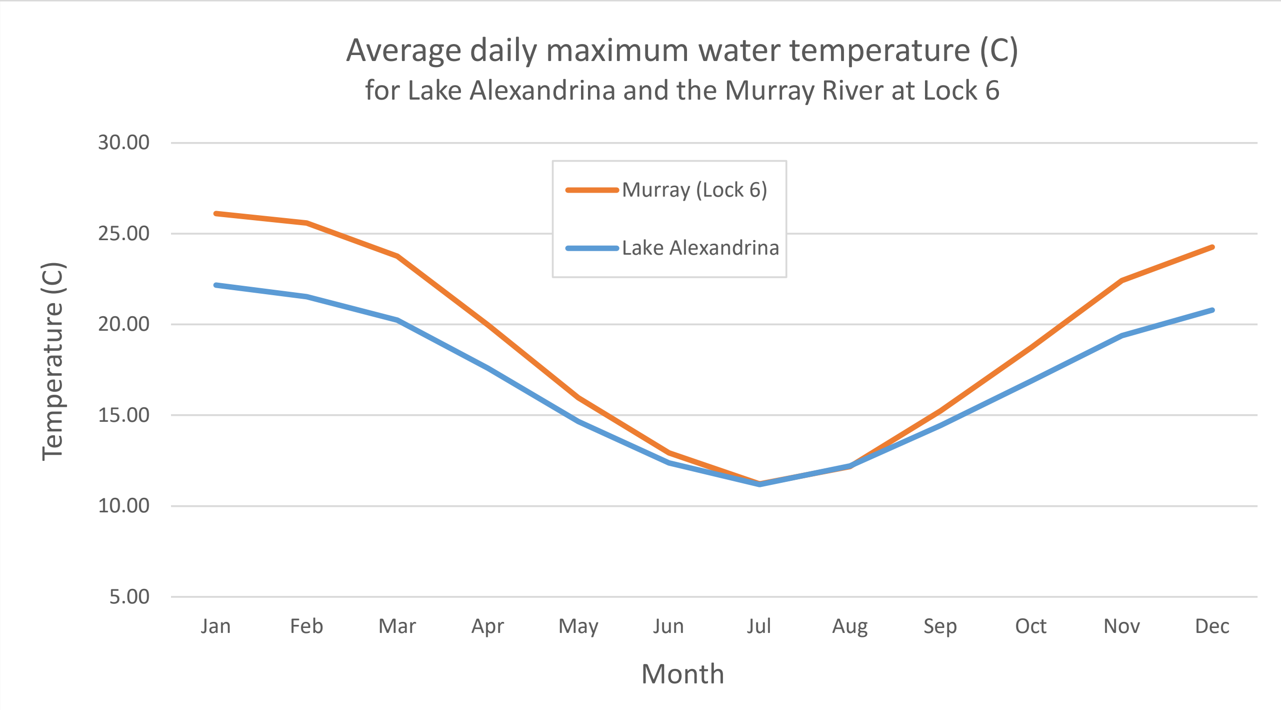

Figure 72 Water temperature within Lake Aexanrina and at Lock 6 in the Murray River

Daily maximum water temperature (C) aggregated to a monthly average, for Lake Alexandrina and Lock 6 in the Murray River (close to Chowilla Floodplain)

Source: raw data obtained from www.waterconnect.sa.gov.au

12.5.6 Exposure pathways and risks for the Lower Lakes and Coorong

A generic assessment for the nine identified exposure pathways was undertaken in Section 11. The discussion below interprets the outcomes from Section 11 in the context of the Lower Lakes and Coorong and their natural assets.

(a) Exposure of native aquatic species to low DO

Likelihood of exposure: under this pathway, hypoxic events are predicated on the accumulation of significant numbers of carp carcasses in a stationary or lentic waterbody. This scenario is plausible in some shallow and partially disconnected wetland areas of the Lower Lakes – in particular, during dry seasons when the winter-spring recharge has not been effective, and the carp in these areas have become relatively more concentrated. While wind-driven carcasses may accumulate on the fringes of the lakes themselves, the wind itself (which is persistent and generally high across the lakes) is likely to aerate the water and prevent an hypoxic or anoxic event. Within the mouth of the Murray River and the Coorong, where the salinity is higher and oxygen therefore less soluble in water, the carp biomass density is likely to be lower and the wind-driven re-aeration higher. Collectively, it is very unlikely that hypoxic conditions would be precipitated within these parts of the ecosystem through the accumulation of carp carcasses. This conclusion was supported by the modelling work of Hipsey *et al.* (2019) who found very little impact of carcass decomposition on DO within the Lower Lakes, even at artificially elevated carp biomass loadings.

Likely consequences of exposure: low DO primarily affects fish and crustaceans. Air-breathing aquatic animals will not be directly affected. Indirectly, low DO may also impact on ecological communities and on the integrity of wetlands. The Lower Lakes and Coorong provide nursery and feeding habitat for a very wide range of native fish – including obligate freshwater, diadromous, euryhaline, estuarine and marine species. Of these, the large-bodied and small-bodied species that cohabit shallow and partially disconnected wetland areas of the Lower Lakes are likely to be the most exposed. The Murray cod is the apex predator, and less likely to venture from deeper waters. Adult and juvenile estuary perch, freshwater catfish, golden perch, silver perch, bony herring and river blackfish may be exposed and, of these, the critically endangered silver perch is likely to be the most vulnerable although the strength or importance of its population within the Lower Lakes and Coorong is uncertain. The Lower Lakes provide important habitat for small-bodied native fish and while these are generally more tolerant of low DO than large-bodied fish, they will nevertheless be stressed or killed by levels lower than approximately 2.5 mg/L. This would include the vulnerable Yarra pygmy perch and the endangered Murray hardyhead. In the Coorong itself, and the Murray Mouth, juvenile mulloway, greenback flounder, black bream and yelloweye mullet may be exposed.

The Lower Lakes and Coorong ecosystem also contains a section of the critically endangered Swamps of the Fleurieu Peninsula, as well as the threatened *Gahnia filum* sedgelands. If a low DO event was precipitated by the accumulation of carp carcasses within one of these ecological communities, then the effects of that on local aquatic water-breathing species would have a negative food-web effect on the community itself.

Summary of risks: the accumulation of carp carcasses in numbers sufficient to result in hypoxic or anoxic conditions is only likely to occur in some shallow and partially disconnected wetland areas of the Lower Lakes – in particular, during dry seasons when the winter-spring recharge has not been effective, and the carp in these areas have become relatively more concentrated. If DO concentration falls sufficiently, then all water-breathing aquatic animals will be affected. Under hypoxic (c.f. anoxic) conditions, the small-bodied and benthic native species will be relatively more tolerant and likely to survive. Murray cod and silver perch are two of the threatened and most exposed species, although the importance of the Lower Lakes and Coorong to either species was not well established. Small-bodied native fish will be exposed when the DO fall below about 2.5 mg/L, and these include the three vulnerable species endemic to the Lower Lakes and Coorong ecosystem. The threatened Swamps of the Fleurieu Peninsula and *Gahnia filum* sedgelands may be exposed to the food-web effects of the death of native and invasive fish, where these encompass affected wetlands.

Mitigation of risks: the mitigation of risks associated with low DO within the Lower Lakes and Coorong will focus on the removal of carp carcasses from shallow wetland areas with a high carp biomass density, and within which an aggressive outbreak had occurred. The overarching goal will be to prevent the DO within a given waterbody falling below about 3 to 4 mg/L or to enable it to return to that level. The removal of carp carcasses may be particularly necessary during dry seasons. The removal of carp carcasses is likely to practically challenging in some parts of the Lower lakes and Coorong ecosystem, and priority would be given to areas of highest ecological significance.

(b) Exposure of native species, livestock and humans to cyanobacterial blooms

Likelihood of exposure: the scenario most likely to result in the accumulation of carp carcasses in numbers sufficient to initiate a widespread cyanobacterial bloom will be the same as the scenario most likely to result in a low DO event – that it, an aggressive spring outbreak of CyHV-3 during a dry season in shallow and partially disconnected wetland areas of the Lower Lakes. In this setting, it is plausible that carp carcasses would accumulate in numbers sufficient to result in a cyanobacterial bloom. By contrast, strong and persistent winds across the body of both lakes are likely to promote aeration and minimise stratification, and thus help to prevent the precipitous drop in DO that typically follows from a cyanobacterial bloom. The carp biomass density within the body of the two lakes is also likely to be lower than would generally be needed to initiate a widespread cyanobacterial bloom. Broadly, this was the result reported by Hipsey *et al.* (2019) whose modelling indicated that while DOC and ammonia levels may accumulate in some parts of a waterbody, and may be slower to dissipate or resolve with water flow or mixing and thus lead to a prolonged period of risk, the likelihood that this would occur in the Coorong is low. This result notwithstanding, it is important to note that cyanobacterial blooms are not uncommon within the Lower Lakes, with the first event reported in 1853.

Likely consequences of exposure: where a cyanobacterial bloom occurs, it is likely to result in the direct exposure of most aquatic species and the indirect exposure of a wide range of terrestrial species – including amphibians and nesting waterbirds. In these situations, harm may be caused through either the direct effect of cyanotoxins (if the strain(s) of cyanobacteria involved are cyanotoxic) or indirectly when the bloom dies off and decomposes, raising the BOD and leading to a precipitous fall in DO. It was concluded within the discussion of this exposure pathway (Section 11.2) that the risks associated with widespread cyanobacterial blooms for large- and small-bodied native fish and crustaceans will largely mirror those associated with low DO. Although not at risk from low DO, a range of marine and diadromous fish may also be exposed risk within the Coorong or through a contaminated plume from the Murray Mouth. These include juvenile mulloway, greenback flounder, black bream and yelloweye mullet. Pipi spawn during October and November and recruit to the beaches on Younghusband and Sir Richard Peninsulas from November onwards, and may be susceptible to nutrient load in the plume from the Murray Mouth. The modelling of Hipsey *et al.* (2019) suggests, however, that the exposure of these species, over-and-above the baseline rate of cyanobacterial blooms that occur in the Coorong and Lower Lakes, is likely to be very low.

In addition to native fish, are risks to waterbirds, frogs and turtles. The risks posed by widespread cyanobacterial blooms to ecological communities and wetlands are also different to those posed by low DO.

Waterbird breeding events within the Lower Lakes and Coorong are amongst the most significant in Australia. Over 185,000 waterbirds (57 species) used the Coorong in January 2016. More than half (over 93,000) of these waterbirds were in the south lagoon, with the balance in the north lagoon. Shorebirds and waterfowl the most common functional groups, although Australian pelicans were also prominent in the south lagoon. Around 46,000 waterbirds used the Murray River estuary, and these were principally shorebirds. Over 85,000 waterbirds used the Lower Lakes in January 2016, although this was less than half the numbers of birds within the Coorong (above). Piscivorous seabirds and waterfowl made up over 85% of the birds within the Lower Lakes. As has been the case in recent years, only small numbers (approximately 700) of migratory shorebirds (stints, sandpipers) used the Lower Lakes in January 2016. Four species listed under the EPBC Act are endemic to the Lower Lakes and Coorong ecosystem, including the critically endangered orange bellied parrot; the endangered Australian painted snipe and the southern Mount Lofty Ranges emu wren; and the vulnerable fairy tern.

A widespread cyanobacterial bloom would impact on the quality of the wetland habitat, as well as the availability of most forms of aquatic food. Even in a dry spring, however, the wetland is likely to be reasonably close to other patent waterholes. If the outbreak of CyHV-3 has not resulted in a similar deterioration of water quality within these waterholes, then many waterbirds will be able to relocate – or to source food from beyond their immediate nesting places. The wetlands and waterholes of the Lower Lakes and Coorong also provide refuge and habitat for many species of songbird, bush-bird and raptor (Table 26). The extent to which these will be exposed to either cyanotoxin (if the species of cyanobacteria is cyanotoxic) or water stress will depend on whether the outbreak of CyHV-3 affects only one or a few wetlands, or most of those in a large part of the ecosystem. The risk drivers for birds exposed to a cyanobacterial bloom will be the same within the Lower Lakes and Coorong as discussed in a generic sense in the pathway description for cyanobacterial blooms (Section 11.2). In particular, the risks will be higher for those functional groups or species whose feeding, foraging, nesting and other behaviours place them in close and regular contact with water. Colonial nesters are also at higher risk, as stressors to these populations are likely to be applied to a large number of birds at a time when harm can fall to adults and the success of recruitment can be threatened. Diet is also important, in particular as cyanotoxins bioaccumulate in animal tissues and regular exposure to affected (living) biota can mean that a toxic threshold is eventually breached.

Approximately 10 species of frog occupy the Lower Lakes and Coorong. These are focussed within wetlands fringing the estuaries of the Finniss River, Currency Creek and the western shore of Lake Alexandrina near Milang, and these locations may also be at risk from a widespread cyanobacterial bloom. The southern bell frog is listed as vulnerable under the EPBC Act. The long-necked turtle, short-necked turtle and the broad-shelled turtle are also endemic to the Lower Lakes and Coorong and would be exposed to cyanotoxin if a widespread bloom was to occur.

The Lower Lakes and Coorong ecosystem also contains a section of the critically endangered Swamps of the Fleurieu Peninsula, as well as the threatened *Gahnia filum* sedgelands. A widespread cyanobacterial bloom that affected these ecological communities could have a marked impact on species diversity and food webs.

Livestock and humans are also susceptible to cyanotoxins, and the risk to both were considered in a generic sense within the description of this exposure pathway (Section 11.2). The Lower Lakes and Coorong include a range of land tenures, and many stock have access to its waters. Cyanobacterial blooms are not uncommon within this ecosystem, and it is understood that managers will be accustomed to monitoring water quality. Although recreational fishing is permitted under permit it is very unlikely that drinking water or fish would be taken for human consumption from an area where mass fish mortalities or destructive cyanobacterial blooms were occurring. This likelihood would be further ameliorated through signage in public places. Caution would extend to the use of contaminated waters for swimming and other recreation, although the malodorous nature of a fish kill is likely to be a sufficient deterrent. On balance there are limited realistic scenarios under which humans or farmed livestock would be significantly exposed to harm as a result of an outbreak of CyHV-3 within the Lower Lakes or Coorong.

Summary of risks: although cyanobacterial blooms are not uncommon within the Lower Lakes and Coorong, this pathway considers the special case of a widespread bloom initiated by the decomposition of a large number of carp carcasses within shallow disconnected wetlands during an aggressive spring outbreak of CyHV-3. A widespread cyanobacterial bloom has the potential to impact on both aquatic and terrestrial biota, as well as on the quality and sustainability of riparian or wetland ecological communities. The exceptionally high profile of breeding and nesting waterbirds within the Lower Lakes and Coorong is likely to focus attention on this group. A widespread cyanobacterial bloom would also affect native fish (in particular, the large-bodied and small-bodied native fish that inhabit shallow wetland areas of the ecosystem), as well as frogs and turtles. Livestock and humans are at risk from cyanotoxins, although are likely to be protected by existing safeguards.

Mitigation of risks: the mitigation of risks associated with widespread cyanobacterial blooms within the Lower Lakes and Coorong would focus on the removal of carp carcasses from places where they had accumulated in sufficient numbers, and across a sufficiently broad area, to be likely to result in the initiation of a bloom. The removal of carp carcasses may be particularly necessary during dry seasons. The removal of carp carcasses is likely to practically challenging in some parts of the Lower lakes and Coorong ecosystem, and priority would be given to areas of highest ecological significance.

(c) Exposure of native species, livestock and humans to the pathogens associated with decomposing fish carcasses

Likelihood of exposure: little information is available about the pathogens that may proliferate in the event of a fish kill, and their possible impacts on native species, livestock or humans. This deficit was discussed in the generic description of this pathway (Section 11.3) and in the underpinning review of the literature (Part II: Section 6.3.4). That aside, it is possible that carp gut flora, spoilage organisms, *E. coli*, *Pseudomonas spp*, *Aeromonas spp* and other opportunistic microorganisms might proliferate and, in doing so, may present a hazard to native aquatic and terrestrial species. This may be plausible for shallow disconnected wetlands within the Lower Lakes and Coorong during a dry spring, when the carp biomass density is relatively higher and an aggressive outbreak of CyHV-3 more likely.

Likely consequences of exposure: although there is substantial uncertainty (and likely variation) around the pathogens that may be released following a substantial fish kill, and their ability to survive in an aquatic environment and cause harm to aquatic or terrestrial biota, it is reasonable to expect that the relative exposure of communities, functional groups and species will be similar to that obtained for widespread cyanobacterial blooms – albeit with a lower likelihood of realising the identified ecological endpoints. Balancing this, it was also noted within the generic evaluation of this exposure pathway (Section 11.3) that the experience obtained from field observations of Australian freshwater waterways is that while substantial fish kills are not uncommon, they have not to-date been linked to subsequent epidemics of waterborne pathogens. Some waterborne microorganisms may affect people and livestock, although people are likely to be protected to a large extent by the innate repulsiveness of a waterbody that contains the detritus of a significant outbreak. Livestock, however, may have less ability to avoid affected drinking water if there are limited or no alternatives. The risks to ecological communities are understandably highest for wetlands, including the critically endangered Swamps of the Fleurieu Peninsula, as well as the threatened *Gahnia filum* sedgelands.

Summary of risks: the shallow disconnected wetlands within the Lower Lakes and Coorong may be at risk during a dry spring from contamination with a range of waterborne microorganisms in the event of an aggressive outbreak of CyHV-3. Although dependent on the pathogen(s) concerned, potentially all aquatic and terrestrial species may be at risk. This would include livestock, and in some settings may include people.

Mitigation of risks: the mitigation of risks associated with proliferating waterborne microorganisms within the Lower Lakes and Coorong will focus on the removal of carp carcasses from within the shallow disconnected wetlands. The removal of carp carcasses may be particularly necessary during dry seasons. The removal of carp carcasses is likely to practically challenging in some parts of the Lower lakes and Coorong ecosystem, and priority would be given to areas of highest ecological significance.

(d) Exposure of native piscivorous species to the removal of a dominant and stable food source

Likelihood of exposure: the risks associated with the accumulation of carp carcasses include a low DO event, a widespread cyanobacterial bloom and the proliferation of waterborne microorganisms. These risks are focussed on settings where carp carcasses are most likely to accumulate – in particular, outbreaks of CyHV-3 in shallow disconnected wetlands in a dry spring. This is because risks associated with the accumulation of carcasses on or around the lakes themselves are less likely to result in these outcomes. The same is not necessarily true of the food-web effects of CyHV-3 – including the removal of a dominant and stable food source (this discussion) and prey-switching (below). Here it is sufficient only that a significant proportion of the population of juvenile or adult carp are removed rapidly from the ecosystem. Because the water temperature within the Lower Lakes the Coorong is likely to be suitable for the transmission of CyHV-3 throughout the spring and summer months, a sustained outbreak (or repeated local outbreaks) that includes the highly-susceptible juvenile carp is plausible. The removal of carp will have the most marked effect on the strictly piscivorous waterbirds – termed in this assessment, the seabirds. Aside from a colony of Australian pelicans in the south lagoon of the Coorong, most piscivorous waterbirds are located within the environs of the Lower Lakes. Here, these species and waterfowl made up approximately 85% of the waterbird population in 2016 – or about 75,000 birds. The extent to which these birds utilise or rely upon adult and juvenile carp is not known – just as there is some considerable uncertainty and difference within the literature as to the carp biomass density in the Lower Lakes – but it is plausible that the sudden removal of carp juveniles in particular might place stress on a waterbird breeding event. The breeding population of waterbirds will be largest during a high-flow season when the lakes and wetlands are maximally inundated. This scenario will also provide the conditions for a significant carp spawning event, and a similarly significant population of juvenile carp.

Likely consequences of exposure: if colonial-nesting and strictly piscivorous waterbird species within the environs of the Lower Lakes are exposed to the sudden removal of stable and dominant food source (juvenile carp) then they may be able to compensate for this by feeding on alternative waterbodies in the vicinity (including the mouth of the Murray River and the Coorong) or by switching to alternative food sources – including frogs, crustaceans, small-bodied native fish and juvenile large-bodied native fish (prey-switching). If these alternatives cannot be achieved adequately, then there may be an impact on the survival rate of chicks and this may in turn impact on the resilience of some populations of piscivorous waterbird species. An impact on breeding waterbirds would in turn have a very marked effect on ecological communities within and in the environs of the Lower Lakes.

Summary of risks: the food-web effects of freshwater fish kills in Australia and elsewhere are not well understood. The Lower Lakes and Coorong, however, provide habitat for one of Australia's most significant waterbird breeding events – and this includes a substantial number of piscivorous seabirds. Aside from a colony of Australian pelicans in the south lagoon of the Coorong, seabirds favour the environs of the Lower Lakes. An aggressive outbreak(s) of CyHV-3 is possible within the lakes, and this might include an outbreak amongst highly-susceptible juvenile carp. Although the extent to which these juvenile carp provide a stable source of food for the chicks of piscivorous seabirds is not known, there is likely to be some utilisation and the sudden removal of juvenile carp might place stress on the recruitment of some seabird species. The effect is likely to be enhanced through the coincidence of large waterbird breeding events and large carp spawning event, both of which are likely to take place in high-flow seasons.

Mitigation of risks: the only mitigation identified for this pathway is the release of farmed juvenile large-bodied and small-bodied native fish species, as these will provide both a source of food for nesting waterbirds and protection for the endemic population of both large-bodied and small-bodied species within the ecosystem (below).

(e) Exposure of native species to increased predation as a result of prey-switching

Likelihood of exposure: the exposure of small-bodied and juvenile large-bodied native fish, crustaceans and turtle eggs and hatchlings to prey-switching is conditional on the exposure of piscivorous species to the sudden removal of a stable food source. This is plausible within the Lower Lakes, where an aggressive outbreak of CyHV-3 in a high-flow spring may coincide with a substantial breeding event for piscivorous seabirds.

Likely consequences of exposure: the native species potentially at risk as a result of prey-switching include the juveniles of large-bodied fish, small-bodied fish, crustaceans, frogs, and turtle eggs and hatchlings (Section 11.5). Murray cod and silver perch juveniles would be amongst the large-bodied native species at risk. In the Coorong itself, and the Murray Mouth, juvenile mulloway, greenback flounder, black bream and yelloweye mullet may be exposed. Golden perch juveniles may be exposed in the Lower Lakes, although the broader geographic distribution of this species renders it inherently more resilient. Freshwater catfish spawn within an elaborate nest, and juveniles could be exposed in some shallower parts of the lake. Any of the small-bodied native fish would also be exposed – in particular, as these favour some of the wetland habitats with substantial seabird colonies. All 10 species of frog are also likely to be quite exposed (including the vulnerable southern bell frog), as these species also pre-exist as components of the diet of many carnivorous (but not strictly piscivorous) waterbirds. The extent to which the eggs or hatchlings of the three turtle species might be targeted is unknown, although it is likely that many carnivorous waterbirds would also opportunistically feed on either or both if a large proportion of carp juveniles was removed.

Summary of risks: the risks associated with this pathway are conditional on the realisation of the prior pathway – that is, stress to populations of (in particular) colonial-nesting piscivorous waterbirds as a result of the removal of juvenile carp. This event is plausible within the Lower Lakes and Coorong. If a loss of juvenile carp was to occur, piscivorous waterbirds are likely to switch to other prey species, or increase their dependence on other prey species. Carnivorous waterbird species that might include juvenile carp in their diet are also likely to switch to other prey species. Of these alternative species, juvenile Murray cod and silver perch, any small-bodied native fish, the 10 species of frogs and three turtles are likely to be most exposed.

Mitigation of risks: the only mitigation identified for this pathway is the release of farmed juvenile small bodied and large-bodied native fish species.

(f) Exposure of native species, livestock and humans to an outbreak of botulism

Likelihood of exposure: an outbreak of type C (or C/D mosaic) botulism can arise in two key ways: (a) through the death of animals carrying spores within their gastrointestinal tracts; and (b) through the germination of spores within the environment (Part II: Section 7.6.1). In both cases, the germination of spores is triggered by the development of anaerobic conditions. For carcasses, this occurs when aerobic organisms exhaust available oxygen. Spores within the environment can germinate following any oxygen-depleting process – including the decomposition of large numbers of fish or bird carcasses, or the decomposition of a cyanobacterial bloom. Both pathways for the development of type C (or C/D mosaic) botulism can be linked to an outbreak of CyHV-3. The first pathway relies on the establishment of the carcass-maggot cycle, and this is a highly probabilistic process – the larger the number of affected carcasses, and the population feeding on maggots, the higher the likelihood that the cycle will be sustained. Aggregations of waterbirds (in particular, insectivorous waterbirds) are ideal for this scenario, and most outbreaks of type C (or C/D mosaic) botulism in Australia have been associated with waterbird breeding and nesting events in freshwater wetlands or at the edges of freshwater waterholes, lagoons, billabongs or shallow lakes. Although the science is not definitive, the presence of saltwater appears to provide a protective effect against most strains of *C. botulinum* (Part II: Section 7.2). The exceptions to this are some strains that appear to have adapted to saltwater, and whose epidemiology is linked inexorably to saltwater ecosystems. The salt-adapted strains are *C. botulinum* type C, which is the form of botulism that this assessment focusses on. That notwithstanding, an aggressive outbreak(s) of CyHV-3 is most likely to occur within the freshwater Lower Lakes and less likely within the brackish mouth of the Murray River or the Coorong (Section 12.5.5). In this setting, most waterbirds piscivorous seabirds and omnivorous (including insects) waterfowl. Although these account for only a third of the breeding or nesting waterbirds within the broader ecosystem of the Lower Lakes and Coorong, it is nevertheless a very significant population – approximately 85,000 waterbirds were identified in the Lower Lakes in 2016. This number (and their density in wetland habitats) is likely to be sufficient for the initiation of a carcass-maggot cycle.

Likely consequences of exposure: although the exposure of birds and other biota that feed on aquatic and other insects is likely to be greatest, the impacts of an outbreak of type C (or C/D mosaic) botulism within the Lower Lakes would extend to most terrestrial and aquatic species.

This would include, in particular, the waterfowl, piscivorous seabirds, large waders and Gruiformes. Insectivorous songbirds and bush-birds would also be at risk, and this would include the critically endangered orange bellied parrot and the endangered southern Mount Lofty Ranges emu wren. Frogs and turtles would also be exposed, including the vulnerable southern bell frog. Any livestock with access to the affected waterbodies. Humans are not susceptible to type C (or C/D mosaic) botulinum toxin.

Summary of risks: the reasons why outbreaks of type C (or C/D mosaic) botulism in wildlife occur in some settings, and not in others with an ostensibly similar profile of risk factors, are not well understood but are likely to reflect the strongly probabilistic nature of outbreak initiation and expansion. An aggressive outbreak of CyHV-3 is mostly likely to occur in the freshwater Lower Lakes. A range of pathways could be relevant to this setting, including the carcass-maggot cycle and the germination of environmental spores following an hypoxic or anoxic event. While the population of breeding or nesting waterbirds within the Lower Lakes is only about a third of the total population breeding within the broader ecosystem, it is nevertheless a very significant number (approximately 85,000 in 2016) – and likely to be sufficient for the initiation of the carcass-maggot cycle and the development of a severe outbreak of type C (or C/d mosaic) botulism.

Mitigation of risks: prevention of an outbreak of type C (or C/D mosaic) botulism will rest on the timely removal of carp and other carcasses – including both fish and waterbirds. The removal of carcasses may be particularly necessary during dry seasons, when the likelihood of an aggressive outbreak of CyHV-3 is highest. The removal of carp carcasses is likely to practically challenging in some parts of the Lower lakes (and Coorong more broadly) and priority would be given to areas of where waterbird breeding and nesting is concentrated.

12.6 Case study 6: Upper Lachlan River Catchment (Abercrombie River)

12.6.1 Physical description of the Upper Lachlan case study site

This case study focussed on the reach of the Abercrombie River between Abercrombie Road, north of Tuena, and the Taralga Road, at the Bummaroo Ford camping ground (Figure 73). The site lies completely within New South Wales. The Abercrombie River rises in Blue Mountains National Park near Mt Werong, and flows northwest to Wyangala Dam. The Lachlan River also flows into Wyangala Dam, although is the lesser of the two rivers at this point in the catchment. Outflow from Wyangala Dam is exclusively to the Lachlan River, which then flows through the Lachlan Valley toward its eventual convergence with the Murrumbidgee River in the Murrumbidgee National Park near Oxley in southwestern New South Wales. The Abercrombie River is generally acknowledged as containing the highest quality water flowing into the Lachlan River system.

The case study site includes a 42 km reach of the Abercrombie River that flows through the three parts of Abercrombie National Park (Figure 74). In February 2006, the New South Wales Parks and Wildlife Service released a Plan of Management for the Abercrombie River National Park (NSW PWS, 2006) that included an overview of the river's physical characteristics and ecological values. Unless otherwise referenced, much of the introductory detail for this case study was adapted from the Plan. Abercrombie River National Park is centred on an area of deeply incised gully systems, in conjunction with prominent ridges and spurs.

There is a large altitudinal difference in the Park, from 1,128 metres in the Felled Timber Creek catchment in the north-east to 500 metres at the Abercrombie River in the south-west. Silent Creek and Retreat River both feed into the Abercrombie River within the Park. Retreat River, which originates north of the park, has two main tributaries, Licking Hole Creek whose catchment is wholly contained within the park and Felled Timber Creek.

Figure 73 Abercrombie River, from Abercrombie Road to the Taralga Road

Source: Google Maps[91]

[91] See: https: //www.google.com/maps/@-34.0821966,149.5264195,51285m/data=!3m1!1e3

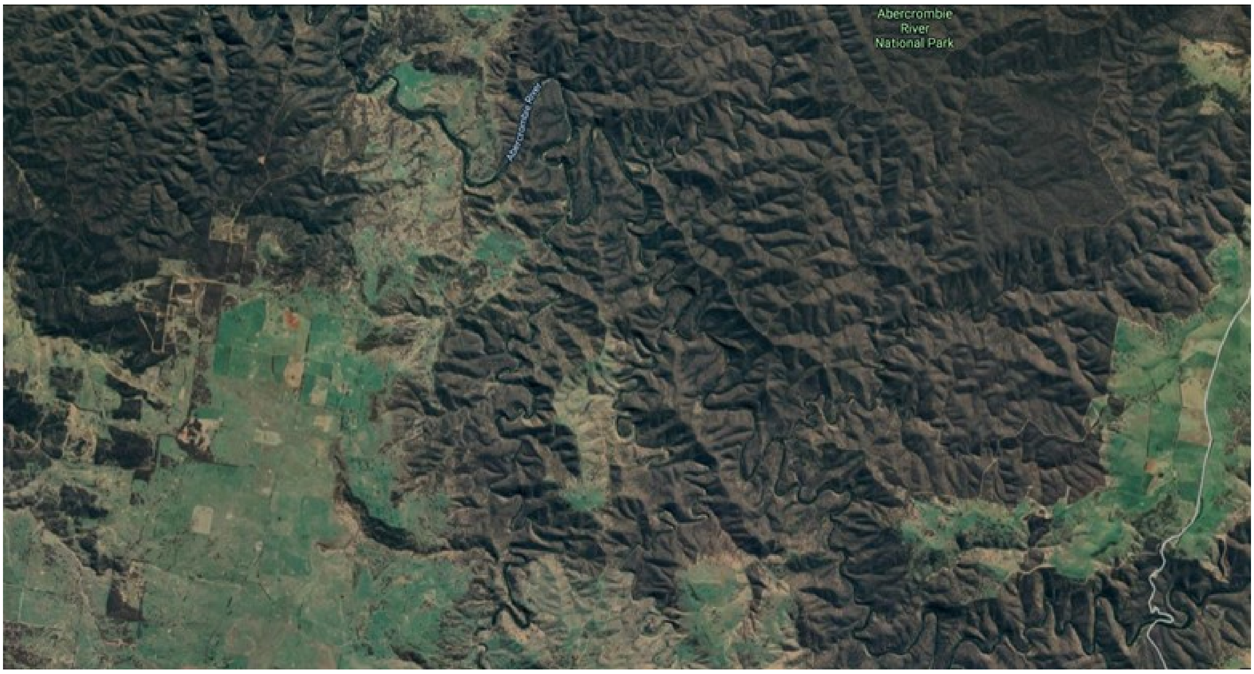

Figure 74 Abercrombie River National Park and the Abercrombie River

Source: Google Maps[92]

12.6.2 Hydrology of the Upper Lachlan (Abercrombie River)

This case study focuses solely on an unregulated reach of the Abercrombie River, in the Upper Lachlan Catchment.

12.6.3 Ecological values of the Upper Lachlan (Abercrombie River)

The Plan of Management for Abercrombie River National Park (NSW PWS, 2006) describes its floral and faunal attributes and key ecological communities.

The Abercrombie River National Park protects an important area of remnant bushland within the south-western Central Tablelands of New South Wales. It conserves a diversity of vegetation communities typical of montane areas, tableland areas and western slopes. It contains the largest remaining intact patch of vegetation typical of the drier parts of the tablelands in the region, and hence is very significant in species conservation within the area. In 1995 the National Parks Association undertook a biodiversity survey of the area, and this included a detailed vegetation survey. The survey highlighted high levels of species richness, particularly for bryophytes and liverworts, in a comparatively dry area. It has been suggested that there may be some correlation between this feature and a limestone influence in the soil. In 1998 a vegetation survey of the whole park was carried out by the NPWS as part of a Comprehensive Regional Assessment of the region. These surveys identified sixteen distinct plant communities that exist within the park (see Appendix 1).

[92] See: https: //www.google.com/maps/@-34.1512333,149.6383682,12842m/data=!3m1!1e3

These plant communities can be divided into three groups based on their regional significance:

- **Group 1**: Vegetation typical of wet, high altitude sites. These vegetation types are significant because suitable conditions for their occurrence are limited, hence they only occupy a small area within the park. Examples include the Northern Plateau moist fern / herb / grass forest found above 1,000 metres, which occurs in only 0.14% of the park, dominated by brown barrel (*Eucalyptus fastigata*), mountain gum (*Eucalyptus dalrympleana*), bitter pea (*Daviesia ulicifolia*) and bracken (*Pteridium esculentum*). These vegetation communities have been much reduced in the area since the establishment of extensive areas of pine plantations and grazing.
- **Group 2**: Vegetation limited in occurrence due to a lack of suitable conditions. Examples include; riparian Acacia/ shrub/ grass/ herb forest dominated by river oak (*Casuarina cunninghamiana*) is limited to alluvial soils along permanent watercourses. Western Slopes dry grass forest, typified by Blakely's red gum, yellow box and clustered wallaby grass (*Danthonia racemosa*), is limited to relatively fertile soils and falls within the white box / yellow box / Blakely's red gum community.
- **Group 3**: these communities are typically widespread in the drier parts of the tablelands but have been reasonably extensively cleared (34-66%). The park is one of the main areas where these communities are conserved.

The Abercrombie River National Park provides important habitat for at least 90 native bird species. Many of these species are migratory or nomadic. The powerful owl (*Ninox strenua*) and glossy black-cockatoo (*Calyptorhynchus lathami*) have both been recorded during surveys. Other species which have been recorded in the area and which are of particular conservation concern include the grey goshawk (*Accipiter novaehollandiae*), and the peregrine falcon (*Falco peregrinus*). The noisy friarbird (*Philemon corniculatus*) has been identified as a species of particular conservation concern due to the clearance of eucalypt forest and woodland habitat of this species. Other sensitive species that may occur in the area include the Australasian bittern, masked owl (*Tyto novaehollandiae*), black bittern (*Ixobrychus flavicollis*), pink robin (*Petroica rodinogaster*), square-tailed Kite (*Lophoictinia isura*), swift parrot and the turquoise parrot (*Neophema pulchella*). Of these birds, the swift parrot is listed under the EPBC Act as critically endangered, while the glossy black cockatoo is endangered and the masked owl is vulnerable.

The Abercrombie River National Park protects a range of aquatic fauna, including the platypus (*Ornithorhynchus anatinus*) and the Gippsland water dragon (*Physignathus leseuerii ssp. howitti*). Eight frog species have been recorded in the park, including the booroolong frog, which is confined in its range to mountain streams of the Great Divide and has almost disappeared from this region. The booroolong frog is listed as endangered under the EPBC Act.

The Abercrombie River and its tributaries provides habitat for endangered trout cod, the critically endangered silver perch, the endangered Macquarie perch, the river blackfish and the Murray crayfish. Farmed juvenile trout cod and Macquarie perch are released into the Abercrombie River. The population of trout cod endemic within the Macquarie River may be entirely stocked. The degradation of native riparian vegetation (Figure 75) has been identified as a key threatening process under the Fisheries Management Act 1994. A Threat Abatement Plan has been prepared by New South Wales Fisheries to address this process.

Figure 75 Riparian setting within Abercrombie River National Park

Source: New South Wales National Parks (https: //www.nationalparks.nsw.gov.au/)

12.6.4 Carp biomass density and population dynamics in the Upper Lachlan (Abercrombie River)

The NCCP project team modelling carp biomass density across Australia found that the density of carp decreased dramatically from a high value of approximately 340 kg/ha within Wyangala Dam, to approximately 120 kg/ha at the crossing of the Tuena Rd (and the start of this case study site). Quickly after that, the carp biomass density within the Abercrombie River dwindles toward a very low value of approximately 40 kg/ha. The carp biomass density within the reach of the river that lies within the ecologically-sensitive Abercrombie River National Park is between approximately 50 and 75 kg/ha. The carp biomass density in tributary streams leading to the Abercrombie River is as low as 30 kg/ha in places and never higher than the Abercrombie River itself. The carp biomass density downstream of Wyangala has a similar distribution. Between the outflow and Forbes the density is a little over 100 kg/ha, but diminishes after that to values between approximately 50 and 100 kg/ha. Interspersed along its length are ephemeral lakes and impoundments where the density is higher. Koehn *et al.* (2016) also examined the Lachlan River Catchment as a case study, although focussed on the reach downstream of Wyangala Dam. In recognition of the generally lower carp biomass density throughout the catchment, these authors considered 88 kg/ha to be the threshold for environmental impacts in the region.

The population dynamic of carp in the Abercrombie River and its interaction with the much higher biomass density within Wyangala Dam has not been reported on. It is likely that at least some of the carp within Wyangala Dam have been translocated for the purpose of recreational fishing. This is evident from genetic analysis, which showed that some strains of carp within Wyangala Dam differ from those in adjacent waterways (NSW I&I, 2011). This analysis also appeared to suggest that population of carp within Wyangala Dam do not migrate upstream into either the Lachlan or Abercrombie Rivers. This would mean that spawning and recruitment to the Abercrombie River takes place within the river itself – and not in the more favourable habitat downstream in Wyangala Dam. By way of comparison, carp within the Murray River channel and its off-channel waterbodies are known to migrate to key spawning habitats such as the Barmah-Millewa Forest (Case Study 1: Section 12.1). The restriction of carp within the Abercrombie River may help to explain their very low biomass density, and is likely also to mean that the population itself is composed of relatively older (and likely larger) fish.

12.6.5 CyHV-3 outbreak scenarios for the Upper Lachlan (Abercrombie River)

Although an aggressive and visible outbreak of CyHV-3 is possible within the high biomass density of carp in Wyangala Dam, the same is unlikely to be true of the ecologically-sensitive up-stream parts of the Abercrombie River. In these parts of the Abercrombie River, the carp biomass density is quite low (approximately 50 to 75 kg/ha) and spawning aggregations are unlikely. A single small (10 to 100 fish) aggregation event within the region was reported in CarpMap,[93] although the location of this event is questionable as the coordinates place it in farmland close to Taralga. The event was also reported in mid-winter (01 August, 2018) in a very cold and snow-prone part of southern New South Wales. The reasons for winter aggregations of carp are not certain, although may reflect the conservation of warmth or protection during periods of collective inactivity.

Spawning itself will be triggered by a rise in water temperature to approximately 16 to 17C, which also represents what is broadly understood to be lower end of the permissive range for the transmission of CyHV-3 (approximately 18C, Part II: Section 5.3.4). The water temperature within the upper reaches of the Abercrombie River is unlikely to reach 16 to 18C until early summer, but is also unlikely to exceed the upper threshold for the transmission of CyHV-3 (approximately 28C) as summer progresses. The outbreak of CyHV-3 that ensues might present as occasional carcasses at the river's edge or, given scavenging by turtles and crayfish and other animals, might not be detected at all. Carcass accumulations are unlikely as the carp biomass density is low and the water velocity is reasonably constant under most flow scenarios. Possible exceptions to this might include very dry times when the Abercrombie River contracts to a shallow stream connecting deeper waterholes (Figure 76). Even given the concentration of fish within these waterholes, the biomass density of carp is unlikely to be sufficient to result in an aggressive outbreak with visible accumulations of carp carcasses.

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[93] See: https: //carpmap.org.au/carp/content

Figure 76 Low water level in the Abercrombie River

Source: New South Wales National Parks (https: //www.visitnsw.com/)

12.6.6 Exposure pathways and risks for the Upper Lachlan (Abercrombie River)

A generic assessment for the nine identified exposure pathways was undertaken in Section 11. The discussion below interprets the outcomes from Section 11 in the context of the Abercrombie River and its natural assets.

(a) Exposure of native aquatic species to low DO

Likelihood of exposure: under this pathway, hypoxic events are predicated on the accumulation of significant numbers of carp carcasses in a stationary or lentic waterbody. As explained in Section 12.6.5 above, this scenario is not realistic within the upper reaches of the Abercrombie River where the biomass density of carp is low and the water velocity generally moderate to high. Carcasses and carcass materials are likely to be dispersed rapidly, or consumed by carrion-eating aquatic and terrestrial animals. Any deterioration in water quality is likely to be mitigated by the constant flow from upstream. A possible exception to this may be seen in extremely dry seasons, when the Abercrombie River has contracted to a shallow stream connecting deeper waterholes (Figure 76). However, even given the concentration of fish within these waterholes, the biomass density of carp will not be sufficient to result in an aggressive outbreak of CyHV-3 and the initiation of an hypoxic or anoxic event. On balance, the likelihood of exposure by this pathway is negligible and the pathway was not taken forward.

(b) Exposure of native species, livestock and humans to cyanobacterial blooms

Likelihood of exposure: although cyanobacterial blooms are not uncommon within the Abercrombie River, a widespread bloom initiated by the decomposition of carp carcasses would require the accumulation of significant numbers of carp carcasses in a stationary or lentic body of water. As explained above, this scenario is not realistic within the upper reaches of the Abercrombie River where the biomass density of carp is low and the water velocity generally moderate to high. Carcasses and carcass materials are likely to be dispersed rapidly, or consumed by carrion-eating aquatic and terrestrial animals. Any deterioration in water quality is likely to be mitigated by the constant flow from upstream. On balance, the likelihood of exposure by this pathway was considered to be negligible and the pathway was not taken forward.

(c) Exposure of native species, livestock and humans to the pathogens associated with decomposing fish carcasses

Likelihood of exposure: little information is available about the pathogens that may proliferate in the event of a fish kill, and their possible impacts on native species, livestock or humans. This deficit was discussed in the generic description of this pathway (Section 11.3) and in the underpinning review of the literature (Part II: Section 6.3.4). That aside, it is possible that carp gut flora, spoilage organisms, *E. coli*, *Pseudomonas spp*, *Aeromonas spp* and other opportunistic microorganisms might proliferate and, in doing so, may present a hazard to native aquatic and terrestrial species. This is unlikely within the upper reaches of the Abercrombie River, where the biomass density of carp is low and carcass accumulation will be minimal. The velocity of the water through this reach is also likely to disperse and dilute carcass materials and contaminants. These considerations notwithstanding, the uncertainties inherent to this pathway require it to be taken forward.

Likely consequences of exposure: although there is substantial uncertainty (and likely variation) around the pathogens that may be released following a fish kill, and their ability to survive in an aquatic environment and cause harm to aquatic or terrestrial biota, it is reasonable to expect that the relative exposure of communities, functional groups and species will be similar to that obtained for widespread cyanobacterial blooms – albeit with a lower likelihood of realising the identified ecological endpoints. Native fish, frogs and crustaceans would be most at risk, and these include, in particular, the threatened trout cod, silver perch, Macquarie perch and the booroolong frog. The platypus is also an aquatic animal, and may be exposed to waterborne pathogens. The bird species endemic to the upper reaches of the Abercrombie River are mostly bush-birds and are more able and likely to avoid contaminated water. The black bittern may inhabit this reach, although this was not confirmed. Some waterborne microorganisms may affect people and livestock, although people are likely to be protected to a large extent by the innate repulsiveness of a waterbody that contains the detritus of a significant outbreak. Livestock, however, may have less ability to avoid affected drinking water if there are limited or no alternatives. The risks to riparian ecological communities are not likely to be as substantial as they are for wetlands and marshlands, and mostly contained to the food-web effects of harm to exposed aquatic or terrestrial animals. Balancing these considerations, it was also noted within the generic evaluation of this exposure pathway (Section 11.3) that the experience obtained from field observations of Australian freshwater waterways is that while substantial fish kills are not uncommon, they have not to-date been linked to subsequent epidemics of waterborne pathogens.

Summary of risks: although it is very unlikely that native species or communities within the upper reaches of the Abercrombie River would be exposed to waterborne pathogens as a result of an outbreak of CyHV-3, the scale of uncertainties associated with this pathway mean that it remained a consideration. Although dependent on the pathogen(s) concerned, potentially all aquatic and terrestrial species may be at risk. This would include livestock, and in some settings may include people.

Mitigation of risks: the mitigation of risks associated with proliferating waterborne microorganisms within the upper reaches of the Abercrombie River will focus on the removal of carp carcasses. The river passes through steep and relatively inaccessible terrain, and this may present a practical challenge.

(d) Exposure of native piscivorous species to the removal of a dominant and stable food source

Likelihood of exposure: there are no piscivorous species of bird or aquatic animal within the upper reaches of the Abercrombie River that rely on juvenile or adult carp as a stable food source. For this reason, the likelihood of exposure by this pathway was considered to be negligible and the pathway was not taken forward.

(e) Exposure of native species to increased predation as a result of prey-switching

Likelihood of exposure: the exposure of small-bodied and juvenile large-bodied native fish, crustaceans and turtle eggs and hatchlings to prey-switching is conditional on the exposure of piscivorous species to the sudden removal of a stable food source. Because there are no piscivorous species of bird or aquatic animal within the upper reaches of the Abercrombie River that rely on juvenile or adult carp as a stable food source, the sudden removal of juvenile or adult carp would not be associated with a significant food-web effect. On this basis, the pathway was not taken forward.

(f) Exposure of native species, livestock and humans to an outbreak of botulism

Likelihood of exposure: an outbreak of type C (or C/D mosaic) botulism can arise in two key ways: (a) through the death of animals carrying spores within their gastrointestinal tracts; and (b) through the germination of spores within the environment (Part II: Section 7.6.1). In both cases, the germination of spores is triggered by the development of anaerobic conditions. For carcasses, this occurs when aerobic organisms exhaust available oxygen. Spores within the environment can germinate following any oxygen-depleting process – including the decomposition of large numbers of fish or bird carcasses, or the decomposition of a cyanobacterial bloom. Both pathways for the development of type C (or C/D mosaic) botulism can be linked to an outbreak of CyHV-3. The first pathway relies on the establishment of the carcass-maggot cycle, and this is a highly probabilistic process – the larger the number of affected carcasses, and the population feeding on maggots, the higher the likelihood that the cycle will be sustained. Aggregations of waterbirds (in particular, insectivorous waterbirds) are ideal for this scenario, and most outbreaks of type C (or C/D mosaic) botulism in Australia have been associated with waterbird breeding and nesting events in wetlands or at the edges of waterholes, lagoons, billabongs or shallow lakes. These events do not occur within the upper reaches of the Abercrombie River. Alternatively, and perhaps more likely, an outbreak could follow from the development of anoxic conditions within all or part of the waterbody. This might be the result of the BOD created by the decomposition of carcasses, or may follow from the death and decomposition of a cyanobacterial bloom. Because the biomass density of carp in the upper reaches of the Abercrombie River is low, and the velocity of the water generally high, there is no realistic scenario under which an outbreak of CyHV-3 would result in an anoxic event by way of the direct effect of increased BOD or indirectly through the decomposition of a cyanobacterial bloom. Without any plausible exposure scenarios, the pathway was not taken forward.

13 Discussion: ecological risk

13.1 Outbreak scenarios

One of the key outcomes from the review of the literature in Part II of this report, and from the evaluation of exposure pathways and case studies in Part III, was that the character of an outbreak of CyHV-3 in an Australian freshwater waterway is likely to be underpinned by the following considerations: (a) opportunities for close contact between carp; (b) permissive water temperature (including both upper and lower thresholds for the transmission of CyHV-3); and (c) spawning behaviour of carp in a given setting, and the presence of highly-susceptible juvenile carp.

Close contact is perhaps the most significant of these considerations and is maximised during in an aggregation event (Figure 27). A survey (termed CarpMap) was undertaken in 2017-18 to determine (*inter alia*) where and how often members of the public witnessed carp aggregation events.[94] One of the outputs from this survey is reproduced in Figure 77 below and shows that most aggregations occurred in spring. This supports the generally held view that carp aggregate in anticipation of a rise in water temperature to the point where it is sufficient for the commencement of spawning (16 to 17C, Part II: Section 4.2.2). Carp are also known to aggregate in winter (possibly to generate collective warmth or possibly for other reasons) although, in contrast to highly-active spring aggregations, aggregations in winter are passive and submerged and are more likely to be unnoticed.

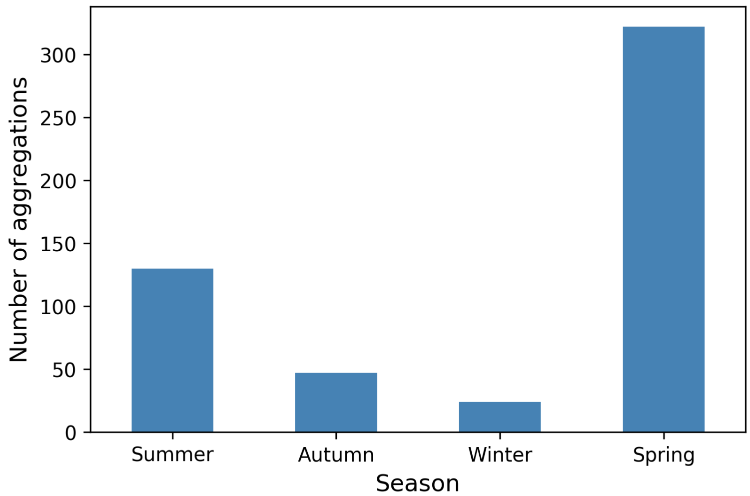

Figure 77 CarpMap aggregation survey results

Source: CarpMap survey, Kerryne Graham (CSIRO Australian Animal Health Laboratory, Geelong)

The spring temperature trigger for spawning in carp approximates the lower threshold for the transmission of CyHV-3 (about 18C, Part II: Section 5.3.4), and it is likely that spring aggregation events will provide the key opportunity for widespread exposure and the initiation of an outbreak

[94] See: https: //carpmap.org.au/carp/content

of CyHV-3. Further transmission is possible during spawning itself, although two constraints may apply. First, as summer progresses, the water temperature in some locations may exceed the upper threshold for transmission and this will then slow or halt the outbreak. Second, the carp biomass density during spawning may be very low in some settings (in particular, where spawning occurs on an inundated floodplain) and this may limit the ongoing propagation of an outbreak if transmission during the original aggregation event did not extend to a sufficiently high proportion of fish. Toward the end of summer, the numbers of highly-susceptible juvenile carp may be high, and this may then trigger a resurgence of the outbreak.

Drawing upon these general principles, outbreak scenarios were described (Section 10) for: (a) wetland or floodplain environments; (b) irrigation reservoirs and other permanent waterbodies; and (c) perennial and seasonally-disconnected riverine environments. Outbreaks in wetland and floodplain settings [a] were examined in case studies based on the Barmah-Millewa Forest, the Chowilla Floodplain and the Lower Lakes and Coorong. Outbreaks in irrigation reservoirs and other permanent waterbodies [b] were examined in the case study based on the Mid-Murray River and Lake Mulwala. Outbreaks in perennial riverine environments [c] were also examined in the case study for the Mid-Murray River and Lake Mulwala, as well as the Abercrombie River (within the Upper Lachlan River Catchment); while outbreaks in seasonally-disconnected riverine environments were examined in the case study based on the Moonie River Catchment.

The characteristics of an outbreak of CyHV-3 in the **Barmah-Millewa Forest** are likely to be determined primarily by the river flow. In a high-flow setting, when the floodplains are inundated, an aggressive outbreak is possible if sufficient transmission occurred during the aggregation of carp prior to spawning. Once carp move out onto the floodplains, the biomass density will fall substantially (a published observation was 22 kg/ha; Brown *et al.*, 2005). Some ongoing transmission is likely, although curtailed by opportunities for contact between infected and susceptible carp. As summer progresses, the outbreak is also likely to be curtailed by rising water temperature on the inundated floodplains. In a lower-flow setting, spawning will be constrained to permanent or semi-permanent off-channel waters. In this setting, the carp biomass density will remain high and the temperature of the deeper water will be less likely to exceed the upper threshold for transmission. An aggressive outbreak is more likely in the lower-flow setting and is also more likely to result in the accumulation of carp carcasses in numbers sufficient to impact on water quality. A lower-flow setting is also, however, less likely to be associated with a significant waterbird breeding and nesting event, and this may limit some of the key ecological risks.

The characteristics of an outbreak of CyHV-3 in the **Chowilla Floodplain** are likely to be broadly similar to those in the Barmah-Millewa Forest, albeit metred by: (a) the high degree of control over flows through the floodplain provided by the Chowilla regulator and associated structures; and (b) the higher water temperature in summer. These differences mean that carp will be able to access floodplains under a wider range of flow scenarios, thus lowering the biomass density at spawning, and that ongoing transmission is more likely to be curtailed in mid-to-late summer. Collectively, an aggressive outbreak of CyHV-3 is less likely in the Chowilla Floodplain than the Barmah-Millewa Forest and is less likely to be associated with the accumulation of carp carcasses in numbers sufficient to result in an impact on water quality. The ability to flush affected parts of the Chowilla Floodplain with some precision is also likely to minimise the impacts of an outbreak, as will the substantially smaller size of waterbird breeding and nesting events.

The characteristics of an outbreak of CyHV-3 in the **mid-Murray River (Lake Mulwala to Tocumwal)** are less likely to differ between high-flow and lower-flow seasons as this reach of the Murray River is regulated to provide reliable downstream water for irrigation and environmental purposes. The reach does, however, include three quite different ecological settings and the characteristics of an outbreak in each of these is likely to differ. The main channel is a lotic and relatively deep-water environment. Its flow varies most in winter and spring, as a result of rainfall within the upstream catchment, but is reasonably stable through the balance of the year – and between years. Carp resident within the main channel are likely to spawn in off-channel waters, or downstream in the Barmah-Millewa Forest ecosystem. An outbreak in the main channel may be aggressive if sufficient transmission occurs during a pre-spawning aggregation event, although is unlikely to result in the accumulation of carcasses in numbers sufficient to impact on water quality. The reach also includes minor off-channel anabranches, billabongs and wetlands, and here both spawning and the accumulation of carp carcasses are more likely. Waterbird breeding events are also more substantial in these settings than in the river channel. The case study also included Lake Mulwala, upstream of the Yarrawonga Weir. This is a significant waterbody (approximately 4,450 ha, with storage capacity of 130,000 ML) supporting a large and diverse waterbird population. It is effectively a permanent waterbody, with substantive flow from both Hume Dam and the confluence with the Ovens River. Lake Mulwala has a range of aquatic and riparian habitats and is likely to support carp spawning events in most years. Given its relatively higher carp biomass density (approximately 251 kg/ha) an aggressive outbreak of CyHV-3 within Lake Mulwala is plausible. The depth and flow of the lake, however, will mean that the accumulation of carp carcasses in numbers sufficient to impact on water quality is less likely.

The characteristics of outbreaks of CyHV-3 within the **Moonie River Catchment** are likely to follow a seasonal pattern based on the transient reconnection of otherwise disconnected waterholes. The water temperature within these waterholes is likely to be suited to the transmission of CyHV-3 during spring and autumn. Summer rains will briefly reconnect the waterholes and allow the river to flow. The contraction of waterholes between flow events results in the concentration of aquatic biota (including carp) and a deterioration in water quality. In spring, prior to summer rains, when the waterholes are maximally contracted, carp biomass density may be high (as a result of concentration) and with it the potential for an aggressive outbreak of CyHV-3 and the accumulation of carcasses in numbers sufficient to impact further on water quality (in particular, DO and the risk of cyanobacterial blooms). These outbreaks may be terminated either by their direct impact on the population of susceptible carp, or by an indirect impact on the ecosystem within each waterhole and its ability to sustain a population of aquatic biota. If this does not occur, then as the water warms in late spring toward the upper threshold for the transmission of CyHV-3, a proportion of infected carp may transition to from being actively infected to latently infected. These and susceptible carp will then be redistributed through the river system with summer flow events. When the flow ceases, the Moonie River will return to a baseline of disconnected waterholes. As the water within these disconnected waterholes cools in late summer, latent infections may then transition once again to active infections (recrudescence) and new outbreaks commence. If summer flows were significant, and substantial recruitment of carp occurred, then autumn outbreaks may be aided by the presence of highly-susceptible juvenile carp. Autumn outbreaks may again be terminated by the outcomes of the outbreak process, or a proportion of infected carp may transition to a latent state. These latently-infected carp will then transition back to active infection when the water warms in spring. In this way, the cycle will continue.

The characteristics of an outbreak of CyHV-3 in the **Lower Lakes and Coorong** are likely to reflect the carp biomass density (in particular locations), the predilection of carp for particular spawning sites and the influence of water temperature. There was substantial variation between the low carp biomass density estimates for the Lower Lakes and Coorong provided by Koehn *et al.* (2016) and the generally high estimates generated by the NCCP project team that modelled carp biomass across Australia (Stuart *et al.*, 2019). It was also difficult to reconcile the extremely low numbers of carp (four carp, as opposed to 86,613 bony herring and 76,729 congolli) reported in the most recently published survey of the fish assemblage at this site (Bice *et al.*, 2016). The estimates provided by Stuart *et al.* (2019) – approximately 325 kg/ha within Lake Alexandrina and the Goolwa Channel, and approximately 385 kg/ha in Lake Albert – were taken forward for the case study, although the uncertainty in these bears ongoing consideration. Carp biomass density is also influenced by the flow through the ecosystem. Although the size of the carp population within the Lower Lakes and Coorong declines in lower-flow seasons, this is outweighed by the contraction of waterbodies, such that the carp biomass density is substantially higher. This in turn is likely to increase the aggressiveness of an outbreak of CyHV-3 and the opportunity for carp carcasses to accumulate in numbers sufficient to result in an impact on water quality. Carp in the Lower Lakes are likely to spawn in shallow areas protected by aquatic vegetation, including sheltered reedy freshwater estuaries and ephemeral wetlands adjacent the ingress of minor creeks. Both lakes are fringed with tall reeds, bulrush or cumbungi, and these would also provide suitable spawning habitat. Carp within the Coorong itself are likely to be drawn principally to submerged vegetation within the seasonally freshened waters near to the barrages. Outbreaks of CyHV-3 are likely to be focussed within these spawning areas and may be spatially disconnected and largely independent of each other. Aggressive outbreaks are possible within the Lower Lakes (in particular), and carcass accumulation in some places may be substantial if assisted by wind. This is more likely to occur during lower-flow seasons, when lower water levels act to concentrate carp and raise the biomass density. The persistent wind that characterises this environment, however, is likely to aerate affected water and minimise the likelihood of hypoxia or anoxia. Outbreaks of CyHV-3 within the Lower Lakes and Coorong are not likely to be curtailed in summer by a high water temperature, although the comparatively low water temperature in spring may limit transmission during pre-spawning aggregations.

The characteristics of an outbreak of CyHV-3 in **upper reaches of the Abercrombie River** (in the Upper Lachlan Catchment) reflect a low carp biomass density and generally cool-to-cold conditions in all seasons other than summer. Aggregations may not occur in this river, which is in general quite shallow. The river is subject to periods of low flow and dwindles at these times to a stream connecting a system of semi-permanent waterholes. In this setting, the carp biomass density may be raised as an artefact of dwindling waterbodies but is nevertheless unlikely to be sufficient to result in an aggressive outbreak of CyHV-3 and the accumulation of carcasses in numbers sufficient to impact on water quality.

The detailed case studies (above) demonstrated the importance of carp biomass density and spawning behaviour, and water flows and temperature, to the likely character of an outbreak of CyHV-3. This includes the aggressiveness of the outbreak, as well as the likelihood that carcasses will accumulate in numbers sufficient to have an impact on water quality. Using these principles, it was possible to extend the evaluation to include a more superficial consideration of three additional cases: (a) the Macquarie Marshes in northwest New South Wales; (b) Kow Swamp, in northern Victoria; and (c) an upstream reach of the Glenelg River in southern Victoria (Figure 78).

Figure 78 Additional minor case study sites

In high-flow seasons, the **Macquarie Marshes** accommodate more than 500,000 waterbirds, most of which are colonial-nesting and include high numbers of cormorants, herons, ibises and spoonbills.[95] Located northwest of Warren, in northwest New South Wales, the Macquarie Marshes are fed by the Macquarie river which forms a complex network of anastomoses within the marshland region and then converges to the north to reform the Macquarie River. The Macquarie Marshes Ramsar site lies within the larger ecosystem and covers approximately 20,000 ha. The carp biomass density within the Macquarie Marshes has been estimated at approximately 56 kg/ha (Stuart *et al.*, 2019) although, like the Moonie River Catchment, this figure is likely to vary

[95] See: https: //www.environment.nsw.gov.au/topics/water/wetlands/internationally-significant-wetlands/macquarie-marshes

substantially as the volume of water within the ecosystem expands with flood events (to a maximum of approximately 131,458 ha) and subsequently contracts (to a minimum of approximately 4,333 ha). The Macquarie Marshes are located in a hot and otherwise semi-arid region of New South Wales, and the water temperature in summer (in particular, on inundated floodplains) is also likely to be broadly similar to that of the Moonie River Catchment. An outbreak of CyHV-3 within the Macquarie Marshes ecosystem is thus likely to follow the general parameters for ephemeral wetlands, such that an aggressive outbreak that resulted in a substantial accumulation of carp carcasses is more likely during drier seasons when carp are concentrated in deeper semi-permanent waters. An aggressive outbreak is possible in a season when the floodplain is inundated, although this is likely to be predicated on effective transmission during pre-spawning aggregations. Under either scenario, the outbreak is likely to diminish in summer as the water temperature exceeds the upper threshold for transmission. A resurgence may be seen in autumn as the water temperature decreases and the highly-susceptible juvenile carp become exposed to the virus.

Kow Swamp is a water storage lake with a circumference of 15 km and a capacity of approximately 51,000 ML, located close to Gunbower and to the Barmah-Millewa Forest. Because it is maintained as a reservoir for irrigators, the extent of inundation is considerably less variable than that of the ephemeral wetlands and floodplains in the same reach of the Murray River. Kow Swamp has a permanent population of both carp and native fish, and native fish numbers are supplemented through restocking (in particular, Murray cod and golden perch).[96] During particularly hot summer weather, the temperature of the shallow water at the fringes of Kow Swamp may exceed the upper threshold for the transmission of CyHV-3, although wind across the lake and the ingress of water from channels to the Murray River are likely to mean that this is less frequent than on the Barmah-Millewa Forest floodplains. The carp biomass density for Kow Swamp was not estimated by Stuart *et al.* (2019) although estimates within channels close by ranged from approximately 115 kg/ha at the edge of the swamp to approximately 140 to 230 kg/ha at Taylor's Creek. The characteristics of an outbreak of CyHV-3 in Kow Swamp are likely to be similar to those of other semi-permanent impoundments, such as Lake Mulwala (above), although the flow of water through Kow Swamp is substantially less than through Lake Mulwala and this may increase the likelihood that carp carcasses will accumulate at the periphery of the swamp in numbers sufficient to result in impacts on water quality.

The **Glenelg River** was included here to provide an example of outbreak dynamics outside the Murray-Darling Basin. The Glenelg River rises in the Grampians and flows through swampland then south to the Rocklands Reservoir (a large impoundment with capacity of approximately 350,000 ML[97]) and onwards with numerous tributaries, to the town of Nelson. Carp are now endemic within the Glenelg river system. The carp biomass density within the Rocklands Reservoir has been estimated at approximately 180 kg/ha, while the biomass density in the Glenelg River downstream from the reservoir is considerably lower – approximately 25 to 75 kg/ha (Stuart *et al.*, 2019). Carp are likely to spawn amongst macrophytes and other aquatic vegetation at the fringes of Rocklands Reservoir, and in similarly lentic riparian habitats along the river channel. The water temperature

[96] See: https: //vfa.vic.gov.au/

[97] See: https: //www.gwmwater.org.au/

within the Rocklands reservoir and the Glenelg River system downstream from the reservoir is less likely to exceed the upper threshold for the transmission of CyHV-3 during summer months. An outbreak of CyHV-3 within the reservoir is likely to follow the general parameters for lakes and other impoundments. The high volume of water within this reservoir, and its depth (approximately 17 metres), mean that although an aggressive outbreak of CyHV-3 is possible it is not likely to result in the accumulation of carp carcasses in numbers sufficient to impact on water quality. The Glenelg River downstream from Rocklands is a substantial and lotic waterbody, and this in combination with a low carp biomass density mean that an aggressive outbreak is unlikely and that there is negligible opportunity for the accumulation of substantial numbers of carp carcasses in places other than local points of entrapment.

These additional cases serve to verify the pivotal role of three key considerations in determining the likely character of an outbreak of CyHV-3 in freshwater settings: (a) opportunities for close contact between carp; (b) permissive water temperature (including both upper and lower thresholds for the transmission of CyHV-3); and (c) spawning behaviour of carp in a given setting, and the presence of highly-susceptible juvenile carp. In this context, the character of an outbreak includes its aggressiveness, which may or may not be reflected in the accumulation of carp carcasses in numbers sufficient to have an impact on water quality.

13.2 Exposure pathways

The ecological risk assessment focussed on nine exposure pathways. Three of these pathways concerned aspects of the species-specificity of CyHV-3, including its possible effects on native aquatic and terrestrial species and on humans. These matters are currently under investigation by the NCCP. A systematic scientific review is in train and may result in a recommendation for further experimental work to be undertaken by the CSIRO Australian Animal Health Laboratory. CSIRO's position given the current state of knowledge about species-specificity is to withhold judgement or definitive assessment of risk until the Australian situation can be clarified. These three pathways were not considered further. The remaining six pathways were taken forward in a series of individual evaluations (Section 11) and were then assessed within the context of each of the six case studies (Section 12).

The **exposure of native aquatic species to low DO** focussed on the assumption that few species are able to tolerate conditions of less than 3 mg/L for prolonged periods. Larvae and young-of-year juveniles may survive at DO as low as 20 percent saturation (for example, 1.8 mg/L at 20C), but growth is likely to be restricted. Within these broad parameters, the Basin Plan[98] targets of more than 50 percent saturation and a DO concentration of at least 4.5 mg/L (at approximately 20C) are widely regarded as appropriate critical values for river channels and anabranch creeks. The experimental work of Walsh *et al.* (2018) confirmed that the decomposition of fish carcasses can result in a low DO and, in some situations, anoxia, and that this may persist for approximately 2 weeks. The biomass density used in the experiments of Walsh *et al.* (2018) was very high, although the action of wind or currents may mean that carcasses accumulate in large numbers in certain places within a waterbody. Additional factors that influence the extent and duration of

[98] See: https: //www.mdba.gov.au/basin-plan/plan-murray-darling-basin

oxygen drawdown include temperature, water quality (in particular, salinity), thermal stratification within the water column and the rate of reaeration through the air-water interface (primarily as a function of wind). It is also important to recognise the marked diurnal variation in DO that is a characteristic of many freshwater settings.

In chief, the impacts of hypoxia or anoxia focus on direct harm to local populations of aquatic native biota, including large-bodied and small-bodied fish, crustaceans, zooplankton and macroinvertebrates. Murray cod, trout cod and silver perch are particularly at risk, and if hypoxia or anoxia was to develop within a significant proportion of an ecosystem that includes these species then this might impact on their sustainability of recovery at a local level. Impacts are not restricted to these species, however, and small-bodied native fish and crustaceans will also be affected. Crustaceans are vulnerable in that their response to low DO is to leave the water, and this makes them exposed to predation and desiccation. Ultimately, low DO could have a short-term impact on the character and diversity of wetland and riparian ecological communities.

The key factor influencing the development of hypoxia or anoxia is the flow scenario. This is because the flow scenario is likely to be linked to the accumulation of carp carcasses, to the extent to which an ecosystem is flushed with oxygenated water, to the proximity of native aquatic biota and, in some cases, to the reproductive biology of individual species. An outbreak in a wetland or waterhole during a lower-flow setting is likely to have a greater impact on DO as carp (and carp carcasses) will be concentrated within available spawning and nursery habitat. Native aquatic biota will be sharing the same constrained waterbodies and are thus more likely to be exposed to low DO. In dry seasons, some waterbodies may also become partially or completely disconnected, minimising opportunities for native biota to move from an affected area. This effect was particularly marked in the case study for the Moonie River Catchment. Here the persistence of disconnected, diminishing and stratified waterholes through spring or autumn can result in a high biomass density of carp and the potential for an aggressive outbreak of CyHV-3. The accumulation of carcasses within these waterholes may then be sufficient to result in exacerbation of likely borderline hypoxic conditions, and the exposure of remnant aquatic native biota. A similar effect was discussed within the case study for the Barmah-Millewa Forest, where lower-flow seasons do not result in the inundation of the broader floodplains and spawning carp are constrained to lentic or stationary off-channel waters. Settings where the volume of water, or its flow, are substantial are less exposed. Examples of the former include Lake Mulwala or the Rockland Reservoir on the Glenelg River; while the latter include the Mid-Murray River channel. Settings characterised by the ongoing re-oxygenation of water through persistent strong winds are also less exposed, and this was illustrated within the case study for the Lower Lakes and Coorong. There are also settings where the carp biomass density is simply insufficient to be likely to result in the substantial accumulation of carcasses, as illustrated within the case study for the Abercrombie River in the Upper Lachlan Catchment.

The treatment of risks associated with the low DO that may accompany an outbreak of CyHV-3 will focus on the timely removal of carp carcasses. The extent to which this will be necessary, and practicable, will vary amongst settings although the overarching goal will be to prevent the DO within a given waterbody falling below about 3 to 4 mg/L or to enable it to return to that level. If the physical removal of carcasses is not practicable, then consideration could in some settings be given to flushing carcass components from a waterbody, or diluting their effect, using environmental watering. The potential for applying this strategy was best illustrated within the case study of the Chowilla Floodplain, where the Chowilla regulator and associated structures enable a fine level of control over the inundation or flushing of particular wetlands or floodplains within the ecosystem – even when the flow within the Murray River channel is comparatively low.

The **exposure of native species, livestock and humans to widespread cyanobacterial blooms** focussed on the elevation of DOC and phosphorous as a result of the decomposition of carp carcasses. This effect was observed in the experimental work of Walsh *et al.* (2018). Once initiated, a cyanobacterial bloom will persist for as long as the conditions remain suitable and substrates remain available. An ongoing source of DOC and phosphorus will be important, as will adequate sunlight to maintain water temperature and to support a high rate of photosynthesis. An abrupt change in conditions (for example, a cool change with cloudy weather) may precipitate an imbalance whereby the respiratory needs of the cyanobacteria for oxygen outweigh the oxygenation of the waterbody through photosynthesis. When this or another imbalance occurs, the cyanobacteria will begin to die-off. Death of cyanobacteria results in aerobic decomposition and a concomitant elevated BOD and, with continued declining photosynthesis (as a result of a reducing volume of living cyanobacteria), to a subsequent precipitous fall in DO – ultimately to the point of anoxia. When this is reached, decomposition will become anaerobic and the prevalence of anaerobic microorganisms such as *C. botulinum* may increase. The fall in DO can be catastrophic to fish and other aquatic biota, and it may be difficult to delineate the secondary deaths associated with this from the deaths directly caused by cyanotoxin (noting that not all cyanobacteria are cyanotoxic). If the key factor triggering the death of cyanobacteria was an exhaustion of DOC or phosphorous, then it is also possible for the decomposing carcasses of fish and other aquatic animals killed by cyanotoxins or low DO to replenish these and to thus initiate a secondary bloom.

In chief, the impacts of a widespread cyanobacterial bloom will focus on direct harm to local populations of aquatic native biota, including large-bodied and small-bodied fish, crustaceans, zooplankton, macroinvertebrates, waterbirds (including native and migratory waterbirds), frogs and freshwater turtles. There may also be an impact on humans and livestock. These impacts may result from the pathogenic effects of cyanotoxin (again, if the strain involved is cyanotoxic), the precipitous drop in DO that follows from the collapse of the bloom, the decline in water quality that will follow from the death and decomposition of cyanobacteria and aquatic biota or the ecosystem-wide impacts of these processes on food webs. The risk profile for native fish and crustaceans is similar to that which is associated with low DO (above). Murray cod, trout cod and silver perch are the most likely of the large-bodied native fish species to be at risk and, in some settings, exposure may interfere with their recovery. The area of occupancy of trout cod, some small-bodied native fish and some crustaceans (in particular, the Murray crayfish) might also be threatened, while the breeding cycle for most aquatic species may be disrupted. The risks for waterbirds are generally high as their feeding, foraging, nesting and other behaviours place them in close and regular contact with water. Colonial nesters are at particularly high risk, as stressors to these populations are likely to be applied concurrently to a large number of birds at a time when harm can fall to both adults and chicks. Diet is also important, noting that cyanotoxins bioaccumulate in animal tissues and that regular low-grade exposure can mean that a toxic threshold is eventually breached. Raptors have an additional layer of exposure inasmuch as their position at the top of the food chain means that they are relatively fewer in number, and that the loss of a small number of individuals could significantly affect the sustainability of a local population. The risks faced by frogs and turtles are reasonably similar, as both spend most of their time in or in proximity to a waterbody and have a limited ability to (safely) avoid affected water. Cyanotoxins affect people and livestock, although people are likely to be protected to a large extent by the innate repulsiveness of a waterbody that contains a significant cyanobacterial bloom, and the signage and other warnings that will generally be given in places of public amenity. Livestock, however, may have less ability to avoid affected drinking water if there are limited or no alternatives. The risks to ecological communities are understandably highest for wetlands and marshlands, although a widespread cyanobacterial bloom affecting a waterbody within (or proximal to) a forest and woodland, or grassland, ecological community, may also impact on species that utilise that water.

The key factor influencing both the likelihood that an outbreak of CyHV-3 will result in a widespread cyanobacterial bloom, and the likely consequences of such an event, is the flow scenario. Under a high-flow scenario, ephemeral lakes, wetlands, floodplains and marshes will be inundated and ecological communities will be maximally dense and diverse. However, whilst the potential for significant harm is more likely in this setting the likelihood that carp carcasses will accumulate in numbers sufficient to initiate a widespread cyanobacterial bloom, is lower. The converse is true of a lower-flow scenario, or an outbreak within a reasonably contained body of water – including some permanent lakes and irrigation reservoirs and some minor riverine environments with connected or disconnected waterholes and billabongs. The evaluation of case studies showed that settings where the risk of low DO is highest are also likely to have a higher risk of widespread cyanobacterial blooms. Thus, the disconnected waterholes through the Moonie River Catchment in a typically dry spring or autumn will have a relatively high risk as the carp biomass density within these waterholes may be high and the water itself is likely to be warm and stratified and seeded with cyanobacteria. A similar risk is likely for ephemeral wetlands, such as the Barmah-Millewa Forest, during a lower-flow season when spawning carp are constrained to lentic or stationary off-channel waters. Settings where the volume of water, or its flow, are substantial, are less exposed. Examples of the former included Lake Mulwala or the Rockland Reservoir on the Glenelg River, while the latter include the Mid-Murray River channel. Settings characterised by the ongoing mixing of water through persistent strong winds may also be less exposed, and this was illustrated within the case study for the Lower Lakes and Coorong. In this case study, however, it was also noted that despite the generally protective effects of wind, cyanobacterial blooms continue to occur in some years. There are also settings where the carp biomass density is simply insufficient to be likely to result in the substantial accumulation of carcasses, as illustrated within the case study for the Abercrombie River in the Upper Lachlan Catchment.

The treatment of risks associated with the development of a widespread cyanobacterial bloom will be similar to the treatment of risks associated with low DO and will focus on the timely removal of carp carcasses. The extent to which this will be necessary, and practicable, will vary amongst settings and is difficult to quantify. Particular consideration should be given to the characteristics of a waterbody that will increase its inherent susceptibility to cyanobacterial blooms. These include the connectedness and velocity of water and its temperature, the extent of stratification and the potential for reaeration. Consideration should also be given to characteristics of ecological communities, functional groups and (in some cases) species that may place them at a higher risk. In particular, these include communities with large colonies of colonial-nesting waterbirds, or fragmented populations of threatened species. If the physical removal of carcasses is not practicable then consideration could in some settings be given to flushing carcass components from a waterbody, or diluting their effect, using environmental watering. The potential for applying this strategy in the context of cyanobacterial blooms was again best illustrated within the case study of the Chowilla Floodplain noting, in particular, the fine level of control over the inundation or flushing of particular wetlands or floodplains that can be achieved using the Chowilla regulator and associated structures.

The **exposure of native species, livestock and humans to the microorganisms associated with decomposing fish carcasses** was characterised by a high degree of uncertainty in respect of the particular waterborne microorganisms that may be involved. Carp gut flora and spoilage organisms may proliferate after a fish kill, as might *E. coli*, some *Pseudomonas spp* and other opportunistic microorganisms. Shiga toxin-producing *E. coli* (STEC) has been isolated from bodies of water (such as ponds and streams), wells and water troughs, and has been found to survive for months in manure and water-trough sediments. *Aeromonas spp* have also been found in irrigation water, rivers, springs, groundwater, estuaries and oceans (in particular where water has been contaminated by sewerage) and are of public health concern. Pera *et al.* (2019) undertook a mesocosm experiment with carp carcass biomass densities ranging from 250 to 6,000 kg/ha. These authors found that coliforms increased in all mesocosms that contained carp carcasses. Counts fell to around zero after about a week, although increased again at around 25 days in the mesocosms containing lower carp biomass densities. The authors also assessed the counts of: (a) signature lake bacteria; (b) environmental copiotroph bacteria; and (c) fish gut signature bacteria. The signature lake bacteria decreased in mesocosms that included the decaying fish. Both environmental copiotroph bacteria and fish gut bacteria increased, although in some cases levels were higher or highest within mesocosm with a lower carp biomass density. These and other waterborne microorganisms may be constrained to waterways directly affected by decomposing carp or may persist in unaffected waters or in waters that have returned to a healthy state following a fish kill. Harm from waterborne microorganisms may result from contact with, or the consumption of, contaminated water. It is also possible that harm may result from the consumption of contaminated prey species – including invertebrates. In either event, the effects of waterborne microorganisms are likely to be focussed on acute harm and the impact that this may have on breeding cycles and population sustainability. These effects were judged likely to be similar to those obtained for widespread cyanobacterial blooms (above) – albeit with a lower likelihood of realising any of the identified ecological endpoints. The rational for specifying lower likelihoods reflected in part the science – in particular, the barriers faced by waterborne microorganisms in establishing a presence in an ecosystem equivalent to a cyanobacterial bloom – as well as the experience obtained from field observations of Australian freshwater waterways, where substantial fish kills are not uncommon but have not to-date been linked to subsequent epidemics of particular waterborne pathogens. This notwithstanding, it was recognised that the risks associated with the proliferation of waterborne microorganisms will be highest in settings such as the Moonie River Catchment during a typically dry spring or autumn, when the risk itself has diminished to a series of disconnected waterholes. The risks will be lower in settings where carp carcasses are unlikely to accumulate, where the flow is sufficient to flush carcass decomposition materials out of the system, or where the volume of water within the affected waterbody will be sufficient to dilute these materials. Treatment options for risks associated with waterborne microorganisms will mirror those put forward for widespread cyanobacterial blooms and focus on the removal of carp carcasses and, in some settings, on the flushing of the system.

The **exposure of native piscivorous species to the removal of a dominant and stable food source** focussed on the role that juvenile carp, in particular, may play as a source of food for the chicks of nesting piscivorous waterbirds. The scenario of primary concern is the aggregation of large numbers of colonial-nesting waterbirds in ephemeral wetlands or semi-permanent waterbodies (including lakes and irrigation reservoirs) where carp have spawned, and where juvenile carp are also present in large numbers. In this setting, it can be expected that juvenile carp will be targeted by waterbirds as an abundant source of food for growing chicks. This notwithstanding, there is a lack of contemporary evidence to clarify the importance of juvenile carp (as opposed to alternative prey) to nesting waterbirds. Fieldwork undertaken in the 1980s downplayed this relationship, although it is clear that the population of carp and its dominance within many of Australia's freshwater waterways have increased substantially since that time. It is also likely that the importance of juvenile carp to nesting waterbirds will vary between settings, and this was drawn out within the case studies. In the Barmah-Millewa Forest, for example, significant waterbird breeding events occur in high-flow seasons when the floodplains are inundated. As this is a significant recruitment site for carp during high-flow seasons, juveniles are also likely to be present in large numbers and it seems probable that, in this setting, the strictly-piscivorous species – as well as some carnivorous or omnivorous species – will take advantage of juvenile carp as an abundant source of food for nesting chicks. Conversely, waterbirds within the channel and anabranch habitats of the Mid-Murray River nest in much lower numbers, and as this is not a major recruitment site for carp, these waterbirds are less likely to have developed a reliance upon juveniles as a source of food for growing chicks.

The effect of suppressing or removing a local population of juvenile carp will also depend to some extent on the proximity of alternative sources of juvenile carp. Gunbower Forest and Kow Swamp, for example, are likely to contain spawning and juvenile carp in seasons when carp recruitment is also occurring within the Barmah-Millewa Forest. These ecosystems are very close to the Barmah-Millewa Forest. If an outbreak CyHV-3 was not sequenced so as to impact concurrently on all waterbodies within the area, then it seems likely that piscivorous waterbirds within the Barmah-Millewa Forest would be able to source at least part of their requirement from these alternative waterbodies. The converse is likely to be true of the Chowilla Floodplain, however, which provides important refuge by virtue of the fact that there are no other large and dependable sources of water within that region.

The importance of this pathway in a particular setting may also be tempered by the timing of the outbreak and the extent to which highly-susceptible juvenile carp are exposed to the virus. An aggressive outbreak that occurs prior to the commencement of spawning may remove a high proportion of gravid females, and logically this will have a follow-on effect on the number of juveniles. Carp, however, are extremely fecund, with large females able to produce as many as two million eggs. Because of this, a more significant impact may follow from the direct exposure of the highly-susceptible juveniles to the virus in early or mid-summer. This may also coincide with the period in which waterbird chicks are growing rapidly and have a high demand for both protein and energy. Timing is also important, however, in respect of water temperature. An aggressive summer outbreak amongst juvenile carp is likely to be constrained in settings where the summer water temperature commonly exceeds the upper threshold for the transmission of CyHV-3 (approximately 28C). This may be the case, for example, in the shallow wetlands of the Chowilla Floodplain, but is less likely to be true of an outbreak amongst juvenile carp in, for example, the Lower Lakes or the Coorong.

The treatment of risks associated with the sudden removal of a dominant and stable food source might include the strategic release of the virus within particular catchments at times when its impact is least likely to result in stress to piscivorous waterbirds. This could extend to identifying where particular species of colonial-nesting piscivorous waterbirds are likely to be nesting during high-flow and lower-flow seasons. It would also be beneficial to consider the reliance that piscivorous birds are likely to have on carp within individul settings, considering the populations of both waterbirds and carp and the breadth of alternative prey species (below). Supplementation of local populations of native fish and crustaceans may also beneficial, and this is also discussed within the context of prey-switching.

The **exposure of native species to increased predation as a result of prey-switching** is conditional on the likelihood that waterbirds will be affected by the sudden removal of a stable food source (above). If waterbirds are affected in this way, then they will inevitably turn to alternative food sources, and this may include juvenile large-bodied native fish, adult and juvenile small-bodied fish, crustaceans, frogs and possibly turtle eggs and hatchlings.

The species of large-bodied native fish whose juveniles co-exist with carp are likely to be the most exposed. Juvenile Murray cod in ephemeral wetland settings will tend to be concentrated in off-channel lotic habitats and are less likely to inhabit inundated floodplains. These juveniles may be exposed during lower-flow seasons, although the population of breeding waterbirds will be significantly lower. Murray cod juveniles in semi-permanent waterbodies, such Kow Swamp and Lake Mulwala (both of which are restocked with juveniles to maintain the population strength) may be relatively more exposed, as in this setting they are more likely to share habitat with juvenile carp. The population of breeding waterbirds may also be significant within these ecosystems – in particular, in dry seasons when the ephemeral wetlands such as the Barmah-Millewa Forest are not inundated. Trout cod are limited to the mid-Murray River and (to a lesser extent) the Barmah-Millewa Forest. The exposure of juvenile trout cod within the Mid-Murray is unlikely to be substantial as neither the populations of breeding waterbirds nor spawning carp are high in this setting. The exposure of juveniles within the Barmah-Millewa Forest is likely to be similar to that of Murray cod, and focussed largely on lower-flow seasons. Silver perch also have a limited distribution, although prefer to spawn over gravel or rocky substrates where relatively few carp are likely to spawn. Macquarie perch are localised within upland riverine environments, such as the Abercrombie River within the Upper Lachlan Catchment, where the carp biomass density is generally low and large waterbird breeding and nesting events do not occur. Golden perch, freshwater catfish and bony herring have wide geographical distributions, and spawn in a wide range of habitats – many of which are likely to overlap with carp. Juvenile freshwater catfish, in particular, may be exposed in settings such as some of the disconnected waterholes of the Moonie River Catchment where the water quality is low and other species are limited largely to carp and goldfish. The small-bodied native fish may be exposed to prey-switching as both adults and juveniles, and this may increase the vulnerability of some locally-important populations. Exposure may also occur in a range of settings, although is likely to be highest in a high-flow season when ephemeral wetlands and floodplains the Barmah-Millewa Forest and the Chowilla Floodplain are inundated and large numbers of carp juveniles and nesting waterbirds are also likely to be present.

Crustaceans (including yabbies and crayfish species) are at highest risk in the situation where water quality is declining, as they are then likely to emerge and be vulnerable to predation. Fragmented populations of threatened frogs are likely to be particularly exposed within ephemeral wetlands during high-flow seasons, and within the marshland at the periphery of semi-permanent lakes and irrigation reservoirs during either high-flow or lower-flow seasons. The eggs and hatchlings of freshwater turtles are already under pressure in many freshwater ecological communities, and this may increase as a result of prey-switching. The impacts of prey-switching on ecological communities is likely to depend on the extent to which waterbirds currently rely upon carp (juveniles in particular) as a stable and dominant source of food and the strength and diversity of native aquatic biota.

The treatment of risks associated with prey-switching are likely to be limited to support for partcular local populations of threatened native species through strategic restocking. Although strategic restocking currently occurs for a range of native species – and at a range of locations – additional stocks of threatened native fish and other native aquatic species could be developed in aquaculture settings prior to release of CyHV-3. Guidelines could also be developed for the prioritisation of restocking through Murray-Darling Basin and elsewhere. Although it is recognised that the results of such releases have not to-date been completely successful (for example, Thiem *et al.*, 2017) it seems likely that some benefit would be obtained in the situation where particular populations within a riverine, wetland or lacustrine ecosystem were likely to sustain a significant and widespread loss. As an embellishment on this approach, consideration may also be given to harvesting adult animals from some existing habitats prior to the release of the virus and placing these within aquaculture breeding facilities. This would enable restocking to occur using wild-caught genetics, which may be more resilient or competitive in their natural settings than farmed fish.

The **exposure of native species, livestock and humans to an outbreak of botulism** focussed on type C (or C/D mosaic). Type E botulism was reviewed in detail, and was included within the evaluation of exposure pathways, but was not taken forward in the case studies. The importance of type E botulism stems from three considerations: (a) it is primarily a disease of fish; (b) it is a dangerous zoonosis; and (c) it has resulted in extremely large outbreaks in North America amongst both fish and waterbirds. *Clostridium botulinum* type E, however, has not to-date been identified within Australia. This includes a published review of human cases of botulism in Australia between 2002 and 2017. Whilst it cannot be excluded definitively, the likelihood that type E botulism would arise in Australia as a result of an outbreak of CyHV-3 was judged to be negligible.

An outbreak of type C (or C/D mosaic) botulism can arise in two key ways: (a) through the death of animals carrying spores within their gastrointestinal tracts; and (b) through the germination of spores within the environment. In both cases, the germination of spores is triggered by the development of anaerobic conditions. For carcasses, this occurs when aerobic organisms exhaust available oxygen. Spores within the environment can germinate following any oxygen-depleting process – including the decomposition of large numbers of fish or bird carcasses, or the decomposition of a cyanobacterial bloom. Both pathways for the development of type C (or C/D mosaic) botulism can be linked to an outbreak of CyHV-3. The first pathway relies on the establishment of the 'carcass-maggot cycle', and this is a highly probabilistic process – the larger the number of affected carcasses, and the population feeding on maggots, the higher the likelihood that the cycle will be sustained. The accumulation of carcasses is likely to be most marked in semi-permanent water bodies (including lakes and irrigation reservoirs), in diminishing waterholes within a riverine catchment and in ephemeral wetlands during lower-flow seasons. An outbreak of CyHV-3 on the fringes of Kow Swamp, for example, would be considered a higher-risk setting for type C (or C/D mosaic) botulism. The same could be said for an outbreak of CyHV-3 in the Moonie River Catchment during a typically dry spring or autumn, or an outbreak in the Barmah-Millewa Forest when the floodplains are not inundated. Of these examples, Kow Swamp is arguably the highest risk given that large numbers of breeding or nesting waterbirds are also likely to be present. The second pathway for the initiation of an outbreak of type C (or C/D mosaic) botulism is based on the germination of environmental spores under hypoxic conditions. Typical initiating events may include low DO events following from the eutrophication of waterbodies, or the death of cyanobacterial blooms. Settings where these events are more likely – again, for example, disconnected waterholes within the Moonie River Catchment during a typically dry spring or autumn – are also more likely to be associated with type C (or C/D mosaic) botulism. Conversely, settings such as the Abercrombie River in the Upper Lachlan Catchment, with a comparatively low biomass density of carp and cool, flowing water, are not likely to be exposed either through the carcass-maggot cycle or as a result of the germination of environmental spores.

In chief, the impacts of type C (or C/D mosaic) botulism focus on direct harm to local populations of aquatic native biota. These may include large-bodied and small-bodied fish, crustaceans, zooplankton, macroinvertebrates, waterbirds (including native and migratory waterbirds), frogs and freshwater turtles. There may also be an impact on livestock, although type C (or C/D mosaic) botulism does not affect people. In each case, exposure to botulinum toxin may result from consuming affected or contaminated water, plant materials, insects, animals or carcass materials. Where an outbreak of botulism has developed as a result of the germination of environmental spores, most species and functional groups will be at risk. Fish-eating birds are usually associated with type E botulism, although pelicans, herons and others have died in in large numbers in type C (or C/D mosaic) outbreaks in North America and may also be exposed. Fish themselves are less sensitive to type C toxin than they are to type E, and their role in type C (or C/D mosaic) outbreaks is more likely to be as a source of spores or as a vector for moving vegetative cells and toxin through the food chain to piscivorous birds. Where an outbreak has developed through the carcass-maggot cycle, then the diet and foraging behaviour of particular groups of waterbirds may make them more or less vulnerable. Filter-feeding and dabbling waterfowl, such as mallards, teal, and shovelers, are at higher risk in this setting, as are probing shorebirds, such as avocets and stilts. Shorebird species that feed near the surface of wetland soils and sediments may be at higher risk than those that probe deeply into the substrate for food. Carrion-eating raptors may be relatively less susceptible or may just be more likely to die at a distance from an affected waterbody and, for that reason, their carcasses may not be so commonly discovered.

The treatment of risks associated with an outbreak of type C (or C/D mosaic) botulism arising from a widespread fish kill will focus on minimising the likelihood that the carcass-maggot cycle will be initiated, and minimising the likelihood of significant hypoxic or anoxic events (and the subsequent germination of environmental spores). In both cases, the key action will be the removal of carcasses – that is, the carcasses of carp that have died from CyHV-3 and the carcasses of carp and other aquatic animals or waterbirds that have died from other processes linked to an outbreak of CyHV-3. If the physical removal of carcasses is not practicable in a given setting, then consideration may also be given to flushing carcasses or decomposed carcass materials from a waterbody, or diluting their effects using environmental watering. Whichever approach is taken, priority should be given to scenarios where the ecosystem at risk includes a high number of waterbirds, as well as threatened aquatic or terrestrial species or communities.

13.3 Outcomes for key functional groups, species and communities

A precis of important functional groups, species and ecological communities was given in Part II: Section 3. The relevance of each exposure pathway to these functional groups, species and communities was evaluated in a generic sense in Part III: Section 11, and in the context of a range of ecosystems in the case studies within Section 12. Key outcomes from these sections are summarised below. These outcomes were taken forward in Part IV as a guide for the assessment of EPBC Act MNES assets.

13.3.1 Large-bodied native fish

Large-bodied native fish are physically, physiologically and ecologically close to carp, and a number of individual species were therefore given particular attention in this assessment.

Murray cod are an exceptionally long-lived species that has undergone a substantial decline since European settlement, and particularly in the last 70 years, and are now listed under the EPBC Act as a vulnerable species. Murray cod utilise a diverse range of habitats from clear rocky streams, such as those found in the upper western slopes of New South Wales and the Australian Capital Territory, to slower-flowing, turbid lowland rivers and billabongs such as those of the lower Darling River. Murray cod are considered a main-channel specialist, with only limited use of anabranches and inundated floodplain habitats. Juveniles less than one year old have been found in main river channels where it appears they settle at a late larval stage. Murray cod migrate upstream prior to spawning in late spring or early summer when the water attains a temperature of between 16 and 21C. Larvae then drift downstream before settling out in suitable protected habitat. Murray cod also occupy many impoundments, including lakes and irrigation reservoirs. Their localisation within these settings is often a result of barriers (regulatory structures) and their populations may be augmented through the release of farmed juveniles. Adult Murray cod are solitary and highly-territorial and will return to their territory following spawning.

The Murray cod's preference for deeper water habitats means that its exposure to the water quality effects of the accumulation of carp carcasses (including low DO, widespread cyanobacterial blooms and waterborne microorganisms) is likely to be highest within impoundments and within ephemeral wetlands during lower-flow seasons. An example of the former might include Kow Swamp, while examples of the latter include the Barmah-Millewa Forest or the Chowilla Floodplain. Murray cod may also be exposed in some riverine settings during dry seasons, when the flow and volume of water have decreased – ultimately to the point of disconnected waterholes. Examples of this may include the anabranches and billabongs of the Mid-Murray River or the waterholes of the Moonie River Catchment. Murray cod are amongst the least tolerant of native fish to low DO and may be particularly vulnerable to this pathway. This would include low DO associated with the decomposition of carp carcasses, as well as the precipitous fall in DO that follows from the die-off of a cyanobacterial bloom. Murray cod juveniles, however, are arguably less exposed to the effects of prey-switching, if this was to follow from the widespread death of juvenile carp. This is because juvenile Murray cod are not generally present in large numbers on inundated floodplains where waterbirds are accustomed to feed on juvenile carp. All species of fish are relatively resistant to the effects of type C (or C/D mosaic) botulinum toxin, although as the apex predator in most freshwater settings Murray cod may be relatively more likely to consume large amounts of toxin.

Trout cod have also declined markedly in distribution and abundance since European settlement, and the single naturally-occurring population is restricted to a small (approximately 120 km) stretch of the Murray River from Yarrawonga Weir to the Barmah-Millewa Forest, and occasionally beyond to Gunbower. The trout cod is listed under the EPBC Act as an endangered species. Trout cod occupy stream positions close to riverbanks in comparatively deep and rapidly moving water with a high abundance of large woody debris. They are believed to form pairs and spawn annually during in late September to late October, when water temperatures are between 14 and 22C. In contrast to Murray cod (above), trout cod do not generally migrate to spawning grounds although some individuals may move from the main channel to off-channel branches or to floodplains in the event of a significant flood.

Although trout cod and Murray cod are physically and physiologically very similar species, three key factors mean that they are likely to be more exposed to the possible effects of an outbreak of CyHV-3: (a) their geographical distribution and population strength are very much more limited than those of Murray cod; (b) they tend not to undertake spawning migrations and are considered a less mobile species; and (c) in the event of floods they may occupy floodplain habitats more frequently than Murray cod. These considerations mean that trout cod may be more likely to encounter a water quality event and less likely to evade it by migrating to cleaner or more oxygenated water. Juvenile trout cod may also be more exposed to prey-switching by piscivorous waterbirds. If they are compromised, their limited geographical distribution may then mean that the sustainability of the species in the wild is less secure.

Silver Perch, although once widespread and abundant throughout most of the Murray-Darling river system are now restricted to a single, sizeable, self-sustaining population in the mid-Murray River from the Yarrawonga Weir to the Torrumbarry Weir, and then to the Euston Weir and downstream. Recent monitoring indicates this population now extends down to the South Australian border and up the lower Darling River. Silver perch are listed under the EPBC Act as a critically endangered species. Silver perch spawn in faster-flowing water and generally over gravel or rock rubble substrates. Spawning is not flood-dependent but does appear to be dependent on suitable flows and suitable flood events do appear to maximise spawning efforts and presumably recruitment. Silver perch are a migratory fish, and their long-distance (generally upstream) movements offset the subsequent downstream drift of eggs, larvae and juveniles.

Silver perch share the predilection of Murray cod for in-channel habitat, albeit with a stronger preference for lotic waterways. This includes their preference for lotic spawning habitats. Silver perch are not generally fond on inundated floodplains, and their limited geographical distribution means that they are not generally found in impoundments (including lakes and irrigation reservoirs) unless farmed juveniles have been introduced to those settings. These considerations mean that the exposure of silver perch to the water quality effects of an outbreak of CyHV-3 is likely to be slightly lower than the exposure of Murray cod. Silver perch are also slightly more tolerant of low DO than Murray cod, although this advantage is unlikely to be protective in most settings where hypoxia is occurring. The most plausible scenario for the exposure of silver perch to the water quality effects of an outbreak of CyHV-3 is the accumulation of large numbers of carp carcasses in off-channel habitats of the Barmah-Millewa Forest during a lower-flow season when the floodplains are not inundated. Although an aggressive outbreak of CyHV-3 is not unlikely in this setting, silver perch are more likely to remain in lotic sections of the main channel or in major anabranch habitats. Likewise, juvenile silver perch are not likely to share nursery habitat with large numbers of juvenile carp and are therefore not likely to have a high exposure in the event of prey-switching by nesting waterbirds. Silver perch and other fish are relatively tolerant of type C (or C/D mosaic) botulinum toxin.

Golden perch are predominantly found in lowland, warmer, turbid, slower-flowing rivers – often in sympatry with Murray cod. Golden perch have unusually broad temperature limits (from 4 to 37C) and unusually high salinity limits for a freshwater fish (up to 33 parts per thousand). There is some evidence that golden perch can adapt to a DO as low as 3 mg/L, in response to prolonged exposure. Outside the breeding season, golden perch are nomadic, with schools of fish occupying home ranges of about 100 metres for weeks or months before relocating to another site where a new home range is established. Upstream movements by both immature and adult fish are stimulated by rises in streamflow, and most movement in the Murray occurs between October and April. Recent research in the Murray River has also suggested that some fish may move downstream to spawn. Water-hardened eggs are large (approximately 3 to 4 mm in diameter), semi-buoyant and drift downstream. Adult fish have been recorded migrating well over 1,000 km when flood conditions allow passage over weirs and other man-made obstructions.

The wide geographical distribution of golden perch means that the species as a whole is less likely to be threatened by the water quality effects of an outbreak of CyHV-3. The same characteristic, however, means that local populations are relatively more likely to encounter these effects. Golden perch within disconnected waterholes in the Moonie River Catchment, for example, will encounter diminishing water quality during a typically dry spring or autumn and this may be exacerbated by an outbreak of CyHV-3 amongst carp. Likewise, golden perch within impoundments such as Kow Swamp may experience a low DO event, a cyanobacterial bloom or the proliferation of waterborne microorganisms following an aggressive outbreak of CyHV-3 and the accumulation of carp carcasses in particular parts of the ecosystem. Although they are a comparatively hardy species, these water quality effects may be sufficiently serious to overwhelm most of the exposed fish. Golden perch do not spawn on inundated floodplains, although juveniles can be found in both channel and inundated floodplain habitats and may thus be exposed to the effect of prey-switching by nesting waterbirds. This would be particularly evident in the event of an aggressive outbreak of CyHV-3 in an inundated ephemeral wetland such as the Barmah-Millewa Forest, the Chowilla Floodplain and the Macquarie Marshes. Golden perch and other fish are relatively tolerant of type C (or C/D mosaic) botulinum toxin.

Macquarie perch are closely related to golden perch (above), although a specialised upland species and localised within the upper reaches of the Mitta Mitta, Ovens, Broken, Campaspe and Goulburn Rivers in northern Victoria and the upper reaches of the Lachlan and Murrumbidgee Rivers in southern New South Wales. A larger translocated population exists in the Yarra River and in Lake Eildon in the Goulburn River catchment. It is also found in low numbers in the Mongarlowe River, where the population is considered likely to be the result of a translocation from the Murray-Darling Basin. The Macquarie perch is listed under the EPBC Act as an endangered species.

Macquarie perch is a riverine, schooling species that prefers clear, deep water and rocky holes. Refuge is also important, and may include aquatic vegetation, large boulders, debris and overhanging banks. Spawning occurs just above riffles (shallow running water), where rivers have a base of rubble (small boulders, pebbles and gravel). The eggs, which are adhesive, stick to the gravel, and newly-hatched yolk sac larvae shelter amongst pebbles. Some fish use the same river each year for spawning. Migrations are undertaken by fish resident in lakes, but not otherwise.

Because the geographical distribution of the Macquarie perch is restricted to lotic upland rivers where the biomass density of carp is also low, there is very little likelihood of exposure to the water quality effects of CyHV-3. This was verified within the case study for the Abercrombie River in the Upper Lachlan Catchment. There is also little likelihood of exposure to prey-switching, given that large waterbird breeding and nesting events are not associated with upland rivers. Macquarie perch and other fish are relatively tolerant of type C (or C/D mosaic) botulinum toxin. On balance, Macquarie perch are arguably one of the least-exposed of the large-bodied native fish to the possible effects of an outbreak of CyHV-3.

Freshwater catfish are a benthic species that prefers slower-flowing streams and lake habitats. Most riverine populations have declined significantly, and although the freshwater catfish is not currently listed under the EPBC Act as a threatened species it is no longer common in many areas where it was formerly abundant. Freshwater catfish spawn in spring and summer when water temperatures are between 20 and 24C and are notable for constructing an elaborate circular or oval nest from pebbles and gravel. The eggs are comparatively large (approximately 3 mm) and non-adhesive and settle into the interstices of the coarse substrate within the nest. The freshwater catfish is a relatively sedentary species and adults show very limited tendency for movement or migration.

As a benthic species, freshwater catfish are adapted to poor water quality – including low DO. This may mean, however, that they are present in settings with poor water quality where other hardy fish, such as carp, are also present. An example of this was discussed in the case study for the Moonie River Catchment, where research has shown that freshwater catfish, carp and goldfish are the most common species remaining in dry seasons in many semi-permanent, disconnected waterholes. These waterholes are exposed to aggressive outbreaks of CyHV-3, including the accumulation of carp carcasses and the potential for a detrimental effect on water quality (both low DO and cyanobacterial blooms). Freshwater catfish may also be exposed to these risks in ephemeral wetlands during lower-flow seasons, where the floodplains are not inundated, and fish and other aquatic animals are concentrated within lentic off-channel waters. Their proximity to carp in many settings may also mean that juveniles are relatively more exposed to prey-switching than are the juveniles of some other large-bodied species. All species of fish are relatively resistant to the effects of type C (or C/D mosaic) botulinum toxin although, as a benthic species, freshwater catfish may be relatively more likely to consume toxin or vegetative cells in sediment or in settled carcass materials.

Bony herring (also bony bream, hairback herring or pyberry) is a medium sized fish that is widespread in lowland rivers but largely absent from upland rivers. Bony herring are not listed as a threatened species under the EPBC Act. Bony herring are a hardy fish, tolerating high temperatures (up to 38C), high turbidity, high salinity (up to at least 39 ppt) and low DO. However, they are not tolerant of low water temperatures and are susceptible to the effects of cold-water pollution. Spawning is generally understood to take place in the still waters of shallow, sandy bays in October to February (depending on water temperature). The Character Description for the Barmah-Millewa Ramsar site, however, describes bony herring as a wetland specialist that spawns and recruits in floodplain wetlands and lakes, anabranches and billabongs during in-channel flows. The disparity may reflect the widespread distribution of bony herring and their versatility within a range of habitats. Daytime upstream movements have been recorded for juveniles and adults in the Murray and Murrumbidgee Rivers, and individuals as small as 22 mm have been recorded migrating. These movements are possibly related to the colonisation of new habitats by juveniles, as well as reproductive movements by adults. Bony herring are an algal detritivore, consuming large quantities of detritus, microalgae and micro-crustaceans. They are also consumed by other fish, such as Murray cod and golden perch, and form a significant part of the diet of piscivorous waterbirds such as cormorants and pelicans.

Although the relative hardiness of bony herring is likely to be protective against some of the water quality effects of an outbreak of CyHV-3 it also likely to mean that they are present in significant numbers in settings where these effects are most likely to be realised. Notably this includes waterholes within turbid lowland river systems where water flow and quality have diminished. Carp are also likely to be concentrated in these settings, and an aggressive outbreak of CyHV-3 may result in the accumulation of carcasses in numbers sufficient to result in either a low DO event or a cyanobacterial bloom. This elevated level of exposure can be balanced against the ubiquity and population strength of bony herring within lowland river systems, and the likelihood that acute impacts on local populations will be rectified by the species' high fecundity and mobility and its ability to colonise suitable habitat. A similar result was obtained for the risk associated with prey-switching. As noted, bony herring provide piscivorous fish with an abundant and established source of food and it would be expected that the removal of juvenile carp from a local population would result in increased predation upon bony herring. This notwithstanding it is also likely that the species' high fecundity and mobility would result in recolonisation and reestablishment of local population strength. All species of fish are relatively resistant to the effects of type C (or C/D mosaic) botulinum toxin although, as an algal detrivore, bony herring may be relatively more likely to consume toxin or vegetative cells in decaying algae. In this situation, intoxicated bony herring may surface and be targeted by piscivorous birds, helping to establish or perpetuate an outbreak of botulism.

Australian grayling is a diadromous fish, spending part of its lifecycle in freshwater and at least part of the larval or juvenile stages in coastal seas. Australian grayling occurs in streams and rivers on the eastern and southern flanks of the Great Dividing Range, from Sydney southwards to the Otway Ranges of Victoria and in Tasmania. It is believed to be absent from the inland Murray-Darling system and is comparatively unlikely to be exposed to the possible effects of an aggressive outbreak of CyHV-3 in carp. The Australian grayling is listed under the EPBC Act as a vulnerable species.

13.3.2 Small-bodied native fish

This is a mixed group including both the wetland or floodplain specialists, and the foraging generalists. Six of the small-bodied native fish whose distribution overlaps with carp are listed under the EPBC Act. Although individuals within this group have a range of adaptations and specialisations, and specialised requirements, lower-flow periods are generally conducive for recruitment while high-flows may render channel habitats unfavourable. A small pulse may nevertheless help to inundate spawning grounds, although this pulse would need to persist for 10 to 14 days to enable the sticky eggs to remain submerged through to the point of hatching. Lateral connection to off-channel habitats is likely to be important to most of these species, especially during higher flows. Because the small-bodied fish are in general quite short-lived and are less likely to migrate substantial distances to escape poor conditions, local populations may be relatively exposed in the event of recruitment failures.

Both the wetland or floodplain specialists and the foraging generalists are relatively more tolerant of low DO than are most of the large-bodied fish, and may survive for some time while the DO concentration remains above about 1 mg/L. Relative tolerance to low DO may mean that the small-bodied native fish as a group are likely to be less affected by the fall in DO that may accompany an outbreak of CyHV-3. Small-bodied native fish will not, however, be more tolerant of cyanotoxins. The highest risk scenario for most small-bodied species will be an aggressive outbreak in a contracted waterway, in a lower-flow setting. This was evident in the case study for the Moonie River Catchment, where spring and autumn are typically dry and the river contracts to a series of disconnected waterholes. An aggressive outbreak of CyHV-3 within these waterholes may result in the accumulation of carp carcasses in numbers sufficient to result in very low DO and an increased risk of a cyanobacterial bloom. In this setting, small-bodied native fish such as the locally endemic olive perchlet and carp gudgeons are likely to be as susceptible as most other fish, and exposure may result in the stress or death of adults or juveniles or the failure of annual recruitment. A similar situation may arise in the Barmah-Millewa Forest in a lower-flow season, where carp and other fish are concentrated in lentic off-channel waters and an aggressive outbreak with substantial accumulation of carcasses is plausible. In this setting, the critically endangered flathead galaxias and endangered Murray hardyhead might be exposed. Both adults and juvenile small-bodied native fish may also be exposed to the effects of prey-switching following the removal of a high proportion of juvenile carp. Exposure to prey-switching is most likely to occur in a high-flow season when ephemeral wetlands and floodplains are inundated, and large numbers of nesting waterbirds are also likely to be present. In the Lower Lakes and Coorong, for example, both Murray hardyhead and the vulnerable Yarra pygmy perch might be exposed. Some threatened species of small-bodied fish are reliant on a limited number of local populations, meaning that impacts on local populations – whether the result of poor water quality or prey-switching – may have broader impacts on species sustainability. The critically endangered flathead galaxias is an example of this, with small populations distributed through the southern reaches of the Murray-Darling system.

13.3.3 Waterbirds

Although waterbirds are not at direct risk from low DO, they are exposed to other aspects of poor water quality – including widespread cyanobacterial blooms and proliferating waterborne microorganisms. Some piscivorous waterbirds may also be exposed in the event of the sudden loss of juvenile carp as a stable source of food for nesting chicks. Botulism is a concern for most waterbirds – in particular, for colonial nesting waterbirds which may be exposed in large numbers.

The seabirds (also termed ‘piscivores’ by some authors, although numerous species within other categories also consume fish) encompass the pelicans, cormorants and darters. Gulls and terns are sometimes included in this group, although most species are more correctly classed as shorebirds (below). Many seabirds are migratory or nomadic, and most are colonial nesters. None of the seabirds whose distribution overlaps with that of carp are listed under the EPBC Act as threatened or listed under international agreements for the protection of migratory species to which Australia is a party.

Adult seabirds spend much of their time on the water, while nests are often constructed from mats of vegetation and either float within wetlands or rest close to the water’s edge. This places them in close contact with water, and with maximum exposure to harmful cyanobacteria or proliferating microorganisms. As colonial-nesting species, large numbers of seabirds may be exposed concurrently. This is a concern for local populations and may also impact on the recruitment and the resilience of individual species more broadly. As they are strictly piscivorous, seabirds are likely to be the most exposed of the waterbird functional groups in the event of the removal of juvenile carp. Although the reliance of seabirds on juvenile carp is not completely understood, it is logical that where the floodplains of ephemeral wetlands are inundated, and carp are recruiting in proximity to large colonies of nesting seabirds, that these birds will be taking advantage of juvenile carp as an abundant source of food for nesting chicks. Where this is the case, the sudden removal of a large proportion of carp juveniles may place stress on both adults and chicks and may also result in increased pressure on alternative prey species (see prey-switching below). This scenario might play out, for example, for Australian pelicans and a range of darters, cormorants and spoonbills within inundated floodplains of wetlands such as the Barmah-Millewa Forest and the Macquarie Marshes, and in parts of the Lower Lakes and Coorong, where large numbers of these and other colonial-nesting seabirds are likely during high-flow seasons. The scenario might also play out within lakes and irrigation reservoirs, such as Kow Swamp.

Although piscivorous birds tend to be associated with type E botulism, in 1996 more than 15,000 piscivorous birds became sick or died from type C botulism at the Salton Sea in southern California. This included almost 15 percent of the broader population of western American white pelicans. Since 1996, type C botulism has recurred annually in piscivorous birds at this location, although the numbers of dead birds have been lower (1,000 to 3,000). In these die-offs at Salton Sea, introduced Mozambique tilapia were thought to harbour the bacteria in their gastrointestinal tracts, where toxin is subsequently formed — perhaps under certain stressful conditions. Fish are less sensitive to type C (or C/D mosaic) botulinum toxin, and in this way may vector toxin or vegetative cells from a source within the environment to piscivorous birds. This pathway is not well tested, although might arise in conjunction with substantial seabird breeding events in places where the biomass density of carp is reasonably high, and deoxygenation (for example, through the decomposition of a large number of carp carcasses, or the collapse of a cyanobacterial bloom) has led to the germination of environmental spores of *C. botulinum*. In these settings, carp could potentially play the role in the development of a widespread botulism outbreak that was occupied by tilapia in California’s Salton Sea.

Shorebirds are principally waders, and feed by probing in the mud or picking items off the surface in both coastal and freshwater environments. Waders include the stilts, snipes, sandpipers, plovers and oystercatchers. The shorebirds also, however, include the gulls and terns and their allies which take fish from the sea. Although the distinction is not always upheld, it is also useful to delineate the shorebirds from the 'large waders' of the order Ciconiiformes (below). Most shorebirds nest individually within a loosely-defined area, although are not considered colonial nesters. Six shorebirds whose distribution overlaps with that of carp are listed under the EPBC Act as critically endangered, while a further two are vulnerable. With the exception of the Australian painted snipe, all of these threatened species are also listed under international agreements for the protection of migratory species to which Australia is a party.

Although adult and nesting shorebirds are also very closely associated with water, they are likely to be less exposed (at a population level) than seabirds to the harmful effects of either a widespread cyanobacterial bloom or proliferating waterborne microorganisms as they are not colonial nesters. This will mean that stresses are more likely to be exerted on individuals, or certain groups of individuals, than on a local population as a whole, and individuals may be more able or likely to take action to avoid such stressors – including seeking alternative nesting sites. The dotterels, stilts, greenshanks, snipes and lapwings that nest within the Barmah-Millewa Forest, for example, will be able to feed throughout the inundated floodplains and may therefore avoid most areas of degraded water quality. Some of these species may also extend their feeding range to include parts of Gunbower or Kow Swamp. Shorebirds may also be found in smaller breeding events, and in some settings where the likelihood that an outbreak of CyHV-3 will result in an impact on water quality is relatively higher. The endangered Australian painted snipe, for example, may be found in the Moonie River Catchment where the likelihood that carp carcasses would accumulate in numbers sufficient to result in a widespread cyanobacterial bloom, or the proliferation of waterborne microorganisms, is relatively high during a typically dry spring or autumn when the river has diminished to disconnected waterholes. A similar scenario might play out in a lower-flow season within the minor anabranches, billabongs and wetlands of the Yarrawonga to Tocumwal reach of the Murray River, where the greater painted snipe may be exposed. Shorebirds have a diet that is broader than fish alone, and this (in conjunction with the fact that they are not colonial nesters) means that they are likely to be less exposed than the strictly piscivorous seabirds in the event of the sudden removal of a stable source of feed (juvenile carp). In the Macquarie Marshes, for example, a late-summer or autumn outbreak of CyHV-3 amongst juvenile carp would not be likely to cause stress to the endangered Australian painted snipe or the black-tailed godwit. The diet and feeding behaviour of shorebirds, however, may place them at a relatively higher risk in the event of an outbreak of type C (or C/D mosaic) botulism that has been initiated or maintained through the carcass-maggot cycle. This would apply across all species of shorebird and may be of particular concern in settings such as the Macquarie Marshes and the Lower Lakes and Coorong where large numbers of waterbirds (including shorebirds) are likely to support the initiation and maintenance of the carcass-maggot cycle.

<u>Waterfowl</u> can be divided loosely into herbivores (including swans and geese) and ducks (which includes the dabbling and diving ducks and grebes). Dabbling ducks feed in shallow water and are more likely to have a diet with more aquatic plants and insects, while diving ducks feed deeper in the water and typically eat more fish or crustaceans. The diet of grebes consists mainly of small fish and aquatic invertebrates. Waterfowl may congregate to nest in loosely-defined areas, but do not form colonies. Although some waterfowl in the northern hemisphere migrate to escape harsh winters, the same does not occur in Australia. None of the waterfowl whose distribution overlaps with that of carp are listed under the EPBC Act as threatened or listed under international agreements for the protection of migratory species to which Australia is a party.

The exposure of waterfowl to the effects of cyanotoxins of waterborne microorganisms is likely to be similar to that of shorebirds, as neither are colonial nesters, and both are closely associated with water. Both waterfowl and shorebirds may also nest (in smaller numbers) in settings where the degredation of water quality is relatively more likely. The black swan, chestnut teal, freckled duck, great crested grebe, grey teal, maned duck and pacific black duck may all be found within the disconnected waterholes of the Moonie River Catchment, for example, where the likelihood that carp carcasses would accumulate in numbers sufficient to result in a widespread cyanobacterial bloom is relatively high during a typically dry spring or autumn. The diet of some waterbirds (in particular, the diving ducks) includes a relatively higher proportion of fish but although these species may be impacted to some extent by the sudden removal of juvenile carp, waterfowl are in most settings likely to be able to source alternative prey. Like the shorebirds, the diet and feeding (foraging) behaviour of some waterbirds may place them at a higher risk in the event of an outbreak of type C (or C/D mosaic) botulism that has been initiated or maintained through the carcass-maggot cycle. Again, this would be of most concern in settings such as the Macquarie Marshes and the Lower Lakes and Coorong which support very large numbers of nesting waterbirds (including waterfowl).

The large waders include the storks, herons, egrets, ibises and spoonbills. These birds are carnivorous with a diet that may include fish, reptiles, amphibians, crustaceans, molluscs and aquatic insects. Many of the large waders are colonial nesters, and many are at least partially migratory. The endangered Australasian bittern is the single large wader, whose distribution overlaps with carp, that is listed under the EPBC Act. None of this subset of the large waders is listed under international agreements for the protection of migratory species to which Australia is a party.

As colonial nesters living in close association with water, the exposure of the large waders to either cyanotoxins or to proliferating microorganisms is likely to be similar to that of seabirds. The Australasian bittern, as well as a range of ibis, egrets and herons, for example, may be exposed in the event of a widespread cyanobacterial bloom within the Lower Lakes or Coorong. The generally broader diet of most waders, however, will mean that they are relatively less exposed than seabirds in the event of the sudden removal of juvenile carp. This would apply to Australasian bitterns nesting within the inundated floodplains of the Barmah-Millewa Forest, which are likely to be able to switch from juvenile carp to juvenile large-bodied native fish, small-bodied native fish, frogs and turtle eggs or hatchlings. The species of large wader that include insects within their diet may, however, be relatively more exposed in the event of an outbreak of type C (or C/D mosaic) botulism that has been initiated or maintained through the carcass-maggot cycle. This would be of most concern in settings such as the Macquarie Marshes and the Lower Lakes and Coorong which support very large numbers of nesting waterbirds; including large colonies of large waders.

The Gruiformes include rails, crakes, coots, moorhens and waterhens. This order of waterbird is very large, with more than 160 recognised species, most of which are omnivorous (with a diet that may include molluscs, frogs, small fish and insects). The order also includes cranes and brolgas, some of which are phenotypically similar to herons and other large waders and share some aspects of behaviour and preferred habitat. Gruiformes are not migratory and are not colonial nesters, although may congregate in loosely defined areas. None of the birds in this category whose distribution overlaps with that of carp is listed under the EPBC Act as threatened, nor listed under international agreements for the protection of migratory species to which Australia is a party.

Some species of Gruiformes nest and forage in wetland environments while others prefer drier environments adjacent to a waterbody. Species that occupy wetlands feed on a range of animal biota, including molluscs, frogs, small fish and insects, as well on aquatic plants. The exposure of these species to the effects of a widespread cyanobacterial bloom or proliferating waterborne microorganisms would be similar to that of many shorebirds (above) and would arise largely from contact with affected water and the consumption of cyanotoxins in aquatic biota. This would apply, for example, to the crakes, rails, native hens, moorhens and coots that nest within the inundated wetlands and floodplains of the Barmah-Millewa Forest during a high-flow season. The exposure of Gruiformes that occupy a more terrestrial habitat might be lower, although many of these will nevertheless nest close to a waterbody and are likely to take some prey from it. Like the shorebirds, exposure of the Gruiformes may be mitigated to some extent by the fact that they are not colonial nesters and thus more able to take individual action to avoid affected areas.

Gruiformes species that occupy wetlands feed on a range of animal biota, including molluscs, frogs, small fish and insects, as well on aquatic plants. The exposure of these species to the effects of the sudden removal of carp as a dominant and stable food source is likely to be very similar to that of the omnivorous subgroups of waterfowl (above). This would apply, for example, to the crakes, rails, native hens, moorhens and swamphens that nest within the Lower Lakes and Coorong. The diet and feeding (foraging) behaviour of some Gruiformes may also place them at a higher risk in the event of an outbreak of type C (or C/D mosaic) botulism that has been initiated or maintained through the carcass-maggot cycle.

While the kingfishers are often associated with waterbodies and are regarded as piscivorous (owing to their name), many species live away from water and have a broader diet that includes small crustaceans, invertebrates and other small prey. The laughing Kookaburra is an example of this. None of the kingfishers whose distribution overlaps with that of carp are listed under the EPBC Act as threatened or listed under international agreements for the protection of migratory species to which Australia is a party. The kingfishers may be exposed to cyanotoxin or proliferating waterborne microorganisms as a result of feeding on affected aquatic biota or drinking contaminated water. This would include, for example, the azure and sacred kingfishers that nest within the Barmah-Millewa Forest ecosystem. Kingfishers are not colonial nesters, however, and their nests are not close to water, and although they are not migratory most kingfishers would generally be able to move away from an affected area. As noted above, the diet of most kingfishers is quite broad, and they are not likely to be impacted by the sudden removal of juvenile carp. Kingfishers inhabiting wetland environments are likely to include insects within their diet, and this would place them at risk in the event of an outbreak of type C (or C/D mosaic) botulism that has been initiated or maintained through the carcass-maggot cycle.

The songbirds are small, perching birds of the order Passeriformes (the passerine birds). With more than 110 families and more than 6,000 identified species, Passeriformes is the largest order of birds and among the most diverse orders of terrestrial vertebrates. The mallee emu wren and the thick-billed grass wren have a distribution that overlaps with carp and are listed under the EPBC Act as endangered. In addition to these, the satin flycatcher and the rufous fantail are listed under international agreements for the protection of migratory species to which Australia is a party. The diet of passerine birds varies amongst species, and may include seeds, insects and small crustaceans. Some passerine birds, such as the clamorous warbler, are true waterbirds. Others may be present in wetlands and other water-focussed ecosystems, whether permanently or as an opportunistic means by which to gain refuge from drought and other environmental stresses. These two groups of passerine birds may be exposed to cyanotoxin as a result of drinking affected water or consuming affected aquatic biota. Those passerines that live in close association with waterbodies, and are considered waterbirds, would be additionally exposed through contact with affected water. These birds may also be relatively less able to move away from an affected area. Within the Moonie River Catchment, for example, passerine birds such as the black-throated finch, eastern yellow robin, golden whistler, grey fantail, grey shrike-thrush, jacky winter, rufous whistler, south-west crested shriketit and willie wagtail may be exposed to cyanotoxin in the event of a widespread bloom that occurs within disconnected waterholes during a typically dry spring or autumn. The passerines do not eat fish and would not be exposed in the event of the sudden removal of juvenile carp. Those passerines that include insects within their diet would be at risk in the event of an outbreak of type C (or C/D mosaic) botulism that has been initiated or maintained through the carcass-maggot cycle. This might occur within one of the smaller wetlands in the Moonie River Catchment, although in this setting the botulism outbreak is more likely to have been initiated (c.f. maintained) by way of the germination of environmental spores than through the carcass-maggot cycle.

The bush-birds are an heterogeneous and informally-defined catchall category that encompasses the range of species that may roost, forage and nest in riparian or floodplain forests (including river red gums) but are not generally considered to be true waterbirds. This group encompasses some of the psittacines (cockatoos, cockatiels, corellas, parrots, rosellas, and budgerigars) as well as some honeyeaters, frogmouths, curlews and others. Four bush-birds whose distribution overlaps with that of carp are listed under the EPBC Act as critically endangered, four are endangered and six are vulnerable. In addition, seven species are listed under international agreements for the protection of migratory species to which Australia is a party. Bush-birds are particularly important to this assessment in that they underpin the listing of several Ramsar wetlands. The vulnerable superb parrot, for example, is referenced in the environmental character descriptions for both the Barmah-Millewa Forest and Macquarie Marshes Ramsar sites; while the regent parrot is referenced in the environmental character description for the Riverland (including parts of the Chowilla Floodplain) Ramsar site.

Although some bush-birds may consume insects contaminated with cyanotoxins or waterborne microorganisms, their exposure will in general be limited to drinking water. None of the bush-birds nest in close proximity to water and most will be more able to move away from an affected area than the generally smaller songbirds (above). That notwithstanding, the critically endangered plains wanderer and vulnerable painted honeyeater, for example, may be exposed to cyanotoxin in the event of a widespread bloom that occurs within a series of waterholes within the Moonie River Catchment during a typically dry spring or autumn. Like the passerines (above) bush-birds do not eat fish and would not be exposed in the event of the sudden removal of juvenile carp. Those bush-birds that include insects within their diet would be at risk in the event of an outbreak of type C (or C/D mosaic) botulism that has been initiated or maintained through the carcass-maggot cycle.

The aquatic raptors include the eastern osprey and white-bellied sea eagle, and these are believed to be the only Australian raptors whose diet regularly includes fish. Both may be categorised as waterbirds. Ospreys feed primarily on live fish, while the white breasted sea eagle consumes both live fish and carrion, as well as birds and other animals. The eastern osprey is listed under international agreements for the protection of migratory species to which Australia is a party. Aside from these two species, a wide range of other raptors may play a key role in wetlands and other water-focussed ecosystems but are not considered to be waterbirds. Some of these are primarily hunters, with a diet that may include aquatic animals, birds, small mammals, crustaceans or insects. Other species will feed on carrion. The red goshawk is the single species of raptor listed under the EPBC Act with a distribution that overlaps with carp.

The exposure of raptors to cyanotoxins would include drinking affected water and consuming affected (terrestrial or aquatic) animals or insects. Importantly, this would extend to carrion. As cyanotoxins can bioaccumulate, repeated low-dose exposures may also result in breaching the toxic threshold. The two piscivorous raptors (the eastern osprey and the white-bellied sea eagle) are likely to be relatively more exposed. Both species are endemic within the ecosystem of the Lower Lakes (Alexandrina and Albert, but excluding the Coorong itself), where an aggressive spring outbreak of CyHV-3 during a dry season in shallow and partially disconnected wetland areas might lead to the accumulation of carp carcasses in numbers sufficient to result in a widespread cyanobacterial bloom. The exposure of raptors (in particular, carrion-eating raptors) to proliferating microorganisms may also be quite high, although the extent of uncertainty in respect of the particular microorganisms involved makes conclusions about the likely impact of this exposure difficult. Neither of the two piscivorous raptors are likely to have a dependence on adult or juvenile carp and would not be exposed in the event of the sudden removal of juvenile carp. This would extend to settings such as the Macquarie Marshes that support very large waterbird breeding and nesting events, and where both the eastern osprey and the white-bellied sea eagle are endemic. Although there is evidence that some raptors are relatively resistant to botulinum toxin – and accepting that this would be a likely evolutionary adaptation for those species that consume principally carrion – there not any conclusive studies that show that this holds for Australian raptors, including the two piscivorous species. Given that, the exposure of raptors to type C (or C/D mosaic) botulism may be relatively high. This would include wetland ecosystems such as the Barmah-Millewa Forest which have large waterbird breeding and nesting events and where raptors (in this case, the white-bellied sea eagle and swamp harrier) are endemic. Given that outbreaks of botulism in wildlife may also be initiated through the germination of environmental spores (above) following a low DO event, raptors in ecosystems such as the Moonie River Catchment (including a range of falcons, kestrels, goshawks, kites, eagles and owls) may also be exposed.

13.3.4 Frogs

Two hundred and eight species of frog are currently recognised within Australia, although some others await description. Of these, two species are listed under the EPBC Act as critically endangered, five are endangered and further five are vulnerable. Frogs occupy a wide variety of habitats, and many have developed highly-specialised adaptations to particular climates, ecological sites and communities. As most frog species are susceptible to dehydration, proximity to water is a general prerequisite. Preferred habitats can include rivers, lakes and wetlands.

Exposure to cyanotoxins as a result of a widespread bloom could arise from consuming affected insects or from contact with and consumption of affected water. Most frogs spawn in spring and summer, and this is likely to coincide with the spawning of carp and an outbreak of CyHV-3. Both eggs and tadpoles may therefore be exposed to cyanotoxins in the event of a widespread bloom. Although frogs have some ability to move between waterbodies, it is relatively unlikely that they would be able to avoid a widespread cyanobacterial bloom. Although less likely, a widespread cyanobacterial bloom within an ephemeral wetland, that coincided with inundation of floodplain habitat, would have the maximum impact on frog populations. In the Chowilla Floodplain, for example, this might include the vulnerable southern bell frog. Notwithstanding this, an aggressive outbreak of CyHV-3 is more likely to lead to a cyanobacterial bloom in a lower-flow setting. If this was to take place within the minor anabranches, billabongs and wetlands of the Yarrawonga to Tocumwal reach of the Murray River, for example, the giant bullfrog and growling grass frog are likely to be exposed. Cyanobacterial blooms might also occur within disconnected waterholes in the Moonie River Catchment during a typically dry spring or autumn, and here the warty water-holding frog would be exposed. Conversely, cyanobacterial blooms are less likely to result from an outbreak of CyHV-3 in settings such as the Abercrombie River (in the Upper Lachlan Catchment) where the endangered booroolong frog might otherwise be exposed.

Frogs may also be exposed to prey-switching, if breeding piscivorous waterbirds feeding substantially on juvenile carp are faced with the sudden removal of this otherwise stable and abundant source of food. In some settings, this might include frog spawn and tadpoles. The banjo frog, common eastern froglet, spotted grass frog, southern bell frog and Bibron's toadlet are endemic to the Lower Lakes and Coorong, and may be exposed to prey-switching in the event of the removal of juvenile carp as this ecosystem also includes very large numbers of breeding piscivorous (also carnivorous and omnivorous) waterbirds. No references were identified to substantiate the occurrence of type C (or C/D mosaic) botulism in frogs, although some early papers about the physiology of botulinum toxin included reference to frog skeletal muscle paralysis. If this translates to exposure in type C (or C/D mosaic) outbreaks of botulism in wildlife, then frogs in most settings may be exposed where either the carcass-maggot cycle or the germination of environmental spores are plausible.

13.3.5 Freshwater turtles

Australia is home to 23 species of freshwater turtle. Of those that have a distribution overlapping that of carp, the Bellinger River snapping turtle is listed under the EPBC Act as critically endangered, while the Bell's turtle is vulnerable. Australia’s freshwater turtles spend most of their time in rivers, lakes, wetlands, ponds and storage dams. Freshwater turtles are either carnivorous (including insects, tadpoles, small fish and crustaceans) or omnivorous, and some species may also feed on carrion.

The exposure of most freshwater turtles to cyanotoxins associated with a widespread cyanobacterial bloom is likely to be similar to that of frogs (above). With the exception of brief periods when seeking a suitable nesting site, turtles spend their lives within a waterbody and feed on aquatic biota. Those species that consume carrion might also be exposed to cyanotoxin that has accumulated within the carcass of a dead fish or bird. Turtles would also be exposed to the broader impacts of a widespread cyanobacterial bloom on water quality and food webs. Although they have a greater ability than frogs to leave the water and move away from an affected area, the high rate of dehydration, predation and death through other causes is likely to mean that this would not greatly increase the survivability of a local population. The broad-shelled turtle, long-necked turtle and Macquarie turtle, for example, might all be exposed if a cyanobacterial bloom was to extend to a number of waterholes within the Moonie River Catchment following an outbreak of CyHV-3 in a typically dry spring or autumn.

The exposure of most freshwater turtles to prey-switching will be limited to eggs and hatchlings, as maturing and adult turtles are relatively protected from predation. The exposure of eggs and hatchlings could, however, have a measurable impact on the sustainability of a local population as both are currently targeted by foxes and other predators. In the Lower Lakes and Coorong, for example, the eggs and hatchlings of the long-necked turtle, short-necked turtle and broad-shelled turtle might all be exposed to prey-switching in the event of the removal of juvenile carp as this ecosystem also includes very large numbers of breeding piscivorous (also carnivorous and omnivorous) waterbirds. A single reference was identified to substantiate the impact of type C botulinum toxin on turtles, and this was a government factsheet and not primary research or a formal review. Taken at face value, however, turtles are likely to be exposed through drinking affected water and consuming affected insects. For some species, this will extend to carrion which would be likely to raise the level of exposure.

13.3.6 Freshwater crustaceans

Australia has a wide range of native freshwater crustaceans, including crayfish, lobsters, prawns, shrimps and crabs, a subset of which inhabit ecosystems that may also include carp. Of these, only the endangered Glenelg spiny freshwater crayfish is listed under the EPBC Act, although the population of Murray crayfish is thought to have declined markedly following the widespread and long-lasting blackwater event within the mid-lower Murray River in 2010-11. A range of other native freshwater crustaceans whose distribution overlaps with carp, including the yabby and freshwater shrimp. Both of the larger species are sensitive to water quality and the addition of sediment, thermal pollution, pollutant discharges and bushfire impacts and prefer permanent rivers and streams where the water flow is moderately fast. Emergence occurs at approximately 2 mg/L, with juveniles having a LC50 of about 2.2 mg/L. Emergence leads to a high fatality rate through dehydration and predation. The yabby is more tolerant than the two larger species to low DO and other forms of poor water quality and for this reason survives well in farm dams. This notwithstanding, both yabbies and freshwater shrimp were observed at the water's edge during the widespread and long-lasting blackwater event of 2010-11 – as noted above, the population of Murray crayfish is thought to have declined markedly following this event.

This observation notwithstanding, it is unlikely that the decomposition of carp carcasses would result in a similarly widespread and enduring drop in DO within the lotic river Murray River channel or within the major anabranches and tributaries favoured by these species. In these settings, Murray crayfish, yabbies and other detrivorous crustaceans are likely to be advantaged by the increase in available food. The risk may be higher in a lower-flow setting, when the water level is lower and local populations of Murray crayfish and other crustaceans may be sequestered in poorly-connected parts of the larger river system. In this scenario, carp biomass is also likely to be more concentrated, potentiating a more aggressive outbreak of CyHV-3 and a more marked accumulation of carp carcasses. In this setting, large and small crustaceans may also be exposed to the toxic effects of a widespread cyanobacterial bloom – or to the precipitous drop in DO that follows from the collapse of the bloom.

Juvenile large crustaceans, and adults and juveniles of the smaller species, are an important component of the diet of many carnivorous and omnivorous waterbird species and may be exposed to increased predation as a result of prey-switching. Exposure may be exacerbated by a decline in water quality (including low DO or a widespread cyanobacterial bloom), as many crustaceans will then emerge from the water and be additionally vulnerable to predation. Crustaceans may be contaminated with botulinum toxin and thus play an important role in the epidemiology of botulism outbreaks, although no references were identified linking type C (or C/D mosaic) botulism with clinical disease in crustaceans.

13.3.7 Ecological communities

The EPBC Act recognises cave, forest, grassland, ground spring, marine, shrubland, wetland and woodland communities. In this assessment, the focus was on communities that may include (or be adjacent to) waterways in which carp are known to be present. These included the forest and woodland communities, grassland communities and wetland and marsh communities. The latter include the assemblage of native flora, fauna and micro-organisms associated with and dependent upon the floodplain and river ecosystem. These are complex aquatic and terrestrial ecosystems of interconnected environmental subunits, and may include river channels, lakes and estuaries, tributaries and streams, floodplain wetlands and woodlands, and groundwater. In ecological terms, wetlands and marshes may best be considered landscape-scale ecological meta-communities whose hydrological connectivity is recognised as being essential to their long-term health. Three wetlands or marsh communities are listed under the EPBC Act. The ecological communities encompassed by the Murray River wetlands (and associated floodplains and groundwater systems, from the junction with the Darling River to the sea) and Macquarie Marshes (and its floodplains) were put forward for listing, although disallowed. The Macquarie marshes (and several other large wetland ecosystems within the Murray-Darling system) have, however, been listed under the Ramsar Convention on Wetlands of International Importance.

Although the possibility of indirect harm to forest, woodland and grassland communities was considered (as noted above), the impacts of CyHV-3 on both listed and unlisted wetland and marsh communities remained the principal focus of the component of the assessment that was concerned with ecological communities. Wetland and marsh communities are fundamentally water-focussed, and those that occur within the distribution of carp could potentially be harmed through any of the exposure pathways considered in this assessment. Harm could result from a diminishment of the quality of habitat, as might follow from a low DO event or a widespread cyanobacterial bloom, or from disruption of food webs, as might follow from the removal of carp juveniles and increased predation of native fish and other aquatic animals. There is also the possibility of an outbreak of botulism which has the potential to result in significant direct harm to most of the higher faunal functional groups within a wetland community, and to considerably disrupt food webs and other aspects of the ecosystem balance.

13.3.8 Livestock

Livestock and companion animals may be exposed to cyanotoxins or proliferating waterborne microorganisms if they drink water from contaminated waterbodies, lick their coats after swimming in such waters, or consume contaminated materials (including carcass components, algal scum or mats). The effects of cyanotoxin are as varied in animals as they are in people, with visible symptoms including vomiting, diarrhoea, seizures and death. The exposure of livestock may be plausible in some settings, as livestock are frequently husbanded in such a way as to depend on a particular source(s) of drinking water. This might be access to a reach of a river or stream, or to a billabong. In dryer seasons, when waterbodies have contracted and an aggressive outbreak of CyHV-3 in carp is more likely, such waterbodies may be affected by a cyanobacterial bloom or contaminated with proliferating microorganisms. In these situations, stock within the area may have no choice but to consume affected water – whether directly from that source or when pumped through a storage and distribution system. Importantly, cyanotoxins are also able to accumulate within animal tissues, meaning that repeated low-dose exposures can eventually reach a point within an animal where the toxic threshold is breached, and clinical symptoms are observed.

Livestock may also be exposed to botulism as a result of an outbreak in wildlife. This could result from predating upon carcass materials that contain preformed toxin in situations when particular soil minerals are lacking or imbalanced (termed 'pica'), or from drinking water that contains free preformed toxin or toxin associated with invertebrates or with suspended vertebrate carcass materials. These pathways might occur where fish or bird carcasses have contaminated a natural water source, such a billabong, a wetland or a creek or river. Alternatively, wild bird (and possibly fish) carcasses may contaminate farm dams or a reservoir from which stock water is obtained. Livestock losses have not been associated with past wildlife outbreaks of type C (or C/D mosaic) botulism in Australia. That notwithstanding, the co-occurrence of mass fish mortalities in different parts of Australia has not occurred to date and, in view of this, it is plausible that in some locations the exposure of livestock through drinking water will occur. Settings such as the Moonie River Catchment during a typically dry spring or autumn, for example, have a relatively higher risk of botulism associated with the germination of environmental spores. Substantial numbers of livestock draw on the disconnected waterholes within this catchment and may be exposed. A similar scenario is plausible for some reaches within the mid-Murray River.

13.3.9 Public health

Exposure to cyanobacteria, cyanotoxins and proliferating waterborne microorganisms may occur through accidental or deliberate ingestion or inhalation of toxin-contaminated water, or through dermal contact during recreational activities (for example, swimming or water-skiing). Exposure to cyanobacterial cells may result in skin irritations, including rashes, hives, swelling or skin blisters. Ingestion of cyanotoxins can also cause more severe health effects such as liver or kidney damage. The extent of this will depend upon the cyanotoxin, and the magnitude, duration and frequency of exposure. For example, short-term exposures to microcystins could cause liver damage, while kidney damage is a key health effect for cylindrospermopsin. Impacts of exposure to waterborne microorganisms is less clear-cut, as very little has been published on the topic of the particular waterborne microorganisms that may be associated with the decomposition of fish carcasses in water. This is despite the marked visibility of public health warnings about the need to ensure that fish and other carcass materials are not present in drinking water. It was suggested in a personal communication that carp gut flora and spoilage organisms may proliferate after a fish kill, as might *E. coli*, some *Pseudomonas spp* and other opportunistic microorganisms. Shiga toxin-producing *E. coli* (STEC) has been isolated from bodies of water (such as ponds and streams), wells and water troughs, and has been found to survive for months in manure and water-trough sediments. *Aeromonas spp* have also been found in irrigation water, rivers, springs, groundwater, estuaries and oceans (in particular where water has been contaminated by sewerage) and are of public health concern. To a greater or lesser extent, all of these organisms have the potential to cause harm to people.

The key mitigating factor in respect of the exposure of people to water that may be contaminated with cyanotoxins or proliferating microorganisms is that the visible effects of widespread carcass decomposition are likely to be apparent, and that both these and the odour of decomposition will deter most water users from undertaking recreational activities or taking fish, crustaceans or drinking water from affected areas. In respect of cyanobacterial blooms, there is also likely to be warning signage in places of public amenity, as these are not uncommon in many Australian freshwater waterways.

13.4 Residual uncertainty

The breadth of this ecological risk assessment was considerable and, without any direct experience of the epidemiology of CyHV-3 in an Australian context, a degree of residual uncertainty was inevitable. In particular, this concerned the likely behaviour of the virus in a range of Australian freshwater settings and key components of the identified exposure pathways. Although largely beyond the scope of this assessment, there was also some residual uncertainty about the likely efficacy and practicality of some mitigations when applied in certain settings.

The likely behaviour of CyHV-3 was encapsulated in the outbreak scenarios discussed in Section 10 and summarised in Section 13.1 (above). Separate scenarios were given for outbreaks in wetlands and floodplains, lakes and reservoirs, and rivers and creeks (Figure 28). In each case, the implications of high-flow and lower-flow seasons were examined. The aggressiveness of an outbreak in a given setting hinged upon opportunities for close contact (principally pre-spawning aggregations and contact during spawning) and water temperature. The role of juvenile carp was also considered. Opportunity for harm to the environment reflected the value and sustainability of the ecological assets at risk in a particular location, as well as the carp biomass density, the connectedness and flow of water through the system, and other factors specific to particular exposure pathways and case studies (below). Although the assumptions underpinning these parts of the ecological risk assessment concurred, in broad terms, with the NCCP's epidemiological modelling project (Durr *et al*., 2019a), it was also recognised that the behaviour of an exotic disease in such diverse and complex settings cannot be predicted with certainty. It is possible, for example, that CyHV-3 will not penetrate local carp populations to the extent envisaged. It is equally possible, however, that the virus will be more successful than expected, or that particular characteristics of its epidemiology (such as the higher sensitivity of juvenile carp) will lead to an impact on carp populations that is more marked than modelling and qualitative assessment have suggested.

As noted, residual uncertainty also existed in respect of the identified exposure pathways. The tolerance of each lifecycle stage of every native water-breathing aquatic species that may be exposed to low DO is not known, although may be inferred with varying degrees of confidence from the literature about (in particular) blackwater events. Similarly, whilst the NCCP water quality modelling studies (Walsh *et al.*, 2018; Hipsey *et al.*, 2019) showed that a dangerously low DO was only likely to occur partially-connected or disconnected waterways, with a very high carp biomass density, there remained a degree of uncertainty about the importance of local conditions. A similar situation existed for widespread cyanobacterial blooms, with inference in that case based on the development and impacts of blooms that have occurred naturally throughout Australian freshwater waterways. Substantial uncertainty also surrounded the assessment of waterborne microorganisms that may be released into waterways with the decomposition of carp carcasses. In this case, uncertainty included the species of microorganisms that are likely to be involved, and their pathogenicity for particular functional groups and native species, as well as the persistence of epidemics within waterways after the dissolution of carcass materials.

In addition to the water quality pathways, substantial uncertainty remained in respect of the impact of CyHV-3 on food webs – in particular, in settings that include large numbers of nesting piscivorous waterbirds. The two key aspects of this scenario included the putative effects of removing a stable and plentiful food source (juvenile carp), and the likelihood that piscivorous waterbirds would then switch to native fish, crustaceans, frogs and turtle eggs and young as an alternative source of food. Very little is currently known about the likelihood, and likely severity, of either pathway, and this was reflected in the conservative estimates.

Botulism in wildlife is considered to be an inherently probabilistic process, with relatively few outbreaks observed in Australia given the ubiquity of spores and the frequent alignment of suitable conditions. Compounding this is a paucity of reports specifically linking fish kills to outbreaks of type C (or C/D mosaic) botulism in waterbirds, despite the fact that substantial fish kills (as a result of blackwater events and other processes) are not uncommon within Australian waterways. This notwithstanding, it was recognised that concurrent outbreaks of CyHV-3 across a catchment or river system have the potential to create a uniquely high-risk scenario – in particular, given the co-occurrence of: (a) carp at a relatively high biomass density; and (b) large numbers of nesting waterbirds. In view of this, conservative estimates were assigned to this pathway. Type E botulism was ruled out of the case studies and assessment of MNES (Part V in Volume 2 of this report) on the grounds that there is no evidence that it exists within Australia. This was considered a practical and realistic standpoint, although it was also noted that there has not been a systematic search for type E *C. botulinum* across Australian waterways, and that none of the experts consulted was willing to state categorically that type E is an exotic strain. The importance of type E is twofold: (a) it is primarily a disease of fish (although waterbirds are severely impacted), and therefore more likely to arise in the context of a widespread and multifocal fish kill; and (b) it is highly-toxic (frequently fatal) to humans.

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Acronyms and abbreviations

Abbreviation or acronym	Full term
ASR	Aquatic surface respiration
AWRA	Australian Water Resources Assessment
AWRC	Australian Water Resources Council
BOD	Biological oxygen demand
Ca	Calcium
CAMBA	China–Australia Migratory Bird Agreement
CDC	United States Centers for Disease Control and Prevention
COAG	Council of Australian Governments
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DO	Dissolved oxygen
DOC	Dissolved organic carbon
EPBC Act	Environment Protection and Biodiversity Conservation Act 1999
FRDC	Fish Research and Development Corporation
FRP	Filtered reactive phosphorus
GMO	Genetically modified organism
HAB	Harmful algal bloom
JAMBA	Japan-Australia Migratory Bird Agreement
MDBA	Murray-Darling Basin Authority
Mg	Magnesium
MNES	EPBC Act Matters of National Environmental Significance
MYXV	Myxoma virus
NCCP	National Carp Control Program

Abbreviation or acronym	Full term
P	Phosphorous
RHDV	Rabbit haemorrhagic disease virus
ROKAMBA	Republic of Korea-Australia Migratory Bird Agreement
TP	Total Phosphorous

Scientific names for cited species

Common name(s)	Scientific name
Australasian bittern	*Botaurus poiciloptilus*
Australian grayling	*Prototroctes maraena*
Australian painted snipe	*Rostratula australis*
Australian smelt	*Retropinna semoni*
Australian white ibis	*Threskiornis moluccus*
Barred galaxias	*Galaxias fuscus*
Bar-tailed godwit (also western Alaskan bar-tailed godwit)	*Limosa lapponica bauera*
Belah	*Casuarina pauper*
Bell’s turtle	*Wollumbinia belli*
Bellinger River snapping turtle	*Wollumbinia georgesi*
Bendee	*Acacia catenulata*
Bitter pea	*Daviesia ulicifolia*
Black bittern	*Ixobrychus flavicollis*
Black box	*Eucalyptus largiflorens*
Black swan	*Cygnus atratus*
Black tee tree	*Melaleuca bracteata*
Black-eared miner	*Manorina melanotis*
Black-oak	*Casuarina pauper*
Black-tailed godwit	*Limosa limosa*
Blakely's red gum	*Eucalyptus blakeleii*
Blue-faced honeyeater	*Entomyzon cyanotis*

Common name(s)	Scientific name
Bony herring (also bony bream, hairback herring or pyberry)	*Nematalosa erebi*
Booroolong frog	*Litoria booroolongensis*
Bracken	*Pteridium esculentum*
Brigalow	*Acacia harpophylla*
Broad-shelled turtle	*Macrochelodina expansa*
Brown barrel gum	*Eucalyptus fastigata*
Bull oak	*Allocasuarina luehmannii*
Carp gudgeon species	*Hypseleotris* spp
Carpet python	*Morelia spilota*
Clamorous warbler	*Acrocephalus stentoreus*
Clustered wallaby grass	*Danthonia racemosa*
Common galaxias	*Galaxias maculatus*
Common greenshank	*Tringa nebularia*
Common sandpiper	*Actitis hypoleucos*
Coolabah tree	*Eucalyptus coolabah*
Cranes rails, crakes, coots, moorhens, swamphens and waterhens	Gruiformes
Crimson-spotted rainbowfish	*Melanotaenia duboulayi*
Curlew sandpiper	*Calidris ferruginea*
CyHV-3 (also Koi herpes virus or KHV)	Cyprinid herpesvirus 3
Cyprus pine	*Callitris glaucophylla*
Double-banded plover	*Charadrius bicinctus*
Dwarf galaxias	*Galaxiella pusilla*
Eastern curlew (also far eastern curlew)	*Numenius madagascariensis*

Common name(s)	Scientific name
Eastern osprey	*Pandion cristatus*
Eastern water dragon	*Intellagama lesueurii*
European carp	*Cyprinus caprio*
European carp (also common carp)	*Cyprinus carpio*
Fat-tailed dunnart	*Sminthopsis crassicaudata*
Feathertail glider	*Acrobates pygmaeus*
Flathead galaxias	*Galaxias rostratus*
Fleay's frog	*Mixophyes fleayi*
Floodplain mussel	*Velesunio ambiguous*
Fork-tailed swift	*Apus pacificus*
Freshwater (also eel-tailed) catfish	*Tandanus tandanus*
Freshwater galaxias species	*Galaxias* spp
Freshwater gudgeon species	*Mogurnda* spp
Freshwater shrimp	*Macrobrachium* spp.
Giant barred frog (also southern barred frog)	*Mixophyes iteratus*
Giant bullfrog	*Limnodynastes interioris*
Giant burrowing frog	*Heleioporus australiacus*
Giles' planigale	*Planigale gilesi*
Gippsland water dragon	*Physignathus leseuerii ssp. howitti*
Glenelg spiny freshwater crayfish	*Euastacus bispinosus*
Glossy black cockatoo	*Calyptorhynchus lathami*
Golden perch	*Macquaria ambigua*
Goldfish	*Carassius auratus*
Great knot	*Calidris tenuirostris*

Common name(s)	**Scientific name**
Green and golden bell frog	*Litoria aurea*
Grey box	*Eucalyptus macrocarpa*
Grey goshawk	*Accipiter novaehollandiae*
Grey teal	*Anas gibberifrons*
Grey wagtail	*Motacilla cinerea*
Growling grass frog (also southern bell frog, green and golden frog and warty swamp frog)	*Litoria raniformis*
Guppy	*Poecilia reticulata*
Hardyhead species	*Craterocephalus* spp.
Hyrtl’s catfish	*Neosilurus hyrtlii*
Koi carp	*Cyprinus carpio haematopterus*
Latham's snipe (also Japanese snipe)	*Gallinago hardwickii*
Laughing kookaburra	*Dacelo novaeguineae*
Lesser sand plover (also Mongolian plover)	*Charadrius mongolus*
Lignum	*Muehlenbeckia florulenta*
Little black cormorant	*Phalacrocorax sulcirostris*
Little curlew / little whimbrel	*Numenius minutus*.
Little tern	*Sterna albifrons sinensis*
Littlejohn's tree frog / heath frog	*Litoria littlejohni*
Long-necked turtle	*Chelodina longicolis*
Loons	Gaviiformes
Macquarie perch	*Macquaria australasica*
Macquarie short-necked turtle	*Emydura macquarii*
Mallee emu wren	*Stipiturus mallee*
Mallee western whipbird	*Psophodes leucogaster leucogaster*

Common name(s)	Scientific name
Malleefowl	*Leipoa ocellata*
Marsh sandpiper (also little greenshank)	*Tringa stagnatilis*.
Masked owl	*Philemon corniculatus*
Molly box	*Eucalyptus pilligaensis*
Mosquito fish	*Gambusia holbrooki*
Mountain gum	*Eucalyptus dalrympleana*
Mozambique tilapia	*Oreochromis mossambicus*
Murray cod	*Maccullochella peelii*
Murray hardyhead	*Craterocephalus fluviatilis*
Murray Rainbowfish	*Melanotaenia fluviatilis*
Murray crayfish	*Euastacus armatus*
Narrow-leafed ironbark	*Eucalyptus crebra*
Noisy friarbird	*Philemon corniculatus*
Northern corroboree frog	*Pseudophryne pengilleyi*
Northern Siberian bar-tailed godwit	*Limosa lapponica menzbieri*
Olive perchlet (also Agassiz's glass fish)	*Ambassis agassizii*
Oriental cuckoo / Horsfield's cuckoo	*Cuculus optatus*
Painted honeyeater	*Grantiella picta*
Pectoral sandpiper	*Calidris melanotos*
Peppermint box	*Eucalyptus odorata*
Peregrine flacon	*Falco peregrinus*
Peron’s tree frog	*Litoria peronii*
Pilchard	*Sardinops sagax*
Pink robin	*Petroica rodinogaster*

Common name(s)	Scientific name
Plains-wanderer	*Pedionomus torquatus*
Platypus	*Ornithorhynchus anatinus*
Poplar box	*Eucalyptus populnea*
Powerful owl	*Ninox strenua*
Rainbow trout	*Oncorhynchus mykiss*
Rainbowfish species	*Melanotaeniidae* spp
Red goshawk	*Erythrotriorchis radiatus*
Red knot	*Calidris canutus*
Redfin perch	*Perca fluviatilis*
Red-lored whistler	*Pachycephala rufogularis*
Red-necked stint	*Calidris ruficollis*
Red-tailed black cockatoo	*Calyptorhynchus banksii graptogyne*
Regent honeyeater	*Anthochaera phrygia*
Regent parrot	*Polytelis anthopeplus monarchoides*
River mussel	*Alathyria jacksoni*
River blackfish	*Gadopsis marmoratus*
River cooba	*Acacia stenophylla*
River oak (also she-oak)	*Cassurina cunninghamiana*
River red gum	*Eucalyptus camaldulensis*
Rosewood	*Alectryon oleifolius*
Ruddy turnstone	*Arenaria interpres*
Ruff (also reeve, a sandpiper)	*Philomachus pugnax*
Rufous fantail	*Rhipidura rufifrons*
Rufous scrub-bird	*Atrichornis rufescens*

Common name(s)	**Scientific name**
Salmon catfish	*Neoarius graeffei*
Satin flycatcher	*Myiagra cyanoleuca*
Sea mullet	*Mugil cephalus*
Sharp-tailed sandpiper	*Calidris acuminate*
Shorebirds	Charadriiformes
Short-finned eel	*Anguilla australis*
Short-headed lamprey	*Mordacia mordax*
Silver perch	*Bidyanus bidyanus*
Silver-leafed ironbark	*Eucalyptus melanophloia*
Songbirds	Passeriformes
Southern corroboree frog	*Pseudophryne corroboree*
Southern purple-spotted gudgeon	*Mogurnda adspersa*
Southern pygmy perch	*Nannoperca australis*
Spangled perch	*Leiopotherapon unicolor*
Spotted mars frog	*Lymnodynastes tasmaniensis*
Spotted tree frog	*Litoria spenceri*
Square-tailed kite	*Lophoictinia isura*
Stuttering frog / southern barred frog	*Mixophyes balbus*.
Sugarwood	*Myoporum platycarpum*
Superb parrot	*Polytelis swainsonii*
Swift parrot	*Lathamus discolour*
Tench	*Tinca tinca*
Thick-billed grass wren	*Amytornis modestus*
Trout cod	*Maccullochella macquariensis*

Common name(s)	Scientific name
Turquoise parrot	*Neophema pulchella*
Unspecked hardyhead	*Craterocephalus stercusmuscarum*
Variegated pygmy perch	*Nannoperca variegate*
Water thyme	*Hydrilla verticillata*
Warty water-holding frog (also rough frog)	*Cyclorana verrucosa*
Waterfowl	Anseriformes
Weeping Myall	*Acacia pendula*
White box	*Eucalyptus albens*
White-bellied sea eagle	*Haliaeetus leucogaster*
White-plumed honeyeater	*Lichenostomus penicillatus*
White-throated needletail	*Hirundapus caudacutus*
White-winged tern (also white-winged black tern)	*Chlidonias leucopterus*
Wilga (also native willow)	*Geijera parviflora*
Yabby	*Cherax destructor*
Yarra pygmy perch	*Nannoperca obscura*
Yellow box	*Eucalyptus melliodora*
Yellow wagtail	*Motacilla flava*
Yellow-billed spoonbill	*Platalea flavipes*
Yellow-spotted tree frog (also yellow-spotted bell frog)	*Litoria castanea*

Appendix A Risk assessment endpoints

A.1 Ecological endpoints

Endpoints for Australia's natural assets or values were termed here 'ecological endpoints' and were adapted from the significant impact criteria provided by the Department of Environment and Energy as a guide for determining whether an action is likely to cause harm to one or more of the Matters of National Environmental Significance – and thus require Ministerial approval under the EPBC Act.[99]

Separate criteria are given for the exposure of each category within the Matters of National Environmental Significance, including listed threatened species and ecological communities and wetlands of international importance. These are discussed below.

Although not relevant to the case studies, additional significant impact criteria are also provided by the Department for actions that may impact on the Commonwealth marine environment, World Heritage Properties or National Heritage Places, and for the assessment of nuclear actions, actions that may impact on the Great Barrier Reef Marine Park and for coal mining or coal-seam gas developments.

A.1.1 Endpoints for listed threatened species and ecological communities

The Department of Environment and Energy provides separate significant impact criteria for each of the following groups of threatened species:

- Critically endangered and endangered species
- Vulnerable species
- Listed migratory species
- Critically endangered and endangered ecological communities.

Although not directly relevant to the case studies, significant impact criteria are also provided for species that are extinct in the wild.

Critically endangered or endangered species: an action is likely to have a significant impact on a critically endangered or endangered species if there is a real chance or possibility that it will:

- Lead to a long-term decrease in the size of a population
- Reduce the area of occupancy of the species
- Fragment an existing population into two or more populations
- Adversely affect habitat critical to the survival of a species

[99] Matters of National Environmental Significance Significant impact guidelines 1.1 Environment Protection and Biodiversity Conservation Act 1999, Commonwealth of Australia 2013

- Disrupt the breeding cycle of a population
- Modify, destroy, remove, isolate or decrease the availability or quality of habitat to the extent that the species is likely to decline
- Result in invasive species that are harmful to a critically endangered or endangered species becoming established in the endangered or critically endangered species' habitat
- Introduce disease that may cause the species to decline
- Interfere with the recovery of the species.

In this context, a population of a species is defined under the EPBC Act as an occurrence of the species in a particular area. In relation to critically endangered, endangered or vulnerable threatened species, occurrences include:

- A geographically distinct regional population, or collection of local populations, or
- A population, or collection of local populations, that occurs within a particular bioregion.

Similarly, an invasive species is an introduced species, and may include introduced (translocated) native species which out-compete native species for space and resources, or species which are predators of native species. An invasive species may harm listed threatened species or ecological communities by direct competition, modification of habitat or predation.

Finally, habitat is considered to be critical to the survival of a species or ecological community if it is necessary:

- For activities such as foraging, breeding, roosting, or dispersal
- For the long-term maintenance of the species or ecological community (including the maintenance of species essential to the survival of the species or ecological community, such as pollinators)
- To maintain genetic diversity and long term evolutionary development
- For the reintroduction of populations or recovery of the species or ecological community.

This may include habitat that has been identified in a recovery plan as being important to the persistence of a species, as well as Critical Habitat maintained under the EPBC Act.

Vulnerable species: an action is likely to have a significant impact on a vulnerable species if there is a real chance or possibility that it will:

- Lead to a long-term decrease in the size of an important population of a species
- Reduce the area of occupancy of an important population
- Fragment an existing important population into two or more populations
- Adversely affect habitat critical to the survival of a species
- Disrupt the breeding cycle of an important population
- Modify, destroy, remove or isolate or decrease the availability or quality of habitat to the extent that the species is likely to decline
- Result in invasive species that are harmful to a vulnerable species becoming established in the vulnerable species' habitat
- Introduce disease that may cause the species to decline

- Interfere substantially with the recovery of the species.

In this context, an important population is one that is necessary for a species' long-term survival and recovery. This may include populations identified as such in recovery plans, or those that are:

- Key source populations either for breeding or dispersal
- Populations that are necessary for maintaining genetic diversity
- Populations that are near the limit of the species range.

Migratory species: an action is likely to have a significant impact on a migratory species if there is a real chance or possibility that it will:

- Substantially modify (including by fragmenting, altering fire regimes, altering nutrient cycles or altering hydrological cycles), destroy or isolate an area of important habitat for a migratory species
- Result in an invasive species that is harmful to the migratory species becoming established in an area of important habitat for the migratory species
- Seriously disrupt the lifecycle (breeding, feeding, migration or resting behaviour) of an ecologically significant proportion of the population of a migratory species.

In this context, an area of important habitat for a migratory species is habitat:

a) Utilised by a migratory species occasionally or periodically within a region that supports an ecologically significant proportion of the population of the species, or

b) Of critical importance to the species at particular life-cycle stages, or

c) Utilised by a migratory species which is at the limit of the species range, or

d) Within an area where the species is declining.

The extent of an ecologically significant proportion of the population will vary with the species. Some factors that may be relevant include the species' population status, genetic distinctiveness and species-specific behavioural patterns (for example, site fidelity and dispersal rates).

The term 'population' in the context of migratory species means the entire population or any geographically separate part of the population, where a significant proportion of this cyclically and predictably crosses one or more national jurisdictional boundaries.

Critically endangered or endangered ecological community: an action is likely to have a significant impact on a critically endangered or endangered ecological community if there is a real chance or possibility that it will:

- Reduce the extent of an ecological community
- Fragment or increase fragmentation of an ecological community, for example by clearing vegetation for roads or transmission lines
- Adversely affect habitat critical to the survival of an ecological community
- Modify or destroy abiotic (non-living) factors (such as water, nutrients, or soil) necessary for an ecological community's survival, including reduction of groundwater levels, or substantial alteration of surface water drainage patterns

- Cause a substantial change in the species composition of an occurrence of an ecological community, including causing a decline or loss of functionally important species, for example through regular burning or flora or fauna harvesting
- Cause a substantial reduction in the quality or integrity of an occurrence of an ecological community, including, but not limited to
  – Assisting invasive species, that are harmful to the ecological community, to become established, or
  – Causing regular mobilisation of fertilisers, herbicides or other chemicals or pollutants into the ecological community which kill or inhibit the growth of species in the ecological community, or interfere with the recovery of an ecological community.

A.1.2 Endpoints for Ramsar wetlands

The ecological endpoints for Ramsar wetlands reflect threats to their ecological character.

In this context, 'ecological character' is the combination of the ecosystem components, processes and benefits or services that characterised the wetland at the time of its designation to the Ramsar List. Descriptions of the ecological character of listed Ramsar wetlands were obtained for this assessment from the Australian wetlands database.[100]

Given the above, an action is likely to have a significant impact on a Ramsar wetland if there is a real chance or possibility that it will result in:

- Areas of the wetland being destroyed or substantially modified
- A substantial and measurable change in the hydrological regime of the wetland, for example, a substantial change to the volume, timing, duration and frequency of ground and surface water flows to and within the wetland
- The habitat or lifecycle of native species, including invertebrate fauna and fish species, dependant upon the wetland being seriously affected
- A substantial and measurable change in the water quality of the wetland – for example, a substantial change in the level of salinity, pollutants, or nutrients in the wetland, or water temperature which may adversely impact on biodiversity, ecological integrity, social amenity or human health
- An invasive species that is harmful to the ecological character of the wetland being established (or an existing invasive species being spread) in the wetland.

A.2 Endpoints for the exposure of people or livestock

Additional endpoints were developed to capture the possible exposure or livestock of people to cyanobacterial toxin, to microorganisms associated with the decomposition of carp and to *Clostridium botulium* toxin.

[100] See: http: //www.environment.gov.au/water/wetlands/database/index.html

Endpoints for livestock:

- Local exposure of livestock to the clinical effects of a pathogenic organism or its toxin
- Establishment of a pathogenic microorganism in livestock in areas not previously affected.

Endpoints for public health:

- Exposure of individuals to the clinical effects of a pathogenic organism or its toxin
- Exposure of groups of individuals or communities to the clinical effects of a pathogenic organism or its toxin.

Appendix B Expert elicitation

B.1 Affiliations of experts approached

State	Affiliation
New South Wales	Australian Bureau of Agricultural and Resource Economics and Sciences
New South Wales	CSIRO
New South Wales	CSU/New South Wales OEH
New South Wales	New South Wales Department of Primary Industry
New South Wales	New South Wales Department of Primary Industry
New South Wales	New South Wales OEH
New South Wales	New South Wales OEH
New South Wales	Private
New South Wales	Private
New South Wales	University of Canberra
New South Wales	University of New South Wales
New South Wales	University of New South Wales
New South Wales	University of New South Wales
New South Wales/Victoria	Australian National University
Queensland/Northern Territory	CSIRO
Queensland/Northern Territory	Queensland Parks and Wildlife
Queensland/Northern Territory	Queensland Parks and Wildlife
Queensland/Northern Territory	University of Queensland
Queensland/Northern Territory	Wetlands International
South Australia	Australian National University
South Australia	Australian Wildlife Conservancy
South Australia	Deakin University
South Australia	Independent
South Australia	South Australian Department of Environment and Water
South Australia	South Australian Department of Environment and Water
South Australia	University of Adelaide
South Australia	University of Adelaide
South Australia	University of Adelaide
Victoria	Arthur Rylah Institute
Victoria	Arthur Rylah Institute

State	Affiliation
Victoria	Federation University Australia
Victoria	Goulburn Broken Catchment Management Authority
Victoria	Independent
Victoria	La Trobe University

B.2 Explanatory notes for experts

About the project

The Commonwealth Scientific and Industrial Research Organisation (CSIRO) is undertaking a wide-ranging ecological risk assessment surrounding the proposed release of the cyprinid herpes virus (CyHV-3) as a biocontrol agent for Australia's carp (*Cyprinus carpio*) population. The assessment is part of a broader program termed the National Carp Control Plan (hereafter referred to as the NCCP).

This exercise focusses on one particular type of ecological asset potentially impacted by CyHV-3 release, namely waterbirds that currently include fish in their diet. The exercise will use experts to characterise the relationship between the impact of the proposed release of the CyHV-3 on carp populations (particularly juvenile survival following spawning events) and waterbird populations. It will develop a model that predicts the likelihood of significant impacts on waterbird breeding and populations as result of the virus release. This model will be used to support a broader ecological risk assessment for the proposed virus release.

What your participation will involve

You have been invited to take part in the research because you have been identified as having the requisite skills to form judgements about changes to native freshwater fish and waterbird populations resulting from specified changes in the abundance of juvenile carp.

Participation in this research will involve completion of a survey, with optional follow-up. The survey provides a number of scenarios outlining different virus outbreak settings (initial epidemic severity, ongoing effect, extent and recurrence) impacting on the survival of juvenile carp in wetland systems. You will be asked to use your expertise to estimate the expected change (relative to baseline) in waterbird breeding success, local waterbird abundance and total waterbird population size. Your estimates will then be used to produce a predictive model, that collectively emulates all expert responses (equally weighted), for estimating the risk of impacts on water bird populations. It is expected that the survey will take 1-2 hours to complete. There will be an opportunity to discuss and clarify the scenario approach to elicitation with other participants (online or by phone), with the option of adjusting your estimates should you wish.

How your information will be used

The information you provide for this research will be used for the purposes of a report for policy makers and journal publications as part of the NCCP. You will not be identified in any of these publications, except in circumstances with regards to follow-up and where you have given written permission for this to occur. Your participation in the project is entirely voluntary and you are free to withdraw from the study at any time and without having to provide a reason for your withdrawal. You may ask for the information you provide to be removed from the study up until the point where de-identification has occurred. This will occur following initial data analysis.

Ethical clearance and contacts

This study has been cleared in accordance with the ethical review processes of CSIRO within the guidelines of the National Statement on Ethical Conduct in Human Research. If you have any questions concerning your participation in the study, feel free to contact the researchers involved peter.caley@csiro.au. Alternatively, any concerns or complaints about the conduct of the study can be raised with CSIRO's Manager of Social Responsibility and Ethics by email at csshrec@csiro.au or on (07) 3833 5693.

Would you like to participate in the survey?

Please select an answer.

Yes

No

Outline of the proposed study and methods of analysis

General

This exercise is aiming to estimate the risk of significant impacts to populations of waterbirds that rely on fish as a non-trivial component of their breeding diet, arising from changes to levels of survival of juvenile carp caused by the release of the cyprinid herpes virus (CyHV-3).

A number of waterbird species are dependent to various degrees on fish populations that utilise wetlands potentially impacted by the release of CyHV-3. Kingsford *et al.* (2017), for example, lists 14 species that are regularly counted within the East Australian Waterbird Surveys and are classified as piscivorous. A further group of species have diets that contain fish to a lesser degree. This exercise will seek to elicit expected impacts at a broad functional group level (detail below). While a more detailed species-level analysis could be performed, this is not tractable for large numbers of species potentially involved.

There are three key uncertainties that are not easily resolved from the available scientific literature:

1. The extent to which waterbirds currently rely on juvenile carp as part of their breeding diet;
2. Whether and by how much populations of native fish and other waterbird prey items might change in response to changes in carp populations, and
3. How waterbird population breeding success and population size will respond to possible changes in food availability or composition.

As the likely behaviour of CyHV-3 outbreaks in wild carp populations, in terms of severity, age-dependent effects, recurrence, and spatiotemporal spread, remain relatively uncertain, we explore this through a range of outbreak scenarios. A recent North American study by Thresher *et al.* (2018) revealed that the long-term impact of the virus on populations of adult carp may be difficult to detect, with a notable absence of recurrent reported die-offs of adult fish. Note, however, that this does not discount the possibility of significant mortality occurring in juvenile carp, which could possibly be an important food source for breeding Australian waterbirds.

This exercise uses an expert elicitation approach to address the current uncertainties. It asks experts to provide estimates about the impact of CyHV-3 outbreak scenarios on aspects of waterbird population dynamics. The scenarios include a range of “cues” relating to the impact of the virus on juvenile carp survival in space and time. We ask the experts to provide their best judgements about changes to waterbird populations occurring using their own implicit weighting and combination of the cues. Regression techniques will be used to relate the scenario covariates to the expert’s responses. This will produce a model over the population of experts. Thus, the expert’s views about a finite set of cases can be extended to a broader range of situations in regard to how the outbreak may unfold if the virus is released.

The framing of these scenarios and each expert’s task in assessing them is still complicated. The risks posed to waterbird populations by a reduction in juvenile carp survival can depend on a sequence of events stretching over a number of years, and in various locations. Australian waterbird species are highly mobile — a waterbird species may successfully undergo a major recruitment event in a location not impacted by CyHV-3 (e.g. either carp not present or the virus not present). The numerical response between waterbird abundance and fish abundance is not well known in general, so what effect curtailing the survival of juvenile carp during spawning events will have on waterbird breeding success and recruitment is unclear. In a recent global search of the literature (McGinness *et al.* 2019) found no studies describing consumer (e.g. waterbird) responses to mass mortality events of fish in freshwater ecosystems. Likewise, the numerical response of other fish species and waterbird prey items to reduced juvenile carp recruitment is uncertain — will it be partially of fully compensatory (or better), and over what time frames will this potentially occur?

In summary, the waterbird system is dynamic and variable in space and time, and the effect of the proposed release of CyHV-3 in terms of changes to the food web is uncertain, as is the importance of juvenile carp to the breeding success of waterbirds.

What follows is some brief background material on these key issues. As an identified expert, we assume you probably know considerably more about some or all of the topics covered, but this material helps standardise the knowledge base across the group of experts.

Background information

Biomass of carp in waterways

Carp now occur in virtually all catchments within the Murray-Darling Basin, and in a number of other coastal catchments (Figure 72. Modelled estimates of Carp biomass density (kg/ha) across a) river systems and b) waterbodies of eastern Australia. Different colours reflect the variation in density of Carp. Reproduced from Stuart *et al.* (2019).

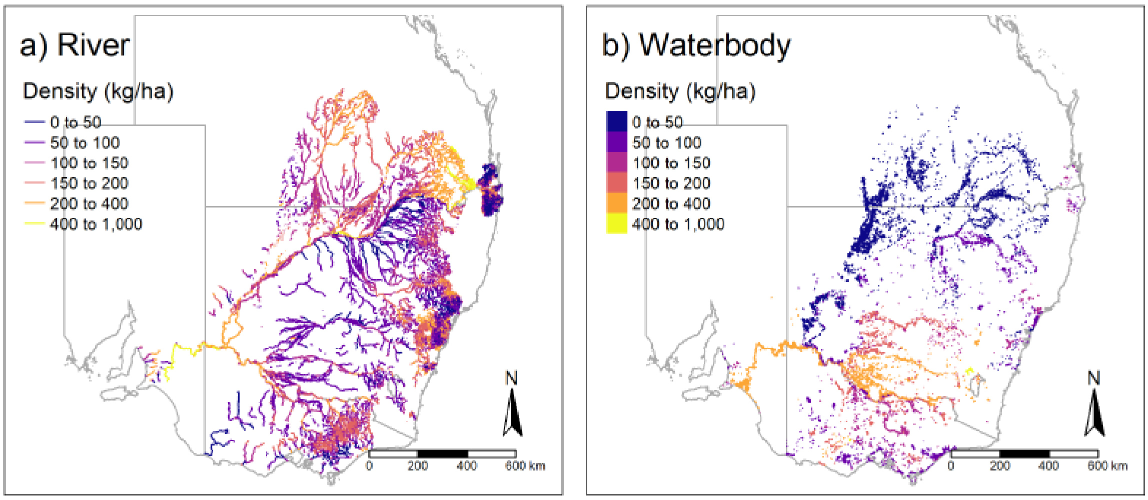

Figure 72. Modelled estimates of Carp biomass density (kg/ha) across a) river systems and b) waterbodies of eastern Australia. Different colours reflect the variation in density of Carp. Reproduced from Stuart *et al.* (2019).

Carp in the diet of waterbirds

There are very few studies that have examined the diet of waterbirds in the presence of the current high and pervasive biomass of carp (McGinness *et al.* 2019). Unsurprisingly given the high proportion of biomass in many waterways that is made up of carp, anecdotal observations and speculation is that carp may make up a large proportion of the fish consumed by piscivorous birds. Published data are, however, scarce. In one more contemporary study, Taylor and Schultz (2008) document the predominance (>90%) of juvenile carp in the diet of the great egret (*Ardea modesta*), in this instance assumed to be sourced from rice paddies and irrigation canals.

Response of native fish to lowered carp abundance

Carp may compete with or impact the habitat of native fish, including bony herring, freshwater catfish, silver perch, Australian smelt, carp gudgeon and flathead galaxias, though the extent of competition is not well quantified. Gehrke *et al.* (2010) reported that large zooplankton, such as Boeckella and Daphnia, increased by more than 10-fold following a 50% reduction in carp within a number of experimental billabongs, and the number of aquatic insects and crustacean species also increased strongly. Similarly, Robertson *et al.* (1997) illustrated the potential of carp predation to alter aquatic micro- and macro-invertebrate communities. It has been noted that carp at all stages of ontogenetic development eat microcrustacea, which are also the primary food source of all larval and most juvenile native freshwater fish species. Kopf *et al.* (2018) used an allometric approach to estimate that the native fish biomass could increase more than twofold in the Murray-Darling Basin if carp populations are reduced to 30% of total fish community biomass. The plasticity in carp's spawning timing gives them a competitive advantage over other native species. Being able to spawn early in a breeding season, their larvae and juveniles gain access to food earlier than native species, which are generally constrained by temperature to spawn later.

Epidemiology of cyprinid herpes virus in carp populations

The epidemic behaviour and impact of CyHV-3 in free-living carp populations in Australia is uncertain, as overseas information either comes from highly intensive aquaculture (fish farms) or freshwater systems with substantially different characteristics (e.g. Thresher *et al.* 2018). For these reasons, for this exercise we are defining the outcomes (epidemic mortality, recurrence interval and synchrony) of the virus release on the survival of juvenile carp arising from carp spawning events (hence saving the expert the difficulty of considering the uncertainty as to what may happen). A separate research project within the NCCP is conducting more detailed assessments of how the virus is likely to behave if released, and these results will be used in conjunction with the results of this exercise to inform the risk assessment.

Things you want to know but aren't specified

There will be other information that an expert may feel they need to perform an assessment. Examples may include the exact size of the carp spawning event being impacted by the virus, the size and occurrence of other spawning events elsewhere, what's happening within the (carp free) Lake Eyre basin, Coastal regions and so on. It is impossible to include such detail, and still be able to undertake an elicitation without having to subject participants to literally thousands of scenarios (to cover off on all the potential variables and their interactions). If there is uncertainty about a particular feature of a scenario the expert should, where possible, average over these uncertain components to produce an expected response. We accept that this may be difficult on some occasions but ask the expert to do this as well as they can.

A note about **botulism**. We ask that you assume that any carp fish kill arising from the release of CyHV-3 **will not** result in an outbreak of botulism. We will clearly state the results based on your elicited opinion are conditional on this assumption.

How will the results be analysed?

Briefly, we will fit a mixed-effects regression model relating the estimated changes to breeding success and population outcomes to predictor variables. The factor "Expert" will be added to the model as a random effect (de-identified). The parameter settings in the scenarios have been chosen to achieve a design that best estimates the parameters and parameter combinations of interest. This approach to elicitation, termed "Point of truth calibration" (Brookes *et al.* 2017), accepts that different experts may have different views — there is no need to seek consensus among participants. The analyses will summarise the expected changes across the group of experts, as well as the overall effect for the scenarios. Once a model that emulates the expert judgement has been developed, it will be used to estimate the effect sizes for new situations, including uncertainty. Note it is possible for the predictions arising from the model of expert judgements to be highly uncertain. That is, if there is no consistent model across the experts, then the predictions will reflect this uncertainty. We will transparently communicate this in our reporting of the results.

General

In no way in this process do we seek to trivialise the complexity of waterbird dynamics to uncertain changes in the food web, or to claim that this approach addresses all risks to waterbirds arising from the proposed CyHV-3 virus release, or that the approach should replace well designed research. The project seeks to summarise current expert opinion in a way that can be used to inform the broader ecological risk assessment, including a number of the Significant Impact Criteria pertaining to assessing Matters of National Environmental Significance under the EPBC Act. We have used this approach in a range of biosecurity problems and its results have always been useful. Additional targeted work is being undertaken separately to manage key risk exposure pathways arising from the proposed release.

Waterbird population risk assessments

For this study we will be providing you with a number of scenarios outlining possible impacts of releasing cyprinid herpes virus (CyHV-3) on the number of juvenile carp recruited from carp spawning events.

Estimating changes to risk endpoints

In these assessments, we ask you, for the given scenario of changes to the number of surviving juvenile carp, to provide your estimate (compared to baseline expectations) of short-term and long-term changes to: (1) Waterbird breeding success; (2) Local waterbird abundance; and (3) Total waterbird abundance. Note these measures are chosen to align with Significant Impact Criteria for assessing Matters of National Significance under the EPBC Act. The measures we ask you to elicit are detailed in Table 1.

Table 1: Risk end-point definitions

Elicitation measure	Temporal scale	Definition
Change to waterbird breeding success	Initial virus outbreak coinciding with major waterbird breeding event	Expected change to the success of a major waterbird breeding event (from baseline expectation) for a typical wetland within the region subject to the initial virus-induced reduction in juvenile carp survival (severity as described in the scenario).
	Longer-term (> 5 years)	Expected change to waterbird recruitment (from baseline expectation) for a typical wetland within the region following the initial then ongoing virus-induced reduction in juvenile carp survival (severity as described in the scenario).
Change in local abundance of waterbird population	Initial virus outbreak coinciding with major waterbird breeding event	Expected change in the abundance of waterbirds within a typical wetland within the region (from baseline expectation), subject to the initial virus-induced reduction in juvenile carp survival (severity as described in the scenario).
	Longer-term (> 5 years)	Expected longer-term change (from baseline expectation) in the abundance of waterbirds within a typical wetland within the region, subject to both the initial then ongoing virus-induced reduction in juvenile carp survival (severity as described in the scenario).
Change to total population size of waterbirds	During initial virus outbreaks coincident with major waterbird breeding events	Expected short-term change (from baseline expectation) in the total (Australia-wide) population of waterbirds potentially utilising wetlands subject to initial carp virus release (severity as described in the scenario).
	Longer-term (> 5 years)	Expected longer-term change (from baseline expectation) in total (Australia-wide) population of waterbirds potentially utilising wetlands subject to ongoing carp virus activity (severity as described in the scenario).

A note on your change estimates

We will ask you to provide your estimate of the expected change in either breeding or population size as a percentage change. If you are more familiar with the finite rate of increase/change (often termed λ), Table

2 provides some examples of equivalence. Note that **any** changes are admissible — the values given in Table 2 are for illustrative purposes only.

Table 2: Brief description of interpretations for example changes presented as either a Percentage change, with the equivalent finite rate of increase

Percentage change	Equivalent finite rate of increase (λ)	Interpretation
-90%	0.1	Population/Breeding reduces by 90% (e.g. from 100 to 10)
-80%	0.2	Population/Breeding reduces by 80% (e.g. from 100 to 20)
-50%	0.5	Population/Breeding halves
-20%	0.8	Population/Breeding reduces by 20% (e.g. from 100 to 80)
0%	1.0	Population/Breeding doesn't change
+20%	1.2	Population/Breeding increases by 20% (e.g. from 100 to 120)
+50%	1.5	Population/Breeding increases by 50% (e.g. from 100 to 150)
+100%	2.0	Population/Breeding doubles
+200%	3.0	Population/Breeding triples

Summary of Variables used in Scenarios

Each scenario contains a set of variables with different values available for each variable. These variables are summarised in Table 3.

Table 3: Scenario variables and their possible values

Variable	**Values**
Functional group	Predominantly piscivorous Variably piscivorous
Wetland region	Southern basin Northern basin
Initial severity/reduction in juvenile carp survival during spawning events [below current baselines]	90%* 50%* 25%*
Ongoing severity/reduction in juvenile carp survival during spawning events [below current baselines]	90%* 50%* 25%*
Proportion of juvenile carp spawning events affected	100% (Every event) 50% (Every second event)
Epidemic synchronisation (within region)	Yes (Perfectly synchronous) No (Unsynchronized)
*Please assume that juvenile carp killed by the virus are **not** available to waterbirds	

Additional variable details

Functional group

There are a range of classifications of water birds into functional groups, used for a range of purposes (e.g. Kingsford *et al.* 2017). Here we are primarily interested in waterbird utilising freshwater habitats, and the extent to which species are reliant on fish whilst breeding. They are:

PREDOMINANTLY PISCIVOROUS — breeding diet approaching 100% reliance on fish. These include the 14 species functionally classified by Kingsford *et al.* (2017) as Piscivores. There are: crested grebe, Australian pelican, darter, great cormorant, pied cormorant, little black cormorant, little pied cormorant, silver gull, whiskered tern, gull-billed tern, Caspian tern, crested tern, lesser crested tern, and white-winged black tern.

VARIABLY PISCIVOROUS — breeding diet typically contains fish at non-trivial level (e.g. yellow spoonbill), that in certain circumstances could be high (e.g. breeding populations of Great Egret).

Wetland region

SOUTHERN BASIN — including Lachlan Catchment southwards & westwards

NORTHERN BASIN — including Macquarie & Bogan Catchments northwards

See https://www.mdba.gov.au/discover-basin/Landscape/geography.

Epidemic recurrence (Proportion of carp spawning events affected over time)

The proportion of major carp spawning/recruitment events within a wetland experiencing a CyHV-3 outbreak (epidemic) over time. A value of 100% would mean all carp spawning events experience a CyHV-3 epidemic (and associated mortality). A value of 50% would mean that on average every second major spawning event at a wetland will be subject to an epidemic. We don’t explore values below 50% as lower values (e.g. 25% or 1 in 4 carp spawning events impacted) would be unlikely to achieve any useful biocontrol of the carp population.

Epidemic synchronisation (within region)

The degree to which CyHV-3 outbreaks in waterbodies within a region are synchronised. For example, if 50% of major carp spawning events are affected (i.e. on average every second spawning event at a wetland suffers a virus outbreak), then these virus outbreaks would be synchronous across wetlands within the region (e.g. either all major spawning events affected during one year, or none are affected). In the case of no synchronous epidemics, there is no spatial synchrony in carp spawning events experiencing virus outbreaks.

Current best knowledge points to outbreaks of infection being driven by aggregations of carp when the water temperature is within the permissive range (21–250C). Within a region (e.g. Southern basin), water temperatures and hydrology are likely similar, meaning synchronous outbreaks may be possible (once the virus is disseminated through the population).

It should be possible at virus release to choose whether to release the virus concurrently (leading to synchronised outbreaks) or not. However, once the virus has disseminated through the population (and persisting with latently infected fish), subsequent outbreaks will be triggered by local conditions, ranging from synchronous (water temperatures permitting) to asynchronous.

Other supporting material follows References

References (please ask if you would like copies emailed to you)

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Example scenario

Consider Scenario L1

Elicitation in words

"Consider a scenario where the released CyHV-3 reduces the number of juvenile carp available to waterbirds by **50% initially**, and by **50%** during all (**100%** of) **subsequent** carp spawning events that are **synchronous** within sub-basin regions.

For a typical wetland in the **southern basin**, thinking about the breeding success and population dynamics of a **predominantly piscivorous** bird species. What do you estimate the change will be to:

- **Breeding success** during an **outbreak of initial severity** coincident with a major breeding event?
- **Breeding success** over the **longer-term** (>5 years)?
- Resident **waterbird abundance** following the **outbreak of initial severity** coincident with a major breeding event?
- Resident **waterbird abundance** over the **longer-term** (>5 years)?

Thinking more broadly (Australia-wide) about the total population size of this **predominantly piscivorous** bird species when CyHV-3 the carp virus behaves in the manner described across the entire range of carp, what do you estimate the change will be to:

- The **total population size** of waterbirds potentially over the **short-term** (1-2 years)?
- The **total population size** of waterbirds over the **longer-term** (>5 years)?"

Entering values

An example of a completed scenario with fictitious (made up) entries is provided in Table 4.

Note to enter a percentage decline you need to precede the value with the minus sign ("-"). To help you, the spreadsheet is colour-coded to have decreases in red and increases in green.

Table 4: Example scenario fictitious entries (circled). Cells coloured yellow are for populating with what you judge to be your best estimate of the percentage change in the measure being elicited

Scenarios Set 1 with example													
Scenario Code	Virus severity (reduction in juvenile carp survival)		Virus recurrence (per carp spawning event) and regional synchrony		Basin region	Piscivory level of waterbird group in question	Your best estimate of % change to waterbird breeding success locally?		Your best estimate of % change to local waterbird population size?		Your best estimate of % change to global waterbird population size?	
	Initial outbreak	Subsequent outbreaks (>5 yrs)	Proportion of spawning events affected	Regional synchronisation of carp virus outbreaks			During initial virus outbreak coincident with major	Longer-term (>5 yrs)	Following initial virus outbreak coincident with major	Longer-term (>5 yrs)	Following initial virus outbreaks coincident with major breeding events	Longer-term (>5 yrs)
H2	50	25	Every event	Yes	Northern	Variably						
I1	50	50	Every second event	No	Southern	Predominantly						
I2	50	50	Every second event	No	Southern	Variably						
J1	50	50	Every second event	Yes	Northern	Predominantly						
J2	50	50	Every second event	Yes	Northern	Variably						
K1	50	50	Every event	No	Northern	Predominantly						
K2	50	50	Every event	No	Northern	Variably						
L1	50	50	Every event	Yes	Southern	Predominantly	-20%	-10%	-5%	0%	-5%	0%
L2	50	50	Every event	Yes	Southern	Variably	-5%	5%	0%	10%	0%	5%
M1	90	50	Every second event	No	Southern	Predominantly						
M2	90	50	Every second event	No	Southern	Variably						
N1	90	50	Every second event	Yes	Northern	Predominantly						
N2	90	50	Every second event	Yes	Northern	Variably						
O1	90	50	Every event	No	Southern	Predominantly						
O2	90	50	Every event	No	Southern	Variably						

Survey scenarios — things that may help (also repeated in Questionnaire Spreadsheet)

Notes on scenarios

- Scenarios that are identical other than for different functional levels of piscivory have the same letter though different suffix (A1, A2, etc.).
- Assume that juvenile carp killed by the virus **are not** available to waterbirds
- For longer-term scenarios assume the virus (CyHV-3) has spread throughout the carp distribution.
- Scenarios generally trend from lowest to highest in terms of impact on juvenile carp survival. You might find it useful to apply your judgement initially to both lowest and highest
- For any particular elicitation, it is helpful to think on how low you realistically think the value could be, following by how high you realistically think the value could be, before arriving at your value of best judgement.
- If you are unable to make an estimate simply leave the cell blank.

Factors you may (or may not) consider – (by no means exhaustive)

- Breeding success — reliance on juvenile carp as part of the diet of the functional group in question?
- Breeding success — the numerical response between waterbird recruitment and juvenile carp abundance (if a significant prey item). What reduction (if any) in the survival of juvenile carp is possible for the food requirements of a waterbird breeding population to remain adequate (i.e. satiated)
- Food web changes — the extent (and speed) to which native prey species for piscivorous waterbirds may recover in response to reduced carp juvenile survival and recruitment
- Total water bird population dynamics — What proportion of the total population of waterbirds are exposed to changes in availability of juvenile carp, whether recruitment from other wetland systems without carp or without CyHV-3 occurs, and to what extent recruitment into the adult population is density-dependent.

CONTACT US

t (1300 363 400
(+61 3 9545 2176
e (csiroenquiries@csiro.au
w (http: //www.csiro.au

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w (http: //www.csiro.au/businessunit

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Insert contact name
t (+61 0 0000 0000
e (first.last@csiro.au
w (http: //www.csiro.au businessunit

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Insert contact name
t (+61 0 0000 0000
e (first.last@csiro.au
w (http: //www.csiro.au businessunit

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Tel: 02 6285 0400
Post: Locked Bag 222, Deakin West ACT 2600

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