Introductory section common to all papers

The National Carp Control Plan (NCCP) is being developed to examine and make recommendations about the feasibility of using a virus to assist in controlling common carp in Australia. The plan is to be developed by December 2019.

This issues paper is one of seven prepared to summarise topics central to the NCCP's development, provide updates on emerging research results, and, where relevant, situate NCCP research within the broader context of scientific literature. Some papers within the series are intended primarily to provide background information or updates, whereas others seek stakeholder input to help shape development of the National Carp Control Plan document. An NCCP engagement report will be completed and published summarising stakeholder input.

The papers draw on results from the NCCP research program, the broader scientific literature, and stakeholder knowledge. Paper topics are:

i. Why and how did the National Carp Control Plan originate?
ii. What is science telling us about the potential use of the carp virus as a biological control agent for carp?
iii. Non-target species susceptibility testing and host-switching risk in carp biocontrol
iv. Water quality and carp biocontrol using Cyprinid herpesvirus 3 (CyHV-3)
v. Managing water quality impacts by carcass management/clean up.
vi. Understanding potential social and economic impacts of carp control
vii. Genetic biocontrol and common carp (provided as final report)

Each of the papers can be read in sequence or singly. Many of the important questions and challenges associated with carp control are multidisciplinary and multifaceted, so cross-referencing between papers is used to direct readers towards more detailed discussions of a particular topic, or to Frequently Asked Questions (FAQs) on the NCCP website (http://www.carp.gov.au/FAQ), when necessary.

Common or European carp (*Cyprinus carpio*, referred to simply as 'carp' in these papers) are an introduced pest fish common throughout a large area of Australia. When carp are abundant, they can damage aquatic ecosystems in several ways, generating environmental, economic and social costs. Carp control initiatives in Australia are therefore based on the general premise that reducing carp numbers below the densities at which they cause environmental damage could result in improved environmental, social, and economic outcomes. While there is evidence for environmental improvements following carp control, these may not eventuate in all ecosystems, follow uniform transition pathways from the 'pre-control' to 'carp controlled' states, or be achieved without activities to address other, non-carp impacts.

Issues Paper 2. What is science telling us about the potential use of the carp virus as a biological control agent for carp?

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1.0. About this paper

This paper summarises and contextualises knowledge emerging from epidemiological modelling under the National Carp Control Plan (NCCP), and from the scientific literature addressing carp virus epidemiology more broadly. By clarifying how the virus is likely to transmit among, and impact upon, Australian carp populations, this knowledge will help to determine whether the carp virus could be a safe and effective carp control option for Australia. If virus release proceeds, knowledge developed from this research will also contribute to development of virus release strategies that maximise effectiveness against carp populations and manage risks. Importantly, the epidemiological modelling results presented here are preliminary; the virus transmission experiments described in section 3.0 could have important implications for carp knockdown, and the research still needs to undergo independent expert review. These preliminary insights are presented to provide stakeholders with early insights into the modelling process and its results.

2.0 Defining epidemiology

Assessing the carp virus's viability as a carp control tool in Australia relies upon the veterinary/medical discipline called epidemiology. The practitioners of this discipline, epidemiologists, study the mechanisms by which disease transmits through, and affects, populations. The *British Medical Journal* defines epidemiology as '…the study of how often diseases occur in different groups of people and why' (note that in this definition, 'animals' could equally be substituted for 'people'). A defining feature of epidemiology is its focus on understanding disease and its impacts at the population level. However, because disease

outbreaks are made up of infections occurring in individuals, epidemiologists must also use knowledge from other branches of medicine or veterinary science that study levels of organisation finer than the population (for example, in cells, tissues, and organs).

Most epidemiologists work on disease prevention and control, in either animal or human populations. However, biological control programs in which the control agent is a pathogen (disease-causing organism) or parasite also use epidemiological knowledge to maximise control effectiveness (McColl et al., 2014). Regardless of whether a program aims to prevent disease in valued populations, or to use it as a pest control or eradication tool, there are four broad approaches to epidemiological research (Thrusfield, 2007). A particular study may follow one of these broad approaches but, more often, elements of some or all may be employed over the course of an investigation. These general approaches can be classified (from Thrusfield, 2007) as:

- *Descriptive (or observational) epidemiology*, which involves observing disease occurrence and recording possible causative factors. A descriptive approach is often used in the initial stages of an epidemiological study to identify potentially useful pathways for further investigation.
- *Analytical epidemiology*, which uses statistical and diagnostic procedures to interpret and explain observations
- *Experimental epidemiology*, which involves the controlled application of various factors to groups of animals such that the effects of those factors in disease transmission, infection, and progression can be distinguished from other, complicating (called 'confounding') variables and assessed. Occasionally, features of the study population may allow 'natural experiments'. For example, isolated cattle populations in the Channel Islands helped epidemiologists identify that the causative agent of bovine spongiform encephalopathy ('mad cow disease') was entering herds through contaminated feed rather than through animal-to-animal contact.
- *Theoretical epidemiology*, in which mathematical models are used to represent, explore, and manipulate the behaviour of environments, the populations that live within them, and the diseases occurring within these contexts.

3.0 Epidemiology in the National Carp Control Plan

3.1 Why we need to use a modelling approach

The NCCP has commissioned an epidemiological modelling project to improve understanding of carp virus dynamics in Australian ecosystems and carp populations. Because it employs a modelling approach, this study fits into the broad category of theoretical epidemiology. However, the modelling process uses data and information collected using analytical, descriptive, and experimental approaches. A modelling approach was selected for the NCCP's epidemiological research because it enabled exploration of virus behaviour in complex, interconnected carp sub-populations, and in river catchments displaying differing flow and temperature regimes. Designing experiments that could encompass the variation through space and time inherent in these complex systems would be costly, time-consuming, and technically difficult. Additionally, the carp virus must remain in a biosecure laboratory until all legislative approvals necessary for its release into the Australian environment have been granted. This restriction limits the potential for experimental research investigating virus

behaviour in natural ecosystems (although some aspects of viral biology can still usefully be investigated in controlled laboratories). In contrast, modelling enables manipulation of various parameters within the modelled system to understand their effects on outcomes. For example, the effects of different carp population densities, water temperatures, flow regimes, and long-term climatic variability (e.g. El Nino vs La Nina years) on the virus's population-level impacts can be modelled. Furthermore, data collected by the NCCP's carp biomass estimation project will be incorporated into the models to improve the realism and usefulness of model predictions.

3.2 Balancing complexity and simplicity in modelling

Natural systems are complex, with many interacting components. Determining the extent to which this complexity needs to be incorporated into a model in order to generate useful predictions is a key decision in the modelling process (Grassly and Fraser, 2008). While simple models can often provide the most useful guides to thinking, oversimplification risks excluding variables that could be important in determining outcomes of interest. Conversely, adding too many variables can increase complexity, making model behaviour difficult to interpret (i.e. the modeller loses the ability to explain why the model has produced particular outputs) (Grassly and Fraser, 2008). In the biological sciences, modelling is often most useful as a guide to thinking rather than as a source of definitive answers (Ögüt, 2001). Expressing interactions between the components of natural systems in mathematical terms forces researchers to think clearly about the nature of these interactions, and thereby highlights areas where understanding is poor and additional data collection may be required. By enabling exploration of diverse scenarios, models can also prompt the development of new hypotheses (testable ideas about the way in which a system works) for investigation (Ögüt, 2001).

3.3 An integrated series of models

While referred to throughout these issues papers as 'epidemiological modelling', this component of the NCCP research program actually consists of a hierarchical series of models incorporating water temperature, inundation and hydrology (water level and river flow) data, habitat suitability metrics (which determine carp abundance) for both juvenile and adult carp, carp aggregation, carp demography (i.e. the processes that occur within populations to drive growth or decline), and epidemiology. Carp biomass estimates generated by the NCCP biomass project integrate into this hierarchy by providing a cross-validation tool for carp abundance estimates generated by the habitat suitability modelling component. Conceptually, the hydrological, habitat suitability, and demographic models create the 'stage' or 'playing field' upon which carp virus epidemiology plays out. Just as the conditions of a sporting field can influence the outcome of a game played on it, so too do these environmental and demographic factors influence the outcome of introducing the virus into carp populations.

3.4 Explaining data-driven modelling

Epidemiological modelling in the NCCP has adopted a 'data-driven' approach. In data-driven modelling, large volumes of real-world data are searched for underlying connections between system variables (Solomatine et al., 2008; Toh and Platt, 2013; Pfeiffer and Stevens, 2015). Data-driven approaches, often termed 'big data', contrast with more traditional, hypothesis-driven approaches to science, which set out to test pre-defined ideas about how the study

system works (Solomatine et al., 2008; Pfeiffer and Stevens, 2015). Data-driven approaches to modelling, in epidemiology and more broadly, have been enabled by increases in both computing power and the prevalence of automated data collection through sensors and other devices connected to the internet (i.e. 'the Internet of Things') (Toh and Platt, 2013). To illustrate the data volumes processed in these studies, an investigation of drugs used to treat angioedema (swelling in deeper skin layers) analysed a sample of 100 million people and 350 million person-years of observation (Toh and Platt, 2013). Because data-driven approaches don't rely on pre-determined ideas about how the study system works, they can be useful when studying complex phenomena that are not amenable to formulation of generalised hypotheses (Pfeiffer and Stevens, 2015). For example, the outcome of interest may have many possible causes (Pfeiffer and Stevens, 2015).

Two examples of data-driven approaches in the NCCP modelling are the delineation of river reaches and identification of carp sub-populations that comprise relatively discrete (i.e. non-mixing) units through time. Disease transmission in complex populations spread across large geographic areas requires understanding connectivity between subpopulations (Parratt et al., 2016). Therefore, it was essential that connectivity between river reaches, and the carp populations inhabiting them, was modelled in a biologically-realistic way. Modellers used a data-driven approach based on river flows to delineate river reaches. Using a data-driven approach in this instance meant that river reaches are defined by a biologically-meaningful attribute rather than being subjectively defined by researchers. Additionally, the code and algorithms used can be applied to any catchment of interest, removing the need to define reaches on a catchment-by-catchment basis. Subsequently, river flow data and carp movement data were combined using a data-driven approach to identify carp subpopulations. In other words, data on river flow and carp movements were allowed to 'speak for themselves' to indicate where and how carp sub-populations could meet and mingle in the real world. In these two examples, data-driven approaches allow a more robust and realistic representation of the real system than could have been achieved if modellers had imposed pre-determined ideas of how the system should work onto the data.

The ability of data-driven approaches to identify previously-unseen patterns has led to predictions that big data will render hypothesis-driven research obsolete (Anderson, 2012). Such claims are, however, hyperbolic (Chiolero, 2013; Pfeiffer and Stevens, 2015). While large datasets can undoubtedly yield new insights, and usually do produce robust conclusions, they are not immune to the problems that can beset observational data in epidemiology (Chiolero, 2013). Observational data are those collected when researchers observe the world as it naturally occurs, rather than under controlled experimental conditions. Big datasets are usually observational in nature.

Confounding is a problem of particular concern to epidemiologists working with observational data, including big data. A confounding variable occurs together with a potential cause of disease, and influences the disease outcome, thereby complicating interpretation of the relative importance of different disease causes. For example, in an epidemiological study investigating the relationship between alcohol consumption and heart disease, smoking would be an important confounding factor, because smoking and alcohol consumption commonly occur together, and both can cause heart disease. To understand the effects of alcohol on heart disease in isolation, investigators would need to 'control' for the effects of

smoking. Confounding can occur in datasets of any size, but some authors have argued that data-driven approaches, with their lack of pre-specified hypotheses and flexible analytical approaches, may be less likely to detect it unless caution is used (Chiolero, 2013). Fortunately, statistical techniques that overcome confounding and other data integrity issues in data-driven approaches are increasing in sophistication, and provide opportunities to exploit the benefits of a larger sample while avoiding potential pitfalls (Toh and Platt, 2013).

Ultimately, data-driven modelling provides epidemiologists with another tool for understanding disease in populations. Uncritical acceptance of big data in epidemiology is as misguided as its outright rejection (Toh and Platt, 2013). Rather, the challenge is to identify ways in which large data-sets can be rigorously assembled and creatively combined to yield new insights into complex systems. In the particular case of the NCCP, data-driven modelling provided the only realistic option for answering applied questions about a system characterised by diverse habitat types, a broadly-distributed and highly-variable target population, and numerous data and knowledge gaps.

3.5 How does the modelling capture variation across carp's Australian distribution?

Modelling the entire Australian distribution of carp would be prohibitive in terms of data requirements and computing power. Therefore, the modelling focuses on five representative catchments (see Table 1).

Table 1: Catchments modelled for combined hydrological, carp habitat suitability, demographic, and epidemiological modelling in the NCCP

Catchment	State(s)	Reason for selection
Lachlan River	NSW	Contains a diversity of habitat types, and the Lachlan's carp populations were thoroughly studied between 2007 and 2009.
Moonie River	Qld	A representative catchment for warmer, higher-temperature habitats in the northern portion of carp's distribution. Additionally, the Moonie River's native fish populations have been well-studied.
Lower Murray River	SA	Carp populations in the lower Murray have been well-studied, providing useful background information for modelling.
Mid Murray River	Vic-NSW	A representative high-flow river; well-studied for carp.
Glenelg River	Vic	A representative coastal river with a geographically-isolated carp population. Additionally, a recent carp movement and population study provided useful background for modelling.

For the catchments listed in Table 1, carp populations, river flows, and climatic conditions have been reconstructed for time intervals of 16 – 28 years (depending upon data availability in each study catchment) enabling modellers to examine the effects of virus introduction under a range of environmental conditions. Against this backdrop of environmental and demographic variability, different epidemiological scenarios relating to transmission rates and mortality in carp populations can be assessed to obtain an understanding of virus behaviour in the highly-variable conditions characteristic of Australian environments and carp populations. Inclusion of this variability is a key feature of the modelling, as insights into carp virus dynamics gained from controlled laboratory experiments will not always be applicable to natural environments (Becker et al., 2018).

The modelling allows exploration of virus impacts on carp populations over 5 – 10 years following virus release. Total reduction in carp density ('total knockdown') is the primary outcome considered by the modelling. However, the extent to which the virus reduces carp populations below threshold densities at which environmental damage may occur (see Issues Paper One for further discussion) is also considered. Because the threshold densities at which damage occurs will vary between ecosystems, and even within an ecosystem through time (see Issues Paper One), three threshold densities – 50, 100, and 150 kg ha^{-1} – were used.

3.6 Applying the modelling

Preliminary epidemiological modelling results have reshaped thinking on likely carp virus disease dynamics in Australian carp populations. When the NCCP began, expectations were for major carp mortalities and rapid disease transmission across large geographic extents (i.e. an epidemic). These ideas accorded with international reports of large carp kills, particularly in aquaculture (e.g. Haenen et al., 2004), and with the high mortalities reported by the initial CSIRO laboratory trials (see issues paper one).

Epidemiological modelling, combined with analysis of Japanese and north American experience with the carp virus, has revealed that a self-propagating carp virus epidemic is unlikely in Australian ecosystems. Rather, outbreaks killing a high proportion of the carp present in an aggregation are only likely when a specific set of climatic and carp behavioural factors coincide. These results suggest that the virus may best be used as a targeted control agent, focussing on schooling (aggregating) carp during relatively narrow seasonal windows of opportunity.

High carp densities are the first of two primary preconditions for a carp virus outbreak. High densities are important because physical contact between infectious and susceptible carp is probably the most effective transmission route for the carp virus (Raj et al., 2011). Infectious individuals are those that have the virus, and are capable of infecting others, while susceptible individuals do not have the virus and are vulnerable to infection. In addition to direct physical contact between infectious and susceptible carp, transmission may also occur when carp contact virus particles either floating in the water column or adhering to sloughed skin cells, mucous, faeces or other materials or organisms (Minamoto et al., 2009; Rakus et al., 2013). Epidemiological modelling in the NCCP currently assumes that transmission requires physical contact. However, experiments testing the relative importance of the direct physical contact and 'water-borne' (including vectoring on mucous and other secretions) transmission pathways are underway. If these experiments indicate that horizontal transmission involving water or other vectors (e.g. shed mucous or other secretions) plays a significant role in virus transmission, this pathway will be included in epidemiological modelling.

The experiments investigating the relative importance of physical contact and horizontal transmission through the water are important, because they will enable modellers to refine their estimates of effective contact rates (denoted by the Greek letter β, 'Beta'), one of the most fundamental rates in epidemiology. The effective contact rate is obtained by multiplying the total number of contacts between infectious and susceptible individuals per unit time, regardless of whether they result in infection, by the risk of infection inherent in these encounters. Effective contact rate is often an important determinant of a disease outbreak's outcome, so obtaining the best possible estimate for this rate is important (e.g. Nkamba et al., 2019).

Water temperature is the second major precondition for an outbreak. The water temperature range within which the carp virus causes disease in carp (the 'permissive range') is variously cited as 18 - 28°C (Michel et al., 2010; Gotesman et al., 2013; Rakus et al., 2013) and 16 - 26°C (Hanson et al., 2016; see discussion in Becker et al., 2018). When carp were infected with the virus in a laboratory at 22°C and maintained at that temperature for 24 hours, followed by a temperature reduction to 11°C over four days, there were no mortalities, no clinical signs of

disease, and no production of infectious virus, but the virus remained physically present in the infected carp (Sunarto et al., 2014). Above 30°C viral replication ceases and the virus becomes harmless to carp (Boutier et al., 2019). Experience with international outbreaks indicates that there is a narrower temperature range, variously cited as 22 - 24°C (Becker et al., 2018; Thresher et al., 2018) and 22 – 26°C (Hanson et al., 2016) that is optimal for infection and disease. Thus, an effective CyHV-3 outbreak probably requires carp to be in close physical contact with each other, and for water temperature to be within the permissive range.

Water temperatures throughout much of carp's Australian distribution tend to rise rapidly through the permissive temperature range in spring or early-mid summer, with the exact timing varying regionally. Rising water temperatures and increasing day lengths in spring and summer are the environmental cues for carp spawning (Smith and Walker, 2004). When responding to these cues, carp migrate towards spawning sites in shallow wetlands, leading to high carp densities at in-stream obstacles like weirs, and in spawning habitats (Smith and Walker, 2004; Stuart and Jones, 2006; Stuart and Conallin, 2018). These spring/summer spawning events provide the carp densities necessary to trigger an outbreak, and tend to occur when temperatures are in the permissive range. Thus, virus release would probably involve actively targeting migrating or spawning carp for infection. As aggregations disperse, the intensity of carp-to-carp contacts necessary to sustain transmission will probably break down, resulting in fade-out of the outbreak. That is, the effective contact rate will reduce below that necessary to sustain the outbreak.

Likely carp knockdown was modelled for a range of virus transmission scenarios. The most biologically-plausible scenarios result in substantial suppression of carp populations in the 5 – 10 years following release. Scenarios with lower effective contact rates, or in which infections that have become latent under non-permissive temperatures (see Box 1 text at end of paper) do not subsequently reactivate with onward transmission, result in lower knockdowns.

These insights into likely carp virus epidemiology in Australian ecosystems indicate how the virus might be used to reduce carp populations while managing risks. The value proposition for biological control often lies at least partly in the self-propagating nature of many biocontrol agents; following release, the agent spreads naturally through the target population, and does its job of reducing numbers with minimal ongoing management (Saunders et al., 2010). In aquatic ecosystems, however, a self-propagating biocontrol agent that causes high mortality rates in the target organism could be a double-edged sword. While such an agent would likely be effective at reducing the target organism's abundance, cleaning up the dead carp resulting from epidemics would potentially be challenging. Because epidemiological modelling for the carp virus is indicating that outbreaks will be geographically-restricted, and only likely when carp aggregation and permissive water temperatures coincide, there may be opportunities to use the carp virus in a targeted way to control carp populations in key areas where densities are above thresholds for ecological damage.

Targeted use of the carp virus could be implemented within broader strategies ranging from staged to broadscale release scenarios. The former option (staged) would involve deploying the virus sequentially in selected locations, while the latter would involve coordinated,

approximately simultaneous, release in multiple locations, within the latitudinal constraints imposed by permissive water temperatures (i.e. recognising that permissive conditions won't occur at the same time across carp's whole Australian range).

The epidemiological and logistical implications of different release strategy options are being considered by NCCP researchers and advisory groups. While a staged approach intuitively seems to allow more focussed use of resources through time, retaining a strong 'surge capacity' to deploy clean-up crews at short notice if unexpected carp kills did occur would still be necessary, so total resourcing requirements may be similar for both the staged and broadscale options over the medium – long terms. Similarly, even a broadscale approach would likely be preceded by one or more 'pilot' releases in relatively contained areas (such as a coastal catchment), to enable operators to refine techniques and 'learn by doing'. Therefore, it is perhaps most accurate to envision the 'staged' and 'broadscale' release approaches as occurring on a continuum, rather then being discrete, mutually-exclusive options.

Carp damage threshold analyses further support a release strategy that actively targets locations with high carp densities for virus release. In two of the study catchments (the Moonie and Glenelg), carp densities rarely exceeded the lower damage threshold of 50 kg ha^{-1}, even without virus release. In the lower and mid Murray catchments, where carp densities were often above the lower threshold for environmental damage, virus release reduced densities below the damage threshold. In the Lachlan catchment, some carp subpopulations were above the lowermost damage threshold and some exceeded it. In the latter, virus release reduced populations below the damage threshold. Thus, targeting locations with high carp densities for virus release is likely to produce relatively greater environmental benefits than a strategy that aimed to introduce the virus into all carp populations, regardless of their density. Additionally, the higher-density populations are most likely to provide the level of carp-to-carp physical contact required to sustain an outbreak capable of killing a high proportion of the carp present. Thus, targeting high-density populations for release may provide an opportunity to maximise both carp knockdown and environmental benefits.

4.0 Can the virus suppress carp populations?

Some scientists have argued that CyHV-3 is unlikely to drive long-term carp population declines (Becker et al., 2018; Marshall et al., 2018; Thresher et al., 2018; Boutier et al., 2019; Kopf et al., 2019). Thresher et al. (2018) reviewed carp abundance before and after carp virus outbreaks in north America, and found that carp had only undergone a sustained decline in one of the five study locations. Kopf et al. (2019) argue that the genetic diversity and patchy distribution of wild carp populations could, in combination with the variable physico-chemical regimes characteristic of natural ecosystems, limit mortality from the virus. Marshall et al. (2018) and Kopf et al. (2019) also note that CyHV-3 may have co-evolved with carp over long periods, and that some Australian carp could consequently possess, or rapidly acquire, resistance to the virus.

Water temperature's central role in carp virus disease dynamics has also led to questions about the virus's effectiveness as a biocontrol agent in the context of the variable water temperature regimes characteristic of many Australian freshwaters. Becker et al. (2018) note that numerous carp will likely be infected by the virus as water temperatures pass through the margins of the permissive range (i.e. as temperatures rise through the upper limits of the permissive range in late spring/early summer, or fall back through its lower limits in autumn). Becker et al. (2018) posit that some of these individuals will survive, developing latent or subclinical infections, and partial, temperature-induced immunity. These individuals could, in turn, expose susceptible carp to the virus at temperatures sub-optimal for disease, leading to the rapid development of herd immunity (Becker et al. 2018). Herd immunity occurs when susceptible individuals are protected from infection by the high proportion of immune, non-infectious individuals around them (Fine, 1993). To develop their arguments, Becker et al. (2018) draw on water temperature data collected below four large dams in the Murray-Darling Basin, observations of carp virus disease and prevalence in Lake Biwa, Japan, and the Australian experience with another fish virus, the iridovirus called Epizootic haematopoietic necrosis virus (EHNV).

The development of immunity resulting from infections occurring outside, or on the margins of, the permissive range differs from the development of true genetic resistance driven by natural selection (Becker et al., 2018). Selection for resistance to a disease occurs because, within any population of organisms, there are individuals who possess genes that make them either resistant to infection by a given pathogen, or capable of defeating disease following initial infection (Lipsitch and Sousa, 2002; Karlsson et al., 2014). When a population encounters a new pathogen for the first time, these resistant individuals remain unaffected, or only develop non-serious disease, while the broader population to which they belong experiences high mortality or debilitating disease that reduces reproductive success (Karlsson et al., 2014). Resistant individuals have more opportunity to reproduce, so the genes that code for resistance spread through the population (Lipsitch and Sousa, 2002; Karlsson et al., 2014). Eventually, most of the population becomes resistant to the disease (Karlsson et al., 2014).

While the basic concept of natural selection is relatively simple, the manner in which selection by infectious diseases plays out in real populations is usually quite complex. This complexity arises because not only does the pathogen apply selective pressure to the host (i.e. by killing non-resistant individuals, or reducing their reproductive success), but the pathogen also

changes (evolves) in response to selective pressures, particularly those related to optimising transmission (Di Giallonardo and Holmes, 2015). Adding to this complexity, the time taken for resistance to develop through natural selection depends upon the proportions of resistant and non-resistant individuals in the population, and the extent to which the resistance genes confer protection against disease (i.e. whether protection is complete or partial) (Lipsitch and Sousa, 2002). Predicting how long genetic resistance will take to emerge for a given host-pathogen system is therefore complex.

Epidemiologists are currently modelling the likely emergence of genetic resistance to the carp virus among Australian carp populations. Initial results indicate that resistance is likely to emerge over relatively long timescales (e.g. 10 – 30 years). Consequently, the overarching strategy for carp biocontrol should involve development and implementation of other carp control measures to capitalise on virus-induced population suppression.

Apart from the role ascribed to water temperature as a driver of herd immunity in the framework of Becker et al. (2018), there are likely to be many Australian freshwater habitats in which temperature rises rapidly through the permissive range in spring and early summer, and remains too high for reliable infection for extended periods. Temperature regimes like this will occur in Qld and northwestern NSW, and in shallow wetlands throughout carp's Australian distribution. High temperatures also create the possibility of 'behavioural fever', wherein infected carp actively seek out warmer water to deactivate virus (Rakus et al., 2017). At the opposite end of the temperature spectrum, coldwater pollution (release of cold water from the deep layers of dams, properly termed 'hypolimnetic release') could result in extended river reaches where temperatures remain below the permissible level for CyHV-3 (Kopf et al., 2019). Coldwater pollution is both more spatially restricted (i.e. to the river reaches below dams) and amenable to management than are high temperatures, but will nonetheless need to be considered when developing CyHV-3 release strategies (Kopf et al., 2019). Consequently, the carp virus may be a targeted, rather than broadscale, biocontrol agent.

5.0 Conclusions

Safe and effective carp biocontrol requires understanding the carp virus's likely behaviour in, and impacts upon, on Australian carp populations, then using this knowledge to develop virus release strategies that maximise effectiveness and manage risks. These goals are essentially challenges in applied epidemiology. Epidemiological research almost always requires knowledge of the study population's abundance, density, and distribution through space and time, so carp biomass estimates are important to this, and many other, facets of the NCCP research program.

The epidemiology of infectious diseases is always contingent upon host demography and behaviour, and upon the physical and climatic features of the environments in which disease plays out. Therefore, a key feature of epidemiological modelling in the NCCP has been developing a sound environmental and carp demography context within which disease dynamics can be explored. In particular, reconstruction of carp populations over extended time period (ranging from 16 – 28 years, depending upon study catchment) provides a powerful tool for assessing the likely impacts of the virus on the carp populations.

Modelling, combined with insights from the broader scientific literature, indicates that the carp virus is only likely to cause major outbreaks when water temperatures in the permissive range combine with carp aggregation events. This concurrence of events is most likely when carp are aggregating to spawn in spring and early summer. Carp aggregation events are important in triggering outbreaks because direct physical contact between carp appears to be the most effective virus transmission pathway, although further work is required to confirm this. The picture of carp virus disease dynamics that has emerged from the epidemiological modelling described here indicates that the carp virus would best suit a highly-targeted virus release strategy, involving identification of aggregation locations and subsequent targeted infection.

Biological control is often favoured as a management approach because biocontrol agents tend to be self-perpetuating within pest populations, and therefore require relatively little management intervention (and hence little expenditure) beyond monitoring (Saunders et al., 2010). In contrast, the epidemiological modelling described here is indicating that the carp virus will require active identification and targeting of carp aggregations for infection. Carp damage threshold analyses support a targeted approach, focussing on areas where carp densities are sufficiently high to cause ecological damage, and aiming to suppress populations below the damage threshold. Targeting areas with high carp densities also increases the likelihood of successfully triggering an outbreak, as the intensity of physical contact between carp necessary to sustain transmission is more attainable where carp are abundant. A targeted release approach may mean that clean-up activities can be more focussed, and potential water quality impacts more manageable, than initially expected, but that effective carp control could require active management over longer timeframes than are usual for a biological control program. Additionally, maintaining capacity to respond to unexpected carp kills will be essential.

Some scientists have raised concerns that carp virus disease dynamics will lead to rapid development of herd immunity. Such an outcome depends upon the capacity of carp with chronic productive or latent infections to transmit the virus to susceptible carp under non-permissive conditions, effectively immunising them. Epidemiologists are currently working with international experts on the carp virus to better understand the potential emergence of this kind of immunity. Further information on carp virus epidemiology, and its implications for virus release, will be provided in updates as work proceeds through 2019.

References

Anderson, C., 2008. The end of theory: The data deluge makes the scientific method obsolete. *Wired Magazine*. Web article https://www.wired.com/2008/06/pb-theory/ last accessed 23/04/2019.

Becker, J.A., Ward, M.P. & Hick, P.M. (2018) An epidemiologic model of koi herpesvirus (KHV) biocontrol for carp in Australia. *Australian Zoologist* DOI: https://doi.org/10.7882/AZ.2018.038

Boutier, M., Donohoe, O., Kopf, R.K., Humphries, P., Becker, J., Marshall, J.C. & Vanderplasschen, A. (2019). Biocontrol of carp: the Australian plan does not stand up to a rational analysis of safety and efficacy. *Frontiers in Microbiology* doi: 10.3389/fmicb.2019.00882 (article provisionally accepted for publication).

Boutier, M., Ronsmans, M., Rakus, K., Jazowiecka-Rakus, J., Vancsok, C., Morvan, L., Peñaranda, M.D., Stone, D.M., Way, K., van Beurden, S.J., Davison, A.J. & Vanderplasschen, A. (2015). Cyprinid herpesvirus 3, an archetype of fish alloherpesviruses. *Advances in Virus Research* 93, 161 – 256. doi: 10.1016/bs.aivir.2015.03.001.

Chiolero, A. (2013). Big data in epidemiology: too big to fail? [letter to the editor]. *Epidemiology* 24, 938 – 939.

Di Giallonardo, F. & Holmes, E.C. (2015). Viral biocontrol: grand experiments in disease emergence and evolution. *Trends in Microbiology* 23, 83 – 90. doi:10.1016/j.tim.2014.10.004.

Eide, K.E., Miller-Morgan, T., Heidel, J.R., Kent, M.L., Bildfell, R.J., LaPatra, S., Watson, G. & Jin, L. (2011). Investigation of koi herpesvirus latency in koi. *Journal of Virology* 85, 4954 – 4962. doi:10.1128/JVI.01384-10

Fine, P.E.M. (1993). Herd immunity: history, theory, practice. *Epidemiologic Reviews* 15, 265 – 302.

Gotesman, M., Kattlun, J., Bergmann, S.M. & El-Matbouli, M. (2013). CyHV-3: the third cyprinid herpesvirus. *Diseases of Aquatic Organisms* 105, 163 – 174. doi: 10.3354/dao02614

Grassly, N.C. & Fraser, C. (2008). Mathematical models of infectious disease transmission. *Nature Reviews Microbiology* doi:10.1038/nrmicro1845.

Grinde, B. (2013). Herpesviruses: latency and reactivation – viral strategies and host response. *Journal of Oral Microbiology* 5, 22766. DOI: http://dx.doi.org/10.3402/jom.v5i0.22766

Haenen, O.L.M., Way, K., Bergmann, S.M. & Ariel, E. (2004). The emergence of koi herpesvirus and its significance to European aquaculture. *Bulletin of the European Association of Fish Pathologists* 24, 293 – 307.

Hanson, L., Doszpoly, A., van Beurden, S., Viadanna, P. & Waltzek, T. (2016). Alloherpesviruses of fish. In *Aquaculture Virology* (Kibenge, F.S.B & Godoy, M.G. eds), pp. 153 – 172). San Diego: Academic Press.

Karlsson, E.K., Kwiatkowski, D.P. & Sabeti, P.C. (2014). Natural selection and infectious disease in human populations. *Nature Reviews Genetics* 15, 379 – 393. doi:10.1038/nrg3734.

Koehn, J.D., Todd, C.R., Zampatti, B.P., Stuart, I.G., Conallin, A., Thwaites, L. & Ye, Q. (2018) Using a population model to inform the management of river flows and invasive carp (*Cyprinus carpio*). *Environmental Management* 61, 432 – 442. DOI 10.1007/s00267-017-0855-y

Kopf, R.K., Boutier, M., Finlayson, C.M., Hodges, K., Humphries, P., King, A., Kingsford, R.T., Marshall, J., McGinness, H.H., Thresher, R. & Vanderplasschen, A. (2019) Biocontrol in Australia: can a carp herpesvirus (CyHV-3) deliver safe and effective ecological restoration? *Biological Invasions* https://doi.org/10.1007/s10530-019-01967-1(0123456789().,-volV() 0123458697().,-volV).

Lipsitch, M. & Sousa, A.O. (2002). Historical intensity of natural selection for resistance to tuberculosis. *Genetics* 161, 1599 – 1607.

Marshall, J., Davison, A.J., Kopf, R.K., Boutier, M., Stevenson, P. & Vanderplasschen, A. (2018) Biocontrol of invasive carp: risks abound. *Science* 359, 877.

McColl, K.A., Cooke, B.D. & Sunarto, A. (2014). Viral biocontrol of invasive vertebrates: lessons from the past applied to cyprinid herpesvirus-3 and carp (*Cyprinus carpio*) control in Australia. *Biological Control* 72, 109 – 117. http://dx.doi.org/10.1016/j.biocontrol.2014.02.014

Michel, B., Fournier, G., Lieffrig, F., Costes, B. & Vanderplasschen, A. (2010). Cyprinid herpesvirus 3. *Emerging Infectious Diseases* 16, 1835 – 1843. DOI: 10.3201/eid1612.100593

Minamoto, T., Honjo, M.N., Uchii, K., Yamanaka, H., Suzuki, A.A., Kohmatsu, Y., Iida, T. & Kawabata, Z. (2009). Detection of cyprinid herpesvirus 3 DNA in river water during and after an outbreak. *Veterinary Microbiology* 135, 261 – 266.

Nkamba, L.N., Manga, T.T., Agouanet, F. & Manyombe, M.L.M. (2019). Mathematical model to assess vaccination and effective contact rate impact in the spread of tuberculosis. *Journal of Biological Dynamics* 13, 26 – 42. DOI: 10.1080/17513758.2018.1563218

Ögüt, H. (2001). Modeling of fish disease dynamics: a new approach to an old problem. *Turkish Journal of Fisheries and Aquatic Sciences* 1, 67 – 74.

Parratt, S.R., Numminen, E. & Laine, A-L. (2016). Infectious disease dynamics in heterogenous landscapes. *Annual Reviews in Ecology and Systematics* 47, 283 – 306. 10.1146/annurev-ecolsys-121415-032321

Pfeiffer, D.U. & Stevens, K.B. (2015). Spatial and temporal epidemiological analysis in the big data era. *Preventive Veterinary Medicine* 122, 213 – 220. http://dx.doi.org/10.1016/j.prevetmed.2015.05.012.

Raj., V.S., Fournier, G., Rakus, K., Ronsmans, M., Ouyang, P., Michel, B., Delforges, C., Costes, B., Farnir, F., Leroy, B., Wattiez, R., Melard, C., Mast, J., Lieffrig, F. & Vanderplasschen, A. (2011). Skin mucus of *Cyprinus carpio* inhibits cyprinid herpesvirus 3 binding to epidermal cells. *Veterinary Research* 42, http://www.veterinaryresearch.org/content/42/1/92 (4 August 2011).

Rakus, K., Ouyang, P., Boutier, M., Ronsmans, M., Reschner, A., Vancsok, C., Jazowiecka-Rakus, J. & Vanderplasschen, A. (2013). Cyprinid herpesvirus 3: an interesting virus for applied and fundamental research. *Veterinary Research* 44, http://www.veterinaryresearch.org/content/44/1/85.

Rakus, K., Ronsmans, M., Forlenza, M., Michiels, T., Wiegertjes, G.F. & Vanderplasschen, A. (2017) Conserved fever pathways across vertebrates: a herpesvirus expressed decoy TNF-α receptor delays behavioural fever in fish. *Cell Host & Microbe* 21, 244 – 253. http://dx.doi.org/10.1016/j.chom.2017.01.010

Reed, A., Izume, S., Dolan, B., LaPatra, S., Kent, M., Dong, J. & Jin, L. (2014). Identification of B cells as a major site for cyprinid herpesvirus 3 (CyHV-3) latency. *Journal of Virology* 88, 9297 – 9309.

Reichert, M., Lukasik, A., Zielenkiewicz, P., Matras, M., Maj-Paluch, J., Stachnik, M. & Borzym, E. (2019). Host microRNA analysis in cyprinid herpesvirus-3 (CyHV-3) infected common carp. *BMC Genomics* 20, https://doi.org/10.1186/s12864-018-5266-9.

Saunders, G., Cooke, B., McColl, K., Shine, R. & Peacock, T. (2010). Modern approaches for the biological control of vertebrate pests: an Australian perspective. *Biological Control* 52, 288 – 295. doi:10.1016/j.biocontrol.2009.06.014

Serquiña, A.K. & Ziegelbauer, J.M. (2016). How herpesviruses pass on their genomes. *Journal of Cell Biology* 216, 2611 – 2613. https://doi.org/10.1083/jcb.201708077

Smith, B.B. & Walker, K.F. (2004). Spawning dynamics of common carp in the River Murray, South Australia, shown by macroscopic and histological staging of gonads. *Journal of Fish Biology* 64, 336 – 354. doi:10.1046/j.1095-8649.2004.00293.x

Solomatine, D., See, L.M. & Abrahart, R.J. (2008). Data-driven modelling: concepts, approaches and experiences. In *Practical hydroinformatics: computational intelligence and technological developments in water applications* (Abrahart, R.J., See, L.M. & Solomatine, D.P., eds), pp. 17 – 30. Berlin: Springer Heidelberg Berlin.

Stuart, I.G. & Conallin, A.J. (2018) Control of globally invasive common carp: an 11-year commercial trial of the Williams' cage. *North American Journal of Fisheries Management* DOI: 10.1002/nafm.10221

Stuart, I., Fanson, B., Lyon, J., Stocks, J., Brooks, S., Norris, A., Thwaites, L., Beitzel, M., Hutchison, M., Ye, Q., Koehn, J. & Bennett, A. (2019). *A National Estimate of Carp Biomass for Australia*. Draft report to the Fisheries Research and Development Corporation, National Carp Control Plan.

Stuart, I.G. & Jones, M. (2006) Large, regulated forest floodplain is an ideal recruitment zone for non-native common carp (*Cyprinus carpio* L.). *Marine and Freshwater Research* 57, 333 – 347.

Sunarto, A., McColl, K.A., St. J. Crane, M., Schat, K.A., Slobedman, B., Barnes, A.C. & Walker, P.J. (2014). Characteristics of cyprinid herpesvirus 3 in different phases of infection: implications for disease transmission and control. *Virus Research* 188, 45 – 53. http://dx.doi.org/10.1016/j.virusres.2014.03.024

Thresher, R.E., Allman, J. & Stremick-Thompson, L. (2018) Impacts of an invasive virus (CyHV-3) on established invasive populations of common carp (*Cyprinus carpio*) in North America. *Biological Invasions*, DOI: https://doi.org/10.1007/s10530-017-1655-2

Thrusfield, M. (2007). *Veterinary Epidemiology Third Edition*. Blackwell Publishing, Oxford, United Kingdom.

Toh, S. & Platt, R. (2013). Is size the next big thing in epidemiology? *Epidemiology* 24, DOI:10.1097/EDE.0b013e31828ac65e

Xu, J-R., Bently, J., Beck, L., Reed, A., Miller-Morgan, T., Heidel, J.R., Kent, M.L., Rockey, D.D. & Jin, L. (2013). *Journal of Virological Methods* 187, 372 – 379. http://dx.doi.org/10.1016/j.jviromet.2012.11.015

Box 1 text: Viral latency explained

Latency and subclinical infection are virologically distinct but, in the particular context of carp biocontrol, have similar epidemiological implications. In virology, 'latency' refers to a strategy used by some viruses, including herpesviruses, to hide from their host's immune system when conditions are unsuitable for active viral replication (Reed et al., 2014; Serquiña and Ziegelbauer, 2016). The exact mechanism viruses use to establish and maintain latency within an infected host varies between viral families (Serquiña and Ziegelbauer, 2016). In herpesvirus latency, the virus forms a circular genetic element called an episome that hides inside host cells, thereby avoiding discovery and attack by the host immune system. Episomes multiply along with the host cells during normal host cell division, but do not replicate by 'hijacking' the host cells (see Issues Paper One for an explanation of viral replication). When conditions again become suitable for the virus to hijack host cells (for example, the host immune system

becomes weakened), the virus emerges from latency and active replication recommences (Reed et al., 2014; Serquiña and Ziegelbauer, 2016). This active replication phase is called the 'lytic' cycle, because this is when the replicating virus particles either 'lyse' (burst open), or bud off from infected cells (Grinde, 2013). Thus, herpesviruses have a latent phase, when the virus is hiding in host cells, and a lytic phase, when the virus is actively replicating (Reed et al., 2014; Boutier et al., 2015; Reichert et al., 2019). Infectious virus is not produced during latent herpesvirus infection, a generalisation that, based on laboratory trials, appears to extend to the carp virus (Sunarto et al., 2014; Hanson et al., 2016).

In contrast to latency, subclinical infection does not involve sequestration of the virus in an episome. Rather, the virus continues to replicate in host cells, but does so at low levels that do not cause clinical signs of disease, and does not 'aggravate' the host immune system into an aggressive response (Grinde, 2013; Sunarto et al., 2014). Thus, subclinical infections are a 'toned down' lytic infection (Sunarto et al., 2014). Subclinical infections are also termed 'chronic productive' infections, because they are persistent through time (chronic) and involve viral replication (so they 'produce' new virus particles).

Carp virus infection can undoubtedly follow a trajectory that is highly indicative of latent and/or subclincal infection. Diseased carp recover when temperatures move out of the permissive range, yet continue to test positive for virus presence, and may subsequently re-develop lytic (and sometimes fatal) infections, with onward transmission to susceptible carp, when temperatures re-enter the permissive range (Sunarto et al., 2014; Boutier et al., 2015). Whether these characteristics indicate true latency, or persistent subclinical infection has not been completely resolved (Michel et al., 2010; Sunarto, 2014). A gene important in controlling latency in mammalian herpesviruses has not been found in fish herpesviruses, potentially indicating chronic productive infection rather than true latency (Sunarto et al., 2014). Conversely, there is evidence that carp white blood cells could be the location where latent virus 'hides' from the host immune system (Michel et al., 2010; Eide et al., 2011; Xu et al., 2012; Reed et al., 2014). Regardless of whether the carp virus exhibits true latency or chronic productive infection, carp in this phase of infection do not appear to produce infectious virus (Sunarto et al., 2014).