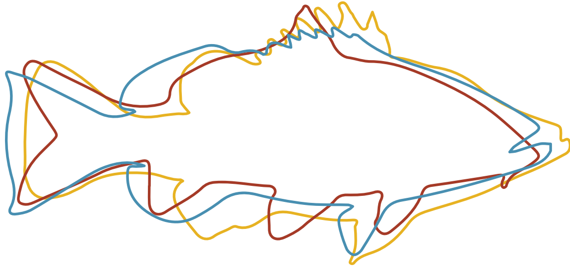
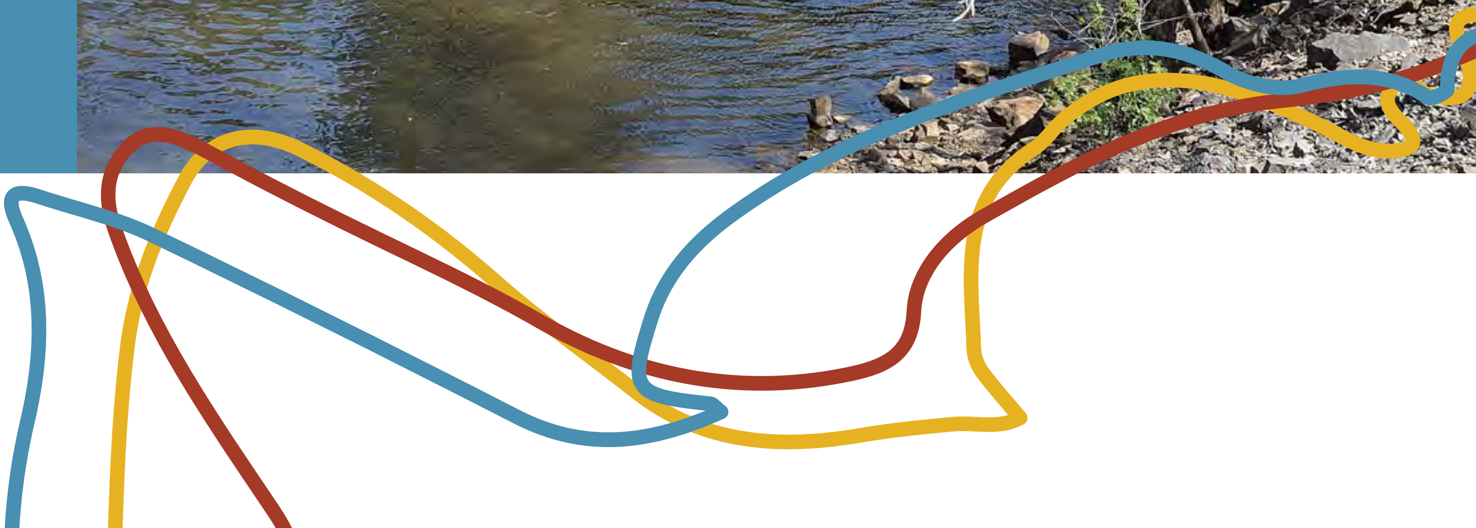
RESEARCH **14** **2017-237**

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



NATIONAL CARP CONTROL PLAN

Risks, costs and water industry response



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
    Appendix 4: Socio-economic impact assessment—tourism sector
    Appendix 5: Stakeholder interviews
    Appendix 6: Socio-economic impact assessment—native fish breeders and growers
    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)

National Carp Control Program

Risks, costs and water industry response

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Executive Summary

The Commonwealth Government has funded a National Carp Control Plan (NCCP) that is considering and evaluating a number of control measures including the release of a strain of Cyprinid Herpes Virus (CyHV-3). The plan includes aspects of research, consultation and economic assessment necessary to adequately addresses risks, including development of complementary control measures and management actions. The large fish kills that would result from a successful virus release presents significant concerns, including impacts on drinking water quality from dead and decaying carp near offtakes or water storages; water infrastructure; aquatic ecosystems; the logistics and costs involved in a large clean-up effort; and the need for further control measures.

There are numerous risks to water utilities if the virus is released in the Murray-Darling Basin (MDB). One of the top risks that were identified in a workshop held in early 2017 was whether the NCCP would negatively affect the ability of utilities to provide high quality drinking water, especially for regional towns. It was unclear whether current treatment plants would have the capacity to remove the organic compounds or taste and odour issues associated with a mass mortality event in the main river channel and whether disinfection would still be manageable. An additional concern was whether any residual by-products might be formed which increased the risk to public health.

Preliminary source water quality impact was investigated by incubating different concentrations of carp biomass in River Murray water in open vessels. This experiment highlighted the potential for measurable impacts on raw water quality even at a low carp biomass. The work warranted further investigation of the impact of decaying carp biomass on the ability of water treatment processes to minimise or eliminate any detrimental effects on water quality.

This report details the water treatment strategy presented by mass carp mortality, considering a range of potential densities based on the most recent NCCP assessments and recently collected data. Conventional alum coagulation, with and without PAC dosing, together with disinfection (chlorine and monochloramine) was investigated. Membrane fouling potential was also briefly considered. Additionally, the impact on product water quality from the perspective of aesthetics and disinfection by-products (regulated and novel) was explored, resulting in recommendations for optimising treatment through appropriate dose rates of coagulant and activated carbon products. The major findings were:

- Two indicator parameters were identified as characteristic of carp-impacted water; free ammonia and peak T_2 fluorescence, related to the presence of the amino acid tryptophan. Fluorescence has potential application as an online sensor, allowing for early detection of carp derived products at the water treatment plant inlet.

- Realistic carp densities between 0.05 and 0.10 kg/m^3, equivalent to 200-400 kg/ha, could be effectively treated by better optimising coagulation for DOC removal, with minimal changed required in disinfectant demand or DBPs.

- Higher carp densities between 0.10 and 0.50 kg/m^3 (400- 2000 kg/ha) could not be treated effectively with coagulation alone and required 30 minutes PAC contact, dosed at levels appropriate for DOC removal (up to 50 mg/L). Although more unidentified DBPs were formed, suggesting some potential for increased health risk, currently regulated DBPs were controlled comfortably within ADWG recommendations.

- Extreme carp densities greater than 1.0 kg/m^3 (4000 kg/ha+) produced high quantities of DOC and ammonia that would be acutely toxic to all aquatic life, along with highly offensive odours. This would result in water that is untreatable using existing treatment infrastructure and could

not realistically meet drinking water guidelines. In this unlikely situation, the only logical strategy would be to cease treatment and avoid access until source water quality improves.

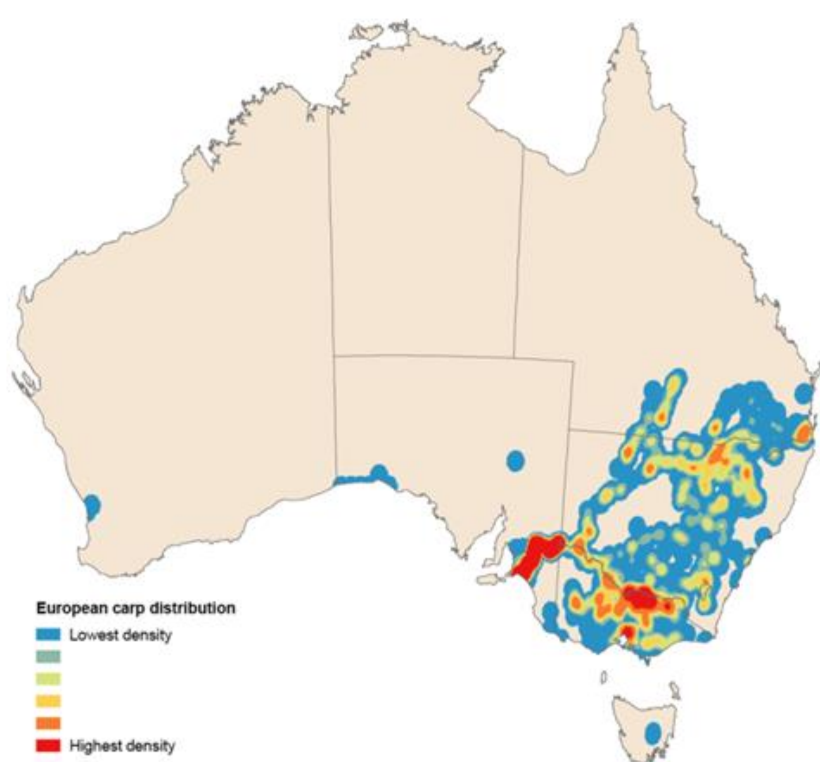
In summary, at currently modelled average carp densities, management of drinking water quality and continued public health protection should be within the capability and capacity of current treatment plant technologies; however, targeted virus delivery and carp clean up strategies will still be important to prevent the extreme scenario and minimise effects on both the river environment and source water quality.

Introduction

1.1 Background and Industry Need

The Commonwealth Government has recently announced the development of a National Carp Control Plan (NCCP) that proposes the release of the Cyprinid herpes virus 3 (CyHV-3) into various bodies of water in Australia, including the Murray- Darling Basin (MDB). It is estimated that carp (*Cyprinus carpio L.*) represent more than 80 percent of the total fish biomass in the Murray Darling Basin; while the distribution and relative density of the species is well documented (Figure 1), the absolute biomass in the MDB has not been substantiated with any real accuracy and estimates range from 0.01 kg/m^2 to 0.3 kg/m^2 (10-3000 kg/ ha Laws et al., 2016). Performing some simple calculations allow us to estimate that the biomass of carp between Lock 4 and the Barrages is between 700 and 21,000 tonnes. The potential mass death of this quantity of fish raises a number of concerns (e.g. impacts to aquatic ecosystems, tourism) but of particular relevance to the water industry, is the potential impact to water quality and issues associated with water treatment and supply.

Figure 1: Distribution of Carp (Cyprinus carpio L.) in Australia (Argent, 2016)



A number of questions can be posed to describe the potential risks to water supply including: Do water treatment plants have the capacity to remove the taste and odour associated with decomposing carp? Will the effectiveness of disinfection be compromised by the load of additional carp-derived organic compounds? What is the potential for the virus to be inadvertently transferred to reservoirs that are supplemented from the River Murray via aqueducts or pipelines? Will the virus be transferred to wastewater lagoons with significant carp populations? What are the potential negative impacts on our business of community perceptions of water tainted by a herpes virus?

The survival of CyHV-3 through water treatment process has been of public concern (Reperant et al., 2016), especially for people within aquarium businesses. Currently, there is no evidence concerning zoonotic transfer of CyHV-3 to humans; the virus being highly specific to carp. Regarding other aquatic life, a recent CSIRO study (McColl et al., 2016) demonstrated that CyHV-3 was not infectious across Australian native fish

(13 species tested, including Murray Cod and various species of perch, eel, catfish and rainbowfish). Additionally, the non-native rainbow trout as well as yabbies were also found to be not susceptible. Other studies have shown that Goldfish are not affected by CyHV-3 (Michel et al., 2010; Matsui et al., 2008). However, Koi carp are highly susceptible.

It is a common practice in Australia to maintain an adequate disinfectant residual throughout the distribution system to ensure water quality for public consumption. Viruses are effectively disinfected by chlorinated water with moderate (0.2-0.5mg/L as Cl_2) chlorine residual within the distribution system which is sufficient to inactivate any CyHV-3 that may survive the treatment process (Kasai et al., 2005). Kitajima et al. (2010) observed 4 log inactivation of waterborne viruses in drinking water from 0.5mg/L chlorine residual after just 30 seconds contact. Therefore, under normal treatment processes and disinfection practices, drinking water poses a minimal risk to aquarium businesses and fish hobbyists. However, questions regarding removal of tastes and odours, as well as additional loads on existing treatment processes remain.

The NCCP has commissioned research to investigate risks associated with these potential issues, under the guidance of research and strategic management steering groups for impacted Australian states. The main purpose of these steering groups is to provide advice to the NCCP to inform the process of assessing the feasibility of CyHV-3 as a biocontrol agent, and development of a detailed strategic management plan for consideration by government as the first stage of a multi-stage assessment and planning process. Given the integrated nature and complexity of the biological, ecological, economic, and social factors involved, the NCCP Research program, must consider a range of outcomes and activities. Examples of the factors involved include a range of carp biomass densities, variable effectivity of the virus depending on the demographics of the carp population, and a range of effort that may be committed to the clean-up operation (removal of dead carp before decomposition), in order to effectively mitigate identified risks. To effectively inform risk assessment and management planning, investigative direction must be clearly and logically defined as part of this project. This includes informed representations by the water industry in answer to the following:

1) at what residual carp concentration water supply operations are untenable and 2) what the likely additional costs associated with the residual carp concentrations below this threshold are.

1.2 Current State of Industry Knowledge

To date, few experiments have been conducted on the impact of rotting carp on water quality (Pera et al., 2018; Carney et al., 2019), and none have investigated impacts on water treatment. Ecological experiments conducted as part of the NCCP research program focused on dissolved oxygen and phosphorus dynamics (Hipsey et al., 2019). In these projects, baseline data for water quality parameter changes were gathered by introducing a range of carp densities, and at a range of ecological complexity. This allowed for inference of the response of water quality in the environment to a range of potential carp inputs, which could then be extrapolated and modelled for eight type representative water bodies. These experiments identify where mass mortality of carp may cause hypoxia and anoxia in parts of the River Murray and inform where risk mitigation efforts can most effectively be committed. Modelling of the main river channel is a difficult and complex task. Although it represents a significant simplification, one outcome suggested that high risk areas are especially associated with high biomass and shallow water and that the bulk of the channel would not become anoxic under most possible carp biomass loading scenarios. Carp biomass aggregation 'hotspots' at specific peripheral channel points were identified based on inputs including carp density (Stuart et al., 2019) and hydrological monitoring data, along with a range of additional biological and environmental inputs.

A Water New South Wales (WaterNSW) risk assessment found the potential release of the virus may have an adverse effect on water quality in storages (Pera et al., 2018; Carney et al., 2019). In the event of a large-scale fish kill because of the release of the CyHV- 3 virus within affected reservoirs, there is the potential for lowered water quality, operational impacts and a loss of public confidence as a result of decaying fish. It

was predicted that depending on the carp biomass within the reservoir there would be the potential for a significant increase in nutrients which could lead to bacterial and phytoplankton blooms, including potentially toxic species. This could then lead to various treatment impacts.

To assess the risk at different hazard levels (carp biomass loadings), a small-scale fish kill was conducted by WaterNSW to mimic the virus in outdoor mesocosms within Prospect Reservoir. Mesocosms were initially filled with ~2000 L of water in small pools that roughly equated to 0, 250, 500, 1000, 1200, 2300 and 6000 kg of dead carp per hectare (~ 0 - 1.5 kg/m^3). An extra treatment of 6000 kg/ha was used to simulate what would happen to water quality if fish were removed after 8 days to simulate a clean-up. In summary the study found the introduction of dead fish at biomass levels between 250-6000 kg/ha firstly resulted in the prevalence of an anoxic environment that persisted between 1 to 12 days. This long-term anoxic environment would have adverse effects to ecological communities and may cause a secondary kill of non-targeted fauna. This was followed by an increase in nutrients corresponding with carp biomass and then an increase in algal biomass. The algal biomass persisted for up to a month and the scale and the length of the water quality change depended on the biomass loading. Their experiments revealed large shifts in dissolved oxygen dynamics associated with both heterotrophic decomposition and nutrient impacts on phytoplankton productivity; followed by increased concentrations of taste and odour compounds, which are non-toxic but aesthetically undesirable.

Pre-project experiments conducted by SA Water in River Murray water and a range of carp densities also demonstrated the potential for serious impairment of raw water quality. These carp biomass incubation investigations conducted prior to this project (unpublished) indicated a critical parameter would be ammonia, produced from the degradation of proteins, and its significant impact on both environmental toxicity and disinfection. WTPs operating either chlorination or chloramination may need to achieve breakpoint (oxidation of ammonia to free nitrogen) in order to establish appropriate residuals. Breakpoint requires chlorine to ammonia ratios in excess of 10:1 and may easily exceed the capacity of current dosing infrastructure at many WTPs. At a biomass concentration of 2000 kg/ha, increases in the concentrations of DOC and free ammonia were approximately 14mg/L and 4 mg/L, respectively, which is of significant concern from water treatment and disinfection perspectives.

Evaluating the potential impact of a mass carp mortality event must consider a range of variables. The extant carp biomass varies considerably between water bodies (Stuart et al., 2019) and differing levels of resources are likely to be deployed for clean-up operations. In the WaterNSW study, timing of the removal of dead fish was shown to be critical to maintaining good water quality. Fish removed within the first 2-3 days of mortality had minimal to no impact on water quality when compared to leaving the fish to rot naturally. An immediate clean up response was recommended to prevent water quality impacts on the environment and water treatment. In reality, response is likely to be driven by ownership of the water body, local risks and sensitivities. Because of these practical considerations, it is possible that water treatment plants may experience residual carp concentrations varying from undetectable levels up to 1000 kg/ha (Stuart et al., 2019). To cover this range, testing of six concentrations of residual carp per water treatment configuration was undertaken in the SA Water study. These concentrations were selected to ensure adequate information was collected to inform steering committee submissions and cover the potential range of carp densities predicted within concurrent NCCP projects (Stuart et al., 2019).

1.3 Advancing Knowledge

This project was designed to advance the water industry knowledge by developing a framework to assess potential carp impacts and conducting much needed experiments into appropriate water treatment responses to mass fish mortality events. It was structured as follows:

Cost of Water Treatment Operations: These experiments were designed to estimate the amounts of additional coagulants and disinfectants and additional chemical (i.e. PAC) required per gram of residual carp, and to define what the upper tolerable threshold of residual carp will be for a range of water treatment facilities. Experimental investigation of the range of water treatment conditions was simulated

at laboratory scale as described in section 2 of this report. Investigation of the potential health and aesthetic issues included analysis of regulated and novel disinfection by-products using both quantitative analytical tests, as well as cell bioassays that provide more ‘health consequence’- based assessment precluding specific knowledge of DBPs present. For aesthetic screening of product water, the assessment was limited to odour characterisation. Although due care is taken, test samples produced within a laboratory may potentially be exposed to hazardous chemical and biological cross-contamination from other concurrent activities and are generally not accepted for taste testing to minimise any risk.

Development of Communication Material informed by the Research Outcomes: Material informing our customers of how we are proactive and engaged in the process and protecting their health, financial and aesthetic interests through an assessment of potential risks, costs, and capacity of utility response to carp biomass inputs. This includes a spreadsheet that will allow the user to estimate what the likely additional costs of water treatment will be, for a range of residual carp scenarios. A range of treatment options will be covered, including conventional coagulation/ sedimentation and coagulation with direct microfiltration, both with the option of added PAC. Because the cost of water treatment chemicals is dependent on location and individual supply contracts, these will be required user inputs to allow representative costing to be obtained. Additionally, fact sheets will be made available explaining how utilities will manage water quality risks, assist in state response action planning and information for the public about how the risks of a carp virus release will be minimised through careful management.

1.4 Project Aims

The purpose of the project was to better understand the risks to water production from large fish-kills and to ensure some effective strategies for continued drinking water treatment plant operation. Although the impact of fish mortality on product water quality and the cost of water treatment will be influenced by the interplay of a number of factors, the specific purpose of this investigation was to determine:

(a.) An indicative threshold of residual carp breakdown product that can impair product water quality in the absence of improved/enhanced/optimised water treatment.

(b.) The quantity of additional water treatment chemicals that will be required to maintain product water quality (health and aesthetic characteristics).

(c.) The threshold of carp biomass loading at which treatment is no longer effective and drinking water quality standards would be exceeded.

(d.) If carp derived disinfection by-products will be novel and pose a risk of adverse health impacts.

1.5 Glossary

Table 1: Terms used in this document

Term	Description
2-MIB	2-Methyl-isoborneol
ACH	Aluminium Chlorohydrate
ADWGs	Australian Drinking Water Guidelines
Alum	Aluminium Sulphate as $Al_2(SO_4)_3.18H_2O$
AOX	Absorbable Organic Halogen
APHA	Australian Public Health Association
DBP	Disinfection By-product
DOC	Dissolved Organic Carbon
EC_{50}	Half Maximum Effective Concentration
FEEM	Fluorescence Excitation-Emission Matrix
HAA9	Haloacetic acids (9 chloro- and bromo-substituted analogues)
HLB	Hydrophilic-lipophilic balance
HU	Hazen Units
IBCs	Intermediate Bulking Containers
MDB	Murray Darling Basin
MF	Microfiltration
MMS	Methyl Methanesulfonate
NCCP	National Carp Control Program
NDMA	N-Nitrosodimethylamine
NTU	Nephelometric Turbidity Units
PAC	Powdered Activated Carbon
PES	Poly-ether sulphone
SA Water	South Australian Water Corporation
SDS	Simulated Distribution System
SPE	Solid Phase Extraction
THM4	Trihalomethanes (4 chloro- and bromo-substituted analogues)
TKN	Total Kjeldahl Nitrogen
TOC	Total Organic Carbon
TMP	Transmembrane Pressure
uAOX	Unidentified AOX (non-regulated DBPs)
UV	Ultraviolet
VBB	Vogel-Bonner broth
Water RA	Water Research Australia
WIL2NS	Human B-lymphocyte-derived cell line
WQ	Water Quality
WTP	Water Treatment Plant

Method

2.1 Carp Concentrates

Carp concentrates were produced by incubating carp carcasses, collected from the fish trap at Lock 1, chilled in an ice slurry and frozen until use, in 1000L of River Murray water over a 14- day period. These surface water incubation experiments were conducted with partial (natural) solar exposure, in open air, but with direct overhead cover (Figure 2). It is acknowledged that sediment biota or fauna that would be present in a mesocosmtype experiment may have reduced carp degradation products through degradative metabolic pathways. Additionally, carp biomass used was from healthy fish caught in traps, which may have degraded slower than carp terminated following CyHV-3 infection and associated physical symptoms. It was intended that these preparations would represent a worst-case scenario where natural environmental processes did not attenuate the effects of the degradation. Carp concentrate was produced at biomass densities of 5.0 kg/m^3 and 0.5 kg/m^3 allowing for dilution to cover the range of realistic carp densities likely to be encountered. Carp concentration (biomass density) range was intended to be based upon the best assessments of normal environmental carp density, but also to account for significant post-mortem accumulation of fish biomass as a result of air or water currents. As a result, a broad range between 0 and 1.0 kg/m^3 was assessed.

Six carp densities were assessed to determine the relationship between WQ parameters and the carp density and whether those relationships could be described as linear or non-linear.

Figure 2: Carp concentrate production in IBCs at River Murray Lock 1

2.2 Physicochemical analyses

Determinations of UV and visible absorbance are easily performed for semi-quantification and/or characterisation of natural organic matter (NOM) and these have been widely adopted by the drinking water industry. UV absorbance at λ254nm has been extensively used as a surrogate parameter to monitor

the concentration of NOM. In addition, UV tends to give a measure of unsaturated bonds that are potential sites with which coagulants or disinfectants can react. Colour (456nm) and UV absorbance at 254 nm (UV254) were measured following filtration through a 0.45µm membrane using a 5 cm and 1 cm quartz cell respectively, on an Evolution 60 Spectrophotometer (Thermo Scientific, USA) reported as Hazen units (HU) and Abs/cm respectively. Turbidity measurements were conducted on a TU5200 Laboratory Turbidimeter (Hach, USA) with results given in nephelometric turbidity units (NTU). Dissolved organic carbon (DOC) measurement (the amount of organic material smaller than 0.45µm) has shown strong relationships with coagulant demand, chlorine demand and disinfection by-product formation. As a quantitative measure, it allows direct comparison between different samples regardless of source, location or time of sampling. Combined with other parameters such as UV λ254nm absorbance and Colour λ456nm absorbance, the character of organics can be assessed (SUVA and Specific colour). DOC was measured following filtration through a 0.45µm membrane using a Sievers 900 Total Organic Carbon Analyser (GE Analytical Instruments, USA). Conductivity was measured using a platinum cell and conductivity meter. Alkalinity was determined by titration according to Standard Method 2320B (APHA et al., 2005). Aluminium, iron and manganese were all analysed by Inductively Coupled Plasma Mass Spectrometry (Method 3125B, APHA et al., 2005). Prior to analysis, samples were filtered through a 0.45µm membrane filter and made up to the 10 mL mark with filtrate; concentrated nitric acid (100 µL) and concentrated hydrochloric acid (100 µL) was then added to the samples to dissolve.

2.3 Treatability assessments

Coagulation tests were conducted using alum, dosed as $Al_2(SO_4)_3.18H_2O$. While alternative coagulants such as ACH or ferric chloride may vary in efficiency at different applied conditions (eg. pH or temperature), the underlying mechanisms of charge neutralisation, co-precipitation and physical enmeshment are the same regardless.

WTC-Coag™ is a coagulant dose prediction model co-developed by SA Water and the University of South Australia that can provide dosing guidance for optimising DOC removal using easily obtainable raw water quality parameters. This tool provided a basis for the dose range to be used and streamline the examination by removing the need to start with broad dose range jar tests. Doses used represented 80, 85, 90, 95, 100 and 110% coagulable DOC removal predictions. Note that coagulable DOC removal is not the same as total DOC removal and excludes recalcitrant low molecular weight and uncharged fractions that do not participate in coagulation mechanisms.

The jar test conditions used a PB-900 6-paddle gang stirrer (Phipps & Bird, USA) with 1 min @ 200 rpm flash mix, 14 min @ 20 rpm slow mix and 15 min settling in 2L square form Gator Jars.Filtered water was achieved through gravity filtration using 11 µm paper filters (Whatman No.1, UK). Filtration for measurement of dissolved water quality parameters (true colour, UV absorbance and DOC) was achieved by filtration through 0.45 µm PES membrane filters (ANPEL Laboratory Technologies, China).

Assuming WTPs will address the risk of carp product effects by deliberately targeting the highest possible levels of treatment with minimal treated water impacts, the following criteria defined optimally treated water:

- Filtered turbidity <0.1 NTU
- Colour <5 HU
- UV absorbance @ 254 nm <0.090 /cm
- Zeta potential -5 mV $< \zeta < 0$ mV
- >0.02 mg/L DOC reduction per mg/L alum added (point of diminishing returns).

A limited assessment of how the carp products affect membrane filtration was conducted using stirred cell reactors and flat sheet microfiltration or nanofiltration membranes with and without pre-coagulation. These tests represented fixed pressure, dead-end filtration (100% recovery) and therefore can only assess

‘relative’ fouling potential. More complex assessments including crossflow modes, hollow fibre membranes and constant flux operation, as typically run at WTPs was outside of the scope of this project. Flux (as sample volume) was measured after 3 minutes at 50psig applied pressure following dosing at the previously identified optimum conditions and 5 minutes stirring at 200 rpm. Results were then normalised against the treated River Murray water without carp impact to produce a relative response.

2.4 Disinfection assessment

Samples were then disinfected using simulated distribution system (SDS) tests with realistic plant doses of chlorine at ambient temperature. Prior to commencement of the SDS, the 72-hour chlorine demand was calculated for each water quality. The pH of the treated water was adjusted prior to chlorination to target a pH range of 7.0 - 7.5 for chlorination and 8.2 – 8.6 for chloramination. Chlorine was dosed at a concentration to replicate the 72-hour chlorine demand + 0.5 mg/L (as residual), with chlorine concentrations measured at regular intervals.

In addition to the measuring the regulated disinfection by-products, it was important to assess broader measures of DBP formation, including AOX, because the different character of precursors in carp impacted water may produce DBPs not previously encountered or considered. All DBP analyses were conducted on a commercial basis by a NATA and ISO 9001 certified laboratory at the Australian Water Quality Centre (awqc.com.au) using industry standard methods. The limit of reporting and measurement uncertainty for the various DBPs are typically 1-3 ug/L and 15-50%, respectively. Details can be found at https://awqc.com.au/our-services/analytical-services/measurement-uncertainty. Bioassays (cytotoxicity and genotoxicity) assessed whether the final disinfected water has increased capacity to influence health factors as a result of carp degradation products.

2.5 Cell bioassays and toxicity

Unlike chemical analyses, bioassays measure biological effects resulting from exposure to test chemical mixtures rather than individual chemical structures. Toxicity testing, which included mammalian cell cytotoxicity and the bacterial Ames Tests, were undertaken as part of the study. Because the bioassays rely on an acute response, pre-concentration by SPE was necessary, so the results do not suggest the water is inherently toxic; but provide a comparative assessment of whether the health risk is either increased or not. The concentration method and the various toxicity
tests are detailed below.

As the toxicity response of the chlorinated treated waters was below detection all samples needed pre-concentration prior to analysis. Solid phase extraction using OasisR hydrophilic-lipophilic balance (HLB) cartridges (Waters, Sydney, Australia) was used to concentrate water samples. The procedure was modified from previous research (Chapman et al., 2011). Briefly, the OasisR HLB cartridge (20 cm^3 with 1000 mg sorbent) was preconditioned with 10 ml absolute methanol followed by 10 ml Milli-Q water. Using a SUPELCO Visiprep™ SPE Vacuum Manifold (Sigma-Aldrich, MO, USA), 1000 ml of test water was passed through the cartridge. Material adsorbed on the cartridge was eluted in 10 ml absolute methanol and concentrated to dryness using a miVac Duo concentrator™ equipped with miVac SpeedTrap™ and miVac Duo pump™ (Genevac, Pacific Laboratory Products, Australia). The dried material was then resuspended in 125 µL absolute methanol. Carp bioassay samples were concentrated from 1000 mL to 500 µL using Oasis hydrophilic-lipophilic balance (HLB) cartridges to produce a 2000x concentrate in 100% methanol. For the purpose of the assay the samples were then diluted into culture medium with the final concentrate at 40-fold and 80-fold tested. Unless otherwise stated, data from the 80-fold final concentration is presented.

It is acknowledged that the SPE concentration method employed would result in poor recovery of volatile organic compounds (Hankemeier et al., 1998) including the THMs. However, this was not considered to be a limitation because preliminary experiments indicated that the THMs were not active in the bioassays (data not shown). This is consistent with the mixed results for genotoxicity of the THMs found in other studies (Richardson et al., 2008).In a preliminary study of the recovery of HAA-induced WIL2NS cytotoxicity,

it was shown that, depending on the HAA, between 20% and 70% of cytotoxicity is recovered by the Oasis HLB cartridges (data not shown). Recoveries of other non-volatile DBPs (such as the HANs) have not yet been determined (Simmons et al., 2002; Cao et al., 2009). As such, the data could not be interpreted quantitatively in terms of individual DBPs; rather the comparison between samples in a set and the similarly prepared positive and negative controls formed the basis of data interpretation.

Previous work had shown that realistic chlorine residuals were quenched effectively by the cell growth medium (Froscio et al., 2010). Within a basic buffered medium, the effects of chlorine were not notable below 6 mg/L (EC50 was approximately 10 mg/L Cl2). Regardless, chlorinated samples were quenched stoichiometrically with sodium thiosulphate (based on residual Cl2 titration) before toxicity tests were undertaken to minimise any potential impacts from both free chlorine and the quenching agent.

2.5.1 WIL2NS Cytotoxicity of Carp by-product DBPs

WIL2NS (human lymphoblastoid) cells were cultured in RPMI- 1640 medium and supplemented with 10% Foetal Bovine Serum 10 mM HEPES, 1.5 g/L sodium bicarbonate, 0.06 mg/mL Penicillin G and 0.1 mg/mL Streptomycin sulphate. Cells were maintained in vented 75 cm^2 flasks in a humidified CO_2 incubator (37°C, 5% CO_2) and passaged twice weekly with a cell density maintained between 10^5 and 10^6/mL. The day prior to experimentation WIL2NS cells were subcultured. Following 24 hr growth, cells were assessed for their viability and concentration using the trypan blue dye exclusion assay. Provided cell viability was greater than 95%, cells were then prepared in culture medium at a concentration of 5x 10^5 cells/mL.

Costar 96-well clear flat-bottomed plates were used for the cytotoxicity assay and 50 µl of the WIL2NS cell suspension was added to the wells, providing a final concentration of 2.5 x 10^4/well. To allow for cell recovery prior to exposure to samples, plates were then incubated in the CO_2 incubator (37°C, 5% CO_2) for 1 hour. During the cell recovery, sample dilutions were prepared in culture medium of 40x and 80x. As the concentrates are in methanol the most concentrated exposure of 80x would contain 4% methanol.

In order to determine whether the WIL2NS cells were adversely affected, a vehicle control of 4% methanol with cells was also included.

Methyl Methanesulfonate (MMS) was used as a positive control with a series of dilutions conducted to assess the dose response of the cells. At the most concentrated 100 mg/mL MMS the methanol concentration was 2%. Concentrations ranging from 100 mg/mL to 10 mg/mL were used to cover the dose response curve.

The recovered WIL2NS cells were exposed to the test samples for 23 hr in the humidified CO_2 incubator (37°C, 5% CO_2). The Rezazurin viability assay was performed on the cells. The premise of this assay is that viable cells are capable of metabolising non-fluorescent rezazurin into the highly fluorescent resorufin. A working solution of rezazurin was made in culture medium at a concentration of 150 µg/mL, and 50 µL aliquoted to each well. Plates were incubated in the humidified CO_2 incubator (37°C, 5% CO_2) for 2 hr. Fluorescence (530 nm excitation, 580 nm emission) was measured using the VARIOSKAN plate reader. Fluorescence is directly proportional to cell viability. Cell viability exposed to the samples was expressed as a percentage of its fluorescence over the fluorescence of the vehicle control (4% methanol).

2.5.2 Ames genotoxicity of Carp by-product DBPs

Both TA098 and TA100 were used for the bacterial mutagenicity assays. 50x Vogel-Bonner broth (40 mM MgSO4・7H_2O, 476 mM Citric acid, 3 mM K_2HPO_4 and 837 mM $NaNH_4HPO_4$・4H_2O) was prepared and autoclaved at 121°C for 20 mins. 40% w/v glucose and 0.05 mM histidine/biotin were prepared and filtered through 0.45 µm membrane. Strains were cultured in nutrient No.2 supplemented with 25 µg/mL ampicillin at 37°C and stirred at 180 rpm for 18 hrs.

Samples were diluted to the desired concentration with 1x VBB supplemented with 2% w/v glucose. 4-nitro-o-phenylenediamine (4-DNP) and sodium azide were used as positive controls for TA98 and TA100, respectively. The chemical exposure was performed by adding 125 µL to the 24 wells plate, followed by 120 µL of 1x VBB supplemented with 2% w/v glucose and 0.01 mM Histidine/Biotin and 5 µL strain. Each sample was treated in triplicate. Positives, non-treated and vehicle control (negative control) were included in each plate. Plates were incubated at 37°C, 180 rpm for 90 minutes.

Following incubation, 2.75 mL of detection medium (1x VBB supplemented with 2% w/v glucose and 10 µg/mL bromocresol purple. Fifty microlitres of the mixture was dispensed into each of 48 wells in a 384 well plates. The plate was incubated at 37°C for 48 hours (TA98) and 72 hours (TA100) and the number of wells that turned yellow were recorded. The average number of positive wells (yellow) was calculated, and baseline (BL) value was determined from an average of number treatment control (NTC) + 1 standard deviation (SD). Mutagenicity of test samples and positive controls was expressed as fold increase (FI) against the baseline. Samples were considered genotoxic when the FI value was ≥ 2.

Table 2: Combinations describing relationship of water quality with carp densities

Carp concentrations (*biomass densities*)	**6** (0, 0.05, 0.10, 0.25, 0.5 & 1.0 kg/m^3)
Jar tests completed	**8** (6 carp concentrations with alum only & 2 carp impacted alum + PAC)
Settled & direct filtration fouling tests	**9** (6 carp concentrations with alum only & 3 carp impacted alum + PAC)
DBP / toxicology assessment	**7** (acceptable treatment optimums alum and alum + PAC above – not 1.0 kg/m^3)

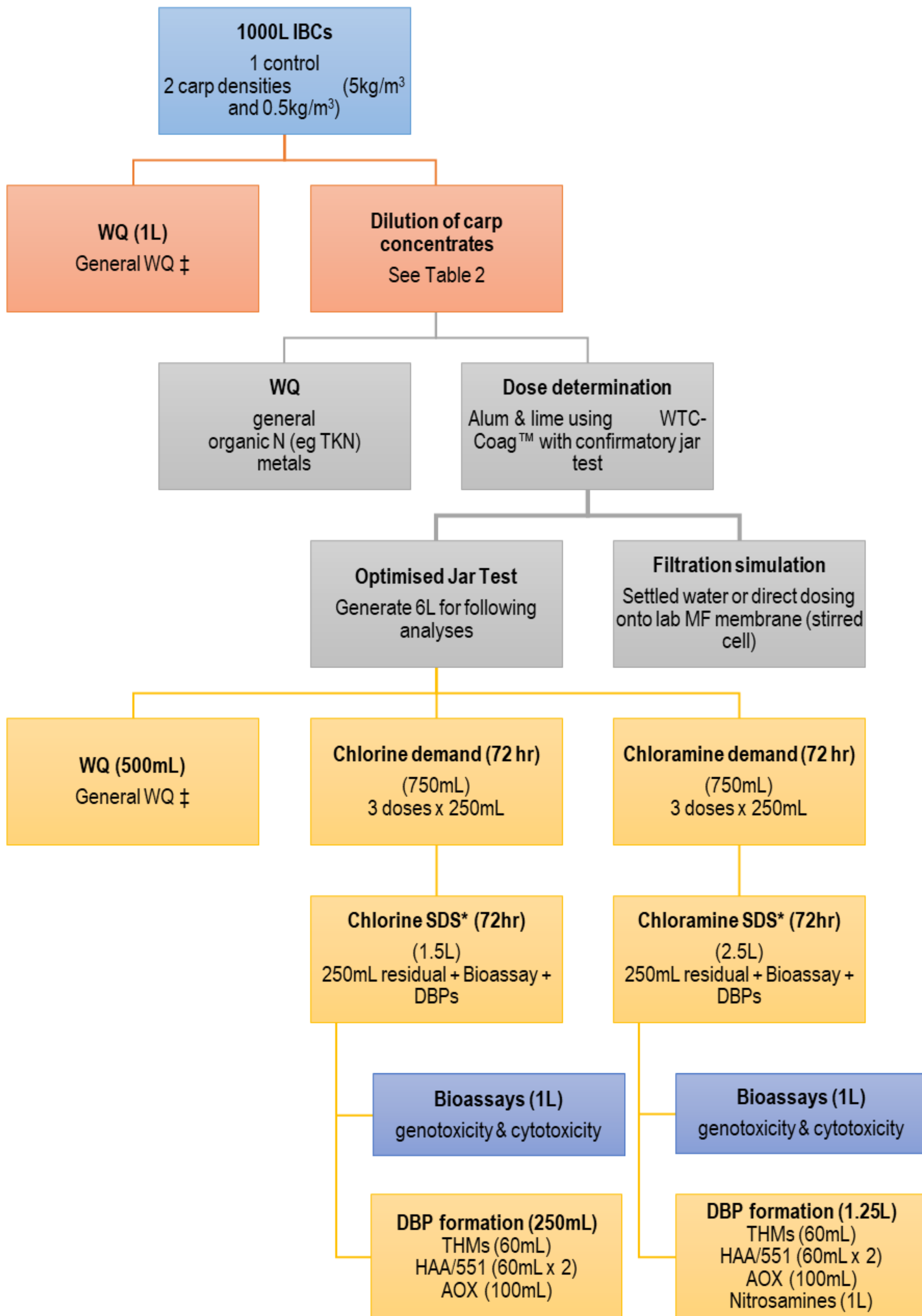


Figure 3: Project work plan and sequence.

3. Results

3.1 Carp concentrate characterisation

Carp concentrates at 5.0 kg/m^3 and 0.5 kg/m^3 commenced production at Lock 1 on 23rd January for Milestone 2 experimental work. After 14-days of environmental exposure, including temperature extremes >40°C, 100 L volumes were sampled from below the surface (to avoid surface floating material and deep sediment) and characterised fully using the nominated suite of tests. Drinking water sources are subject to routine monitoring of many water quality parameters that provide information not just for operational management of the treatment processes, but also for compliance with regulation and measurement against performance targets. The two carp concentrates analysed were found to be of very different character, with concentrations of key solutes that were not directly relatable to carp density differences.

In the case of the 0.5 kg/m^3 concentrate, a green algae bloom had established in the production vessel as a result of the increased nutrient availability from decayed carp together with direct sunlight exposure. While algae may have been present in the early stages of fish biomass decay in the 5.0 kg/m^3 vessel, any bloom activity did not persist till the sampling date due to the high ammonia concentration (127 mg/L) which was more than sufficient to be acutely toxic to all aquatic life, including algae. While this difference will certainly have impacted on water quality, it is a predictable and realistic outcome in the River Murray as a result of any nutrient release during Spring or Summer, so it was accepted as a realistic scenario that could be experienced as a result of the carp virus release.

Table 3: Water quality parameters of background River Murray water and carp degradation product concentrates after 14-days environmental decay. Highlighted values indicate notable variation from base River Murray water (column 2).

Water Quality	River Murray (Anstey Hill)	Carp Density 0.5 kg/m^3	Carp Density 5.0 kg/m^3
pH	7.3	9.8	7.1
Temperature (°C)	21.4	21.9	22.3
Conductivity (µS/cm)	234	253	1021
Colour (HU)	7	32	89
UV absorbance @ 254mn (/cm)	0.065	0.151	0.353
DOC (mg/L)	3.3	10.0	25.5
TOC (mg/L)	n/a	16.1	136
Turbidity (NTU)	46.9	19.8	68.4
Ammonia (mg/L)	0.02	1.4	127
Aluminium - Soluble (mg/L)	0.021	0.019	0.003
Aluminium - Total (mg/L)	9.855	0.202	1.080
Iron - Soluble (mg/L)	0.0115	0.1202	0.1070
Iron - Total (mg/L)	4.849	0.2612	1.885
Manganese - Soluble (mg/L)	0.0055	0.0018	0.0158

Manganese - Total (mg/L)	0.0476	0.0081	0.0330
Nitrate + Nitrite as N (mg/L)	0.138	0.162	0.013
Organic N (TKN) as N (mg/L)	0.51	4.69	115

Two characteristic water quality parameters were observed that indicated the presence of significant carp biomass and could be used as early warning indicators of carp impact:

(a) Ammonia, which was present at a manageable 1.4 mg/L in the 0.5 kg/m^3 concentrate, and 127 mg/L in the extreme 5.0 kg/m^3 concentrate. At such high ammonia levels, the water is acutely toxic in the environment and effectively unfit as source water for drinking water treatment.

(b) Aromatic protein-like fluorescence (defined by peak T_2 excitation/emission λ225/λ350nm) which indicates the presence of the amino acid tryptophan, present as a result of carp protein breakdown (Figure 5). Figure 6 shows a quantitative comparison of peak intensities where 'neat' refers to undiluted measurement, while 'corrected' data was diluted for measurement within the operating window of the instrument and then re-multiplied during data processing for direct comparison. Because fluorescence is a non-destructive, spectroscopic technique, it has possibility of being used in an online monitor that could be positioned at the entry point to the water treatment plant as a 'trigger' for carp management plan activation.

Additionally, two water quality parameters increased as a result of carp degradation products. It should be noted that these are strongly influenced by normal climactic events in the Murray- Darling basin, such as droughts and floods and therefore may not be distinguishable as being specific to carp impacts:

(a) Conductivity increased only marginally at 0.5 kg/m^3 carp density (+8%), but much more significantly in the 5.0 kg/m^3 (+336%). Given that realistic carp densities are expected to be well below 0.5kg/m^3 (Stuart et al., 2019), it is unlikely this would be a reliable indicator.

(b) Organic matter concentrations (DOC and TOC) increased considerably even at the lower of the two carp densities, however the high TOC of 136 mg/L in the 5.0 kg/m^3 carp density is most problematic, as this would continue to break down to form additional DOC over time. Break down kinetic rate will depend greatly on seasonal factors such as temperature and the health and level of biological diversity and activity in any given location.

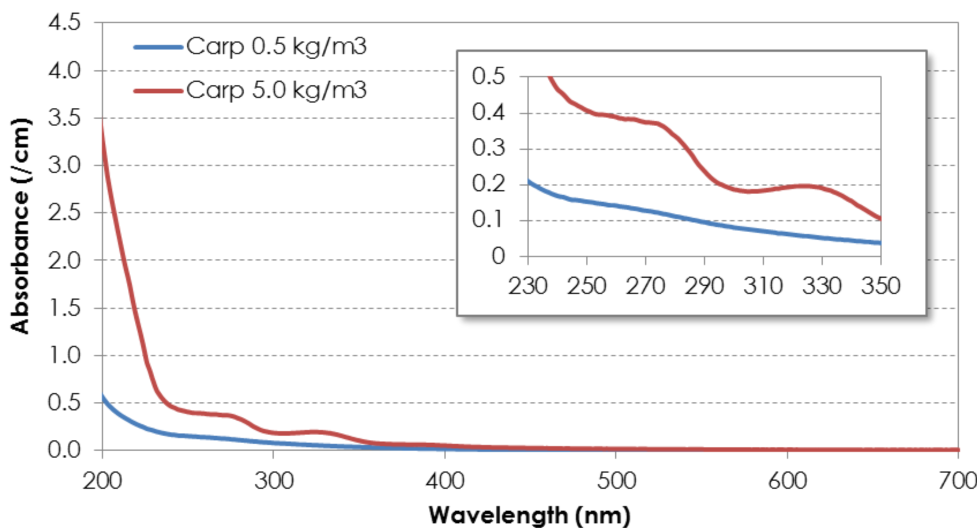


Figure 4: UV/visible absorbance scan 200-700nm with highlighted features (inset)

Assessment of UV-visible absorbance showed some peaks and troughs in the 5.0 kg/m^3 carp concentrate (Figure 4), however there was a lack of specificity, with increased absorbance in wavelength regions that are similarly descriptive of natural organic matter, nucleic acids and aromatic amino acids, indicating that UV absorbance is not a specific indicator of carp by-product impacts.

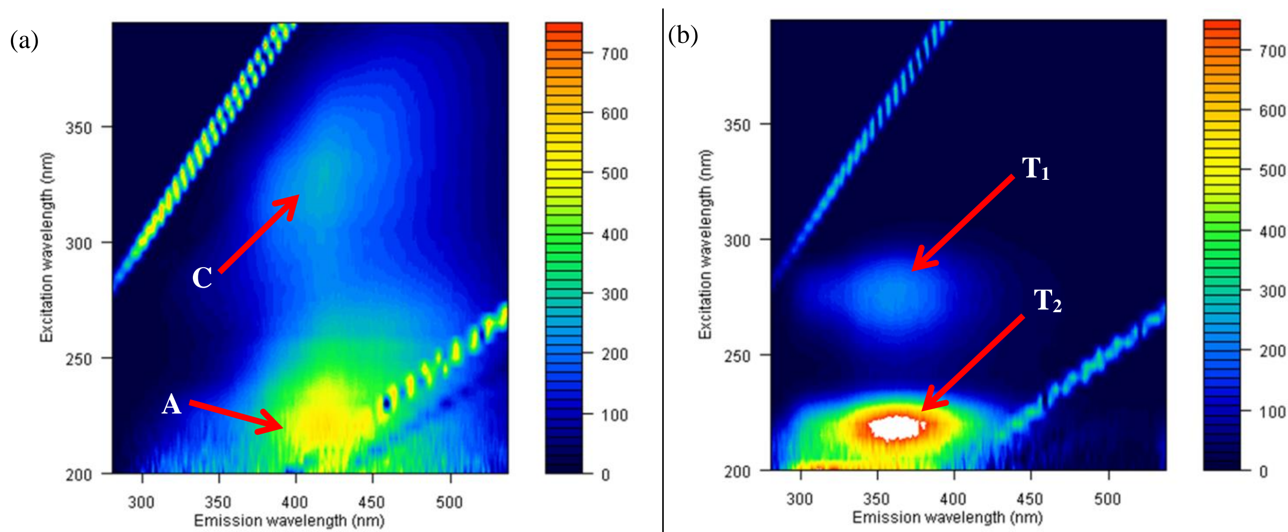


Figure 5: Fluorescence excitation-emission matrix (FEEM) of (a) 0.5 kg/m^3 and (b) 1 in 50 dilution of the 5.0 kg/m^3 carp concentrate. Heat map shows fluorescence unit intensity. White is over-range. A= Fulvic-like; C = Humic-like; T_1 & T_2 = Protein-like.

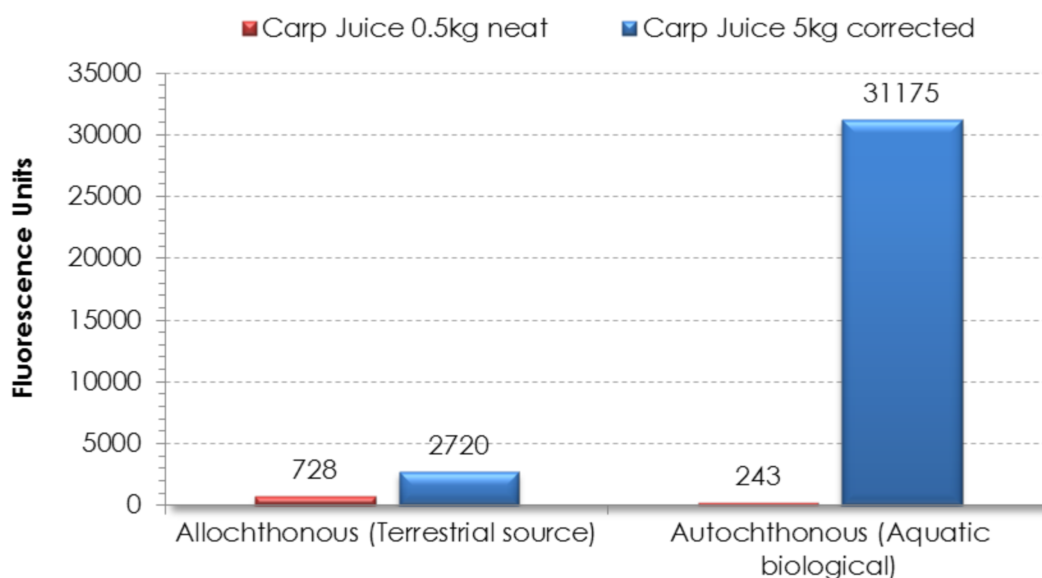


Figure 6: Fluorescence intensity and source allocation for carp concentrates.

3.2 Treatability assessments

Dose determination jar tests on dilutions of the carp concentrates included 0.05, 0.10 and 0.25 kg/m^3 from the 0.50 kg/m^3 concentrate and 1.0 kg/m^3 from the 5.0 kg/m^3 concentrate, providing 6 carp densities for trend evaluation.

Table 4: Initial water quality for examined carp densities

Carp density (kg/m^3)	0	0.05	0.1	0.25	0.5	1.0
pH	7.3	7.4	7.5	8.3	9.8	7.1
Turbidity (NTU)	46.9	43.8	40.1	31.8	19.8	50.9
Conductivity (µS/cm)	234	235	237	244	253	397
Colour (HU)	7	7	10	18	32	18
UV254	0.065	0.067	0.079	0.106	0.151	0.118
DOC (mg/L)	3.3	3.4	4.6	6.3	10	19.3
Ammonia (mg/L)	0.02	0.14	0.27	0.52	1.40	25.40

Results showed that carp densities at the lower (more realistic) concentrations, namely 0.05 and 0.10 kg/m^3, could be treated to equivalent water quality as non-impacted River Murray water using optimised alum coagulation alone (Table 5). Higher carp densities (0.25 kg/m^3 and greater) resulted in alum treated water quality that was poorer than River Murray without carp impacts and required additional treatment. Carp densities of 0.25 and 0.50 kg/m^3 were treated with two-stage treatment using powdered activated carbon (PAC) adsorption followed by alum coagulation to recover treatment performance. This is a treatment option currently available to most River Murray WTPs as a means of removing cyanobacterial taste and odour compounds, and it can also be employed at higher doses for DOC removal for short periods. The carp densities that could not be treated effectively by PAC and alum (1.0 kg/m^3 and higher) are beyond the capability of existing water treatment plants and would be considered nonviable sources of drinking water. Full jar test data for all applied doses is available in Appendix A.

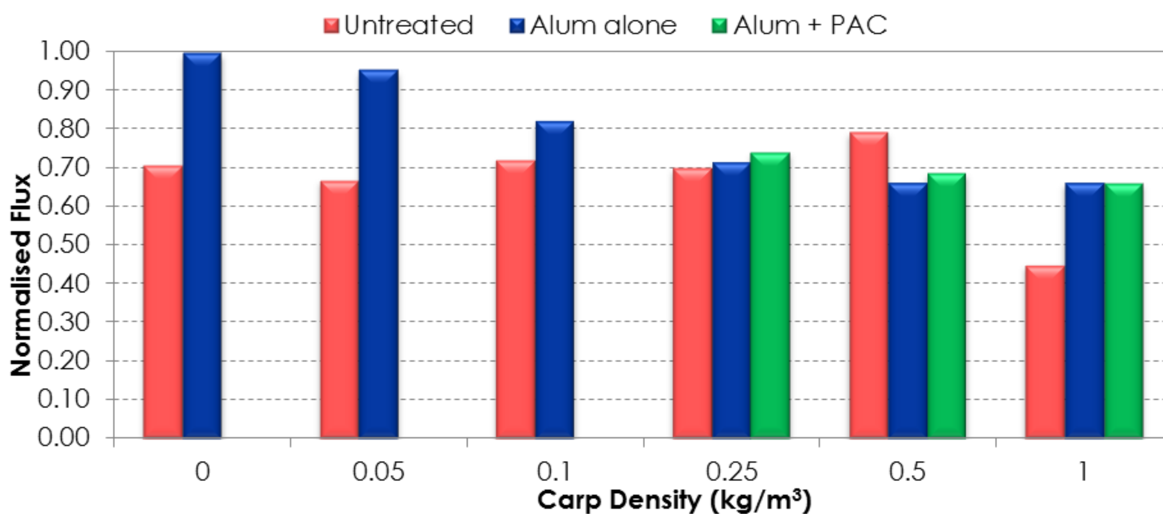


Figure 7: Direct filtration (coagulation + microfiltration) simulation flux, normalised against non-carp impacted River Murray water

As a means of testing membrane fouling potential of the carp impacted waters in a laboratory environment, a simple assessment using microfiltration (0.2 µm pore size) filters in a dead-end stirred cell was undertaken. Results showed less than 20% reduction in flux as a result of realistic carp impacts (0.05 and 0.10 kg/m^3) increasing to approximately 35% at extreme carp impact. Addition of PAC had minimal effect on flux, possibly due to the positive effects of adsorption of organic foulants being offset by the increased filtration resistance of the PAC particles on the filter surface. Because membrane filtration

operates by physical separation mechanism, there is not a direct relationship between flux decline and treated water quality. The impact of flux decline due to increased fouling is mostly reduced operating time between backwashing (cleaning) and increased transmembrane pressure (TMP) requirement to recover flux, which impacts on operating costs for electricity and staff maintenance time, and may shorten the effective lifetime of the membranes, leading to increased and unplanned capital expenditure for early membrane replacement. Alternatively, during challenging treatment periods, the target treated water production rate (volume of treated water produced) may be reduced to minimise damage to the membrane, however the treatment plant must then operate for a greater time period to produce the equivalent required water volume, which also impacts on operating costs.

Figure 8: Stirred cell reactor for direct filtration simulation with 0.5 kg/m³ carp density (alum + PAC treatment)

Table 5: Optimum treatment conditions for each carp density

Carp density (kg/m³)	Alum (mg/L)	PAC (mg/L)	Turbidity (NTU)	Colour (HU)	UV254 (/cm)	DOC (mg/L)	OK
0.00 (River)	52	-	0.10	1	0.032	2.1	✓
0.05	66	-	0.09	2	0.033	2	✓
0.10	84	-	0.19	1	0.032	2.2	✓
0.25	94	-	0.14	2	0.043	2.8	✗
0.25	94	50	0.09	1	0.022	1.7	✓
0.50	114	-	0.15	4	0.059	3.8	✗
0.50	114	50	0.22	2	0.035	2.7	✓
1.0	117	-	0.13	4	0.091	15.3	✗
1.0	117	50	0.22	3	0.051	14.5	✗

Treatment performance was able to be largely recovered in the 0.25 and 0.50 kg/m³ carp densities through the use of high dose PAC adsorption, however at the carp density of 1.0 kg/ m³, the addition of carbon was unable to improve water quality to acceptable standards. When coupled with the high ammonia concentration, which passed the treatment process without attenuation, this water would be non-compliant with ADWGs and unfit for use as drinking water. This is demonstrated graphically in figures 9 and

10 where relationships of key water quality parameters against carp biomass density are shown, with threshold values identifying where treatment is not feasible.

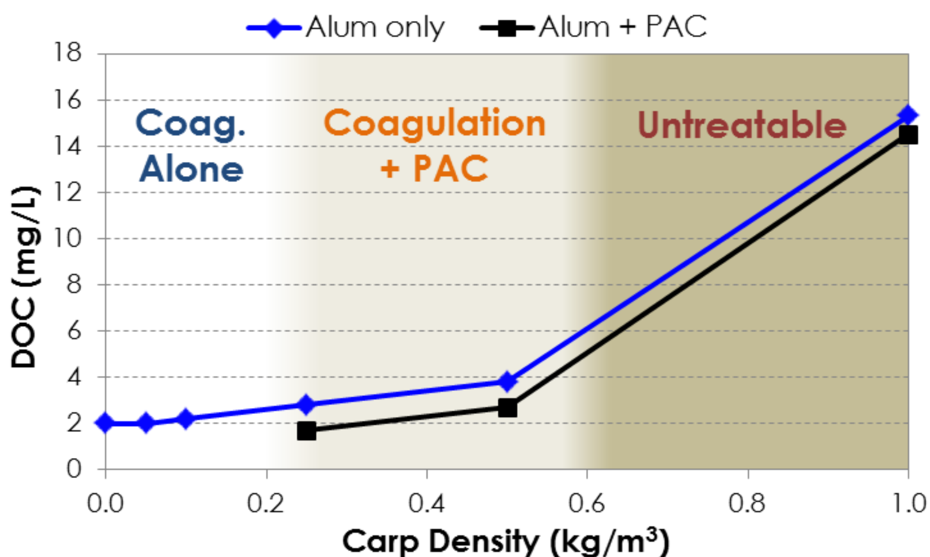


Figure 9: Treatability assessment zones (DOC removal) against Initial carp density

3.3 Disinfection & DBPs

When assessing the impacts of carp degradation products on the effectiveness of treatment plant operation, a key consideration is the effect on disinfection. While treatment is largely responsible for improving aesthetics (clarity, colour, taste and odour), disinfection is the process which maintains public health protection against infectious organisms and therefore cannot afford to be compromised. Changes to disinfection effectiveness can be measured using a number of parameters but can be most simply expressed using chlorine demand and the resulting effects quantified using disinfection by-product analyses. Effectively, chlorine demand is the amount of chlorine consumed in reactions with various organic and inorganic components in the water matrix before a free available residual of chlorine can be created to maintain safety within the distribution system (storage tanks and pipework). High chlorine demand reduces the effectiveness of the disinfectant and increases the cost of disinfection through the requirement for greater initial doses, or additional (booster) doses within the distribution system. Chlorine demands for alum treated waters (Table 6, Appendix B) were closely correlated with product water UV254 and DOC increases resulting from progressively lower treatment effectiveness as carp products concentration increased.

Chlorine demand (mg/L) $= 506 \times UV_{254} - 12.67$ (Eq. 1)
$= 7.79 \times DOC - 12.48$ (Eq. 2)

The relationships were highly linear with correlation factors (r^2) of 0.97 and 0.99 for UV254 and DOC respectively. Through further relationships between UV254, DOC and initial carp density, it should therefore be possible to calculate estimates of the cost of specific carp impacts on the cost of disinfection after treatment. This will be included as part of the spreadsheet calculator, as described in Section 1.3. Correlation factors for alum and PAC treated waters cannot be accurately described due to insufficient data points, given only two carp densities (0.25 and 0.50 kg/m^3) were able to be treated effectively enough to progress to the disinfection simulations, and a minimum of three data points are required for statistical significance.

Table 6: 3-day (72 hr) chlorine and chloramine demand

Carp density (kg/m³) A = alum; P = PAC	0.00 A	0.05 A	0.10 A	0.25 A	0.25 A + P	0.50 A	0.50 A + P
Chlorine demand	2.45	3.56	5.09	8.94	8.27	17.23	16.13
Chloramine demand	0.87	1.06	2.13	1.90	1.74	2.64	2.16

Breakpoint chlorination is a technique that can be applied that uses high doses of chlorine to increasingly substitute the hydrogen ions in the ammonia molecule (NH_4) until the nitrogen is mineralised (as N_2) and dissipated to the gas phase (air), allowing a free chlorine residual to be established. The high ammonia concentration of the 1.0 kg/m³ carp density (25.4 mg/L) would require a theoretical chlorine breakpoint requirement of between 200 and 250 mg/L (8:1 to 10:1 concentration ratio) to eliminate, which is well beyond the dosing capacity of WTPs and the resulting blend of chlorinated organic compounds would be difficult to predict and source specific. Attempts to manage this breakpoint reaction in the precisely controlled environment of the laboratory were unsuccessful, suggesting that in real-world conditions it would be improbable that successful breakpoint chlorination could be achieved. As a consequence, this treated water was not progressed through the disinfection assessment and the recommendation for this inlet water scenario would be to cease treatment until inlet water quality improves and ammonia concentration decreases into a more manageable range (roughly less than 1.5 mg/L). For the remaining treatments (0.50 kg/m³ and lower), the breakpoint reaction was manageable and realistically achieved within treatment plant dose limits (Table 6).

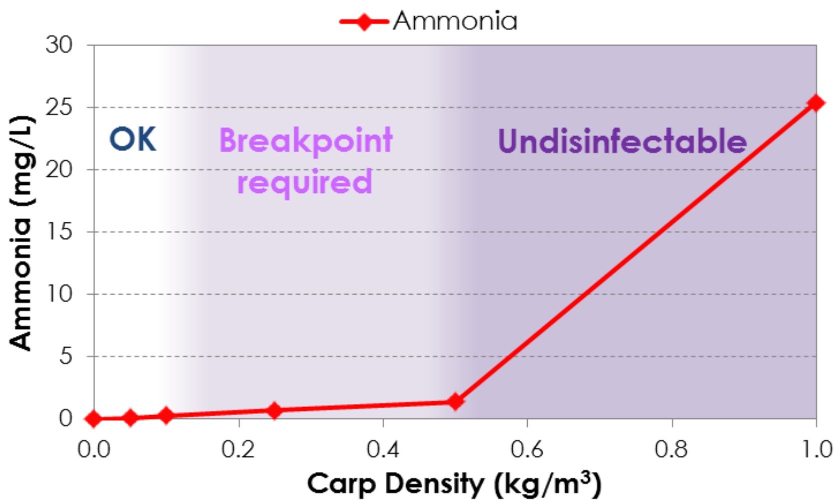


Figure 10: Treatability assessment zones (Ammonia residual) against initial carp density

Regulated DBP formation (Figures 11, 12 & 13) were well below ADWG limits and consistent with variations between product water DOCs (i.e. Treatment scenarios with slightly higher DOC resulted in proportionally greater DBPs). This suggests that despite the differences in character of the carp degradation products (autochthonous, non-humic) compared to typical River Murray organic matter, the basic concentration of organic precursors compounds (measurable by UV absorbance at 254nm and DOC), was still the limiting factors determining actual DBP formation. This is a good result as these parameters are easily and routinely monitored and can aid in driving operational control strategies much easier than more complex characterisation tests.

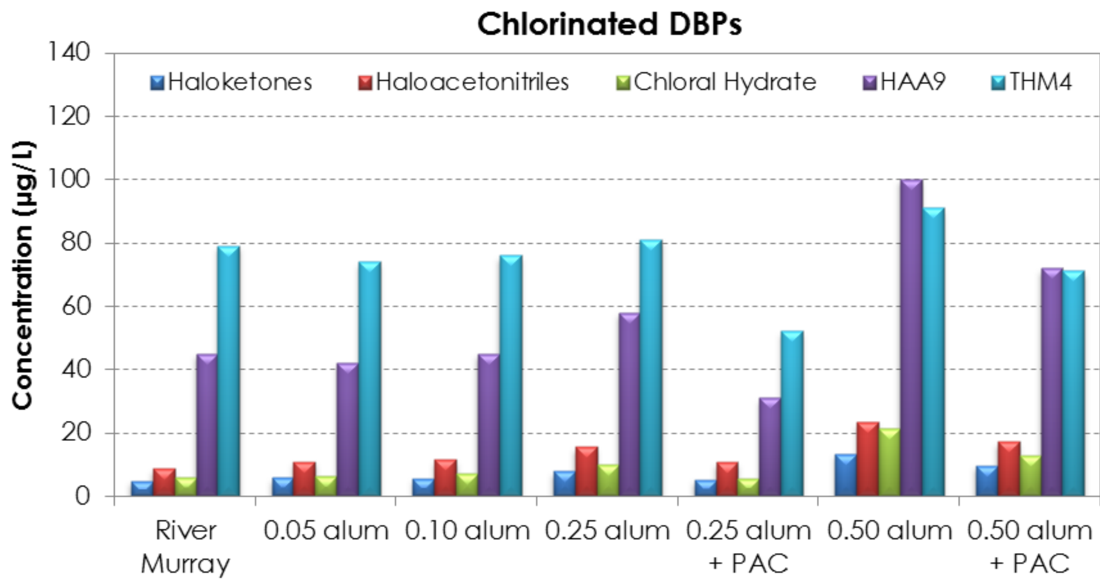


Figure 11: Chlorinated disinfection by-products formed after 72 hrs contact

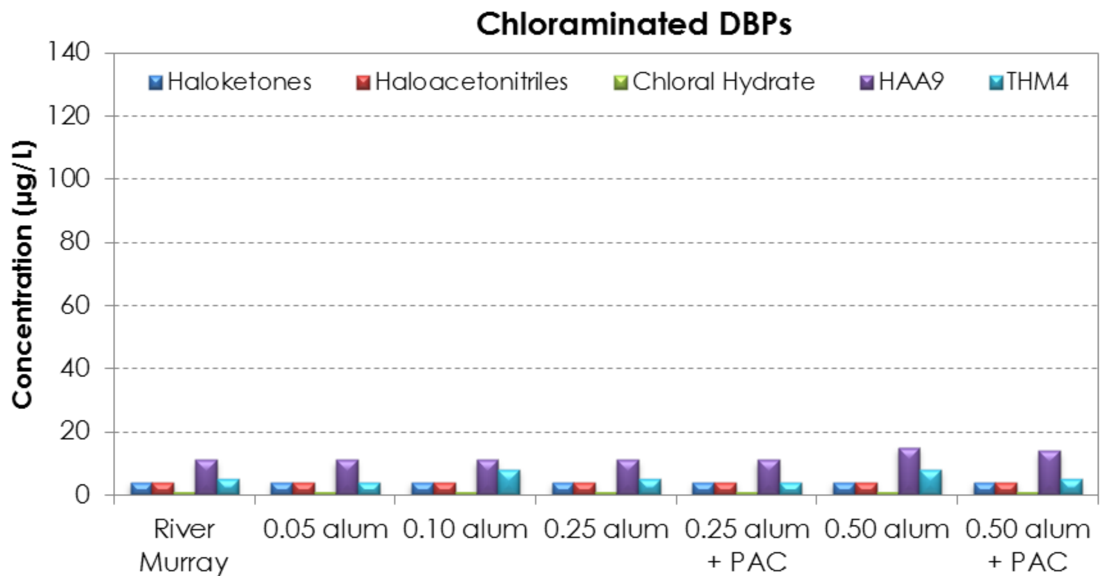


Figure 12: Chloraminated disinfection by-products formed after 72 hrs contact

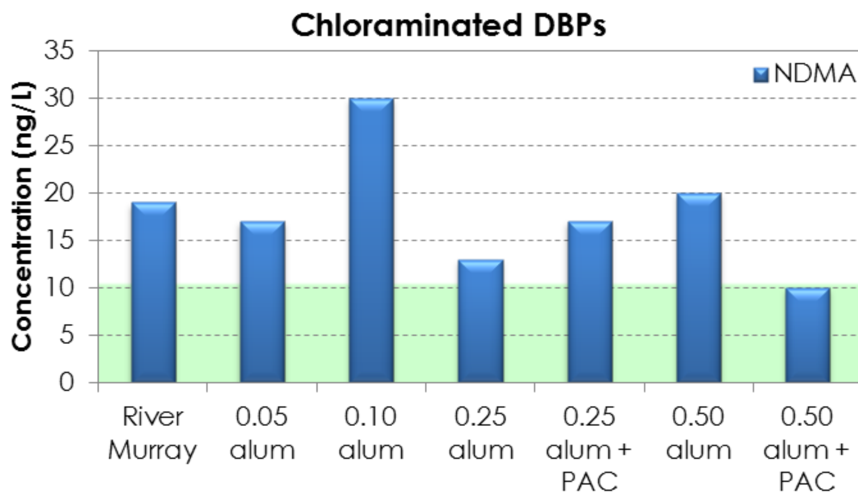


Figure 13: N-Nitrosodimethylamine (NDMA) formation after 72 hrs chloramination

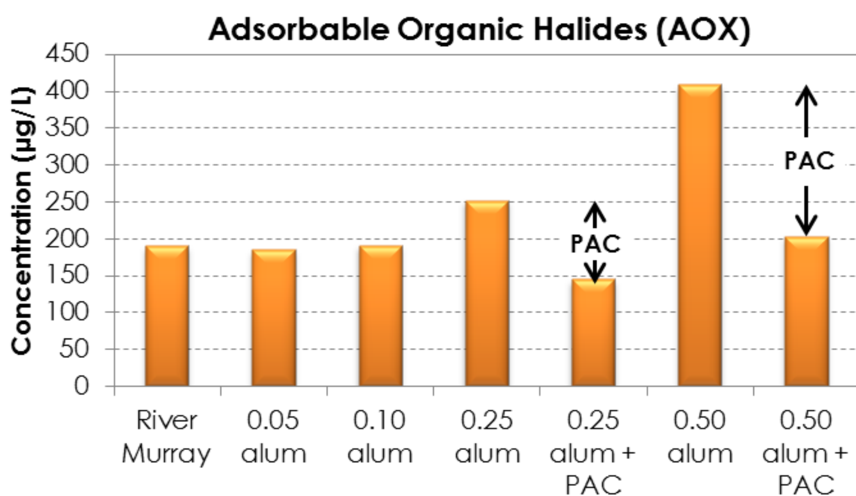


Figure 14: Total halogenated DBPs (highlighting effect of PAC addition)

AOX is encouragingly low and addition of PAC treatment was highly effective for reducing overall DBPs, despite modest DOC reductions (Figure 14). This suggests that DBP precursors were selectively reduced. In both chlorinated and chloraminated disinfection systems, increasing carp degradation products resulted in a reduced proportion of regulated DBP formation (Figure 15) with a corresponding increase in unidentified halogenated DBPs (uAOX). This is important to note, as these DBPs are not measured in routine drinking water quality management and hence monitoring of only the regulated DBPs may give false security that health risk is being adequately managed. Of the identified DBPs, of which there are more than 500 suggested in literature studies (Weinberg et al., 2002), the Australian water industry only reliably monitors 15-30 of the most abundant compounds, with only 7-13 compounds (THMs and HAAs) measured regularly. While the concentration of these can usually be used as surrogates for overall DBP formation to drive treatment and disinfection performance, current scientific understanding is that the causal link between these compounds and community health effects is very weak (Cotruvo and Amato, 2019) and other much less abundant compounds (potentially within the uAOX fraction) may be of significantly greater concern.

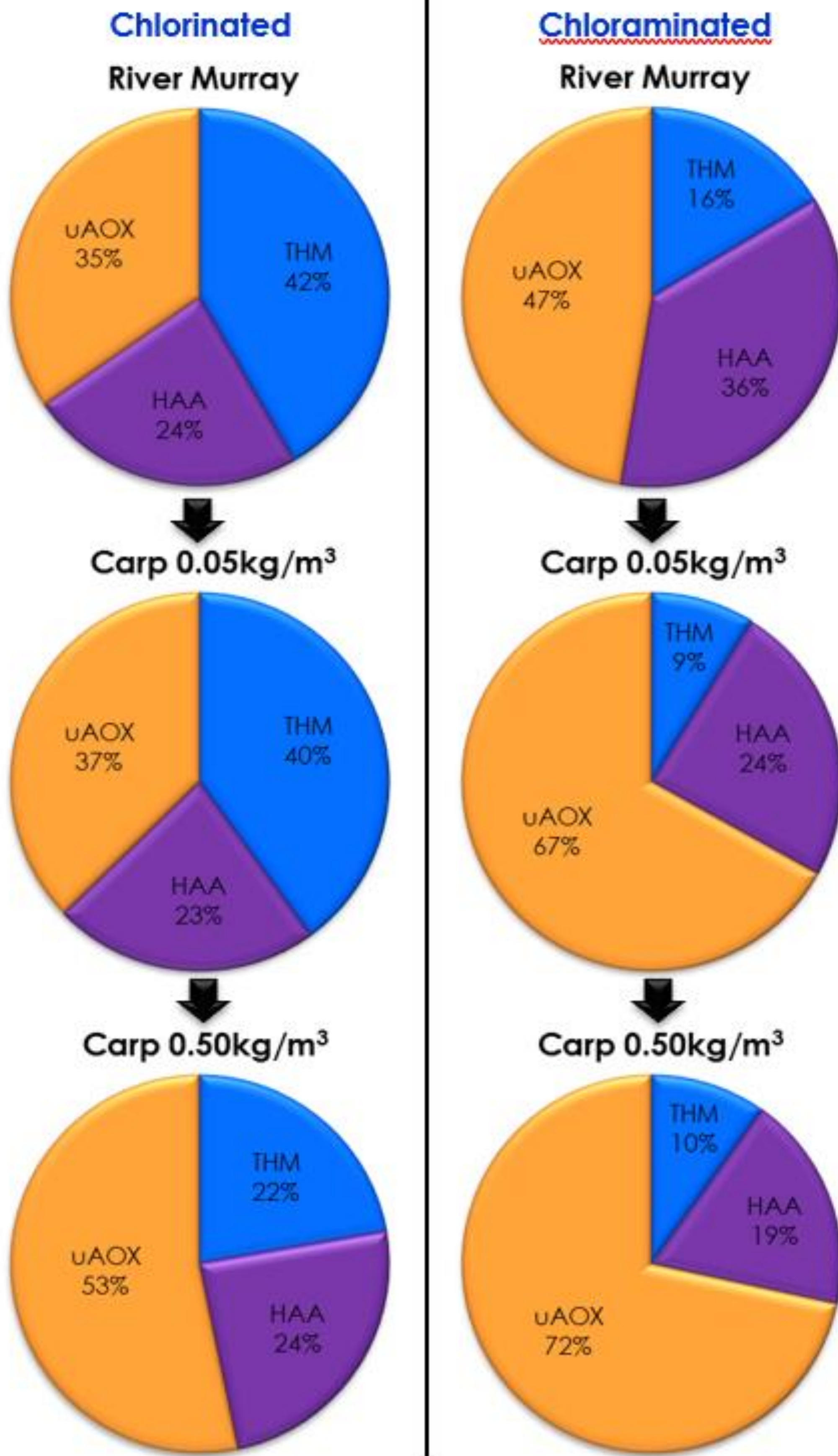


Figure 15: Transition of relative percentages of regulated DBP classes to non-regulated DBPs with increasing initial carp density

3.4 Odour assessment

Both carp concentrates were very different in smell. The low concentration (0.5 kg/m^3) was inoffensive but had subtle musty and earthy undertones (2-MIB or Geosmin) as a result of the green algae bloom that had established in the production vessel. The extreme carp density sample (5.0 kg/m^3) was highly offensive with 'fishy' characteristics from methylamines, as well as rotten meat odours (cadaverine and putresine). These were diluted proportionally in the treatability concentrations and were largely eliminated through alum treatment and filtration, with the exception of the highest carp density (1.0 kg/m^3) that still had detectable odours. The inclusion of PAC in the treatment, as would normally be applied in drinking water processes to adsorb odours, was however effective in all cases.

Taste assessment was not undertaken due to concerns over unknown DBPs and microbiological safety of laboratory treated waters, as discussed in section 1.3.

3.5 Cell Bioassays

In interpretation of cell bioassay results, it is important to consider that there would be no response from the various tests if the treated and disinfected waters were tested without concentration, as any toxic effects of any DBPs present are not detectable at the concentrations produced in drinking water that meets drinking water guidelines. Inherently, the water is not acutely toxic. The guidelines for all drinking water contaminants, including DBPs, are set at levels several orders of magnitude lower than is required to produce any effect in peer-reviewed epidemiological studies (e.g. Cancer outcomes in rodents). This is done deliberately to achieve acceptable risk minimisation over lifetime exposure. Therefore, the treated and disinfected samples were concentrated 80 times in order to produce measurable (non-negative) results between the various carp degradation product concentrations and enable comparative discussion.

Keeping in mind these results relate to highly concentrated samples, the results showed:

- Clear increases of toxicity with chlorinated samples (Figures 16a, 17a & 18a) versus chloraminated samples (Figures 16b,17b & 18b)
- A trend of increasing relative toxicity with increasing carp byproduct concentration in the chlorinated samples (Figures 16a, 17a and 18a)
- A significant beneficial effect of adding powdered activated carbon (PAC) to the treatment for attenuating the toxic effects at a carp loading of 0.25 kg/m^3. This is consistent with both the DOC reduction (Table 5), and the DBP concentrations (Figures 11 and 14).
- The lack of capacity for PAC and coagulation combined to attenuate the effects of carp impacts at 0.50 kg/m^3. The result suggests that at this carp density, the water quality will not only be noticeably diminished, but it may have a negative influence on health if the carp effects are long term (chronic) and consistently high. However, the actual significance of this effect in short term exposure is beyond the scope of this project to estimate and may be negligible.

The outcome of the NCCP project modelling carp densities in the River and associated water bodies (Stuart et al., 2019) suggests that the most realistic loading is at the lower end of the investigated scale (0.05 to 0.10 kg/m^3) which corresponds to approximately 200 to 400 kg of fish biomass per hectare (assuming an average 2 metre depth). In this concentration range, there is little concern for our ability to treat the water effectively and keep it consistently safe. However this may not be the case if the carp degradation product loading is actually higher, which could result if insufficient effort is applied to removing the fish biomass following virus release, leading to extended periods of water quality impact.

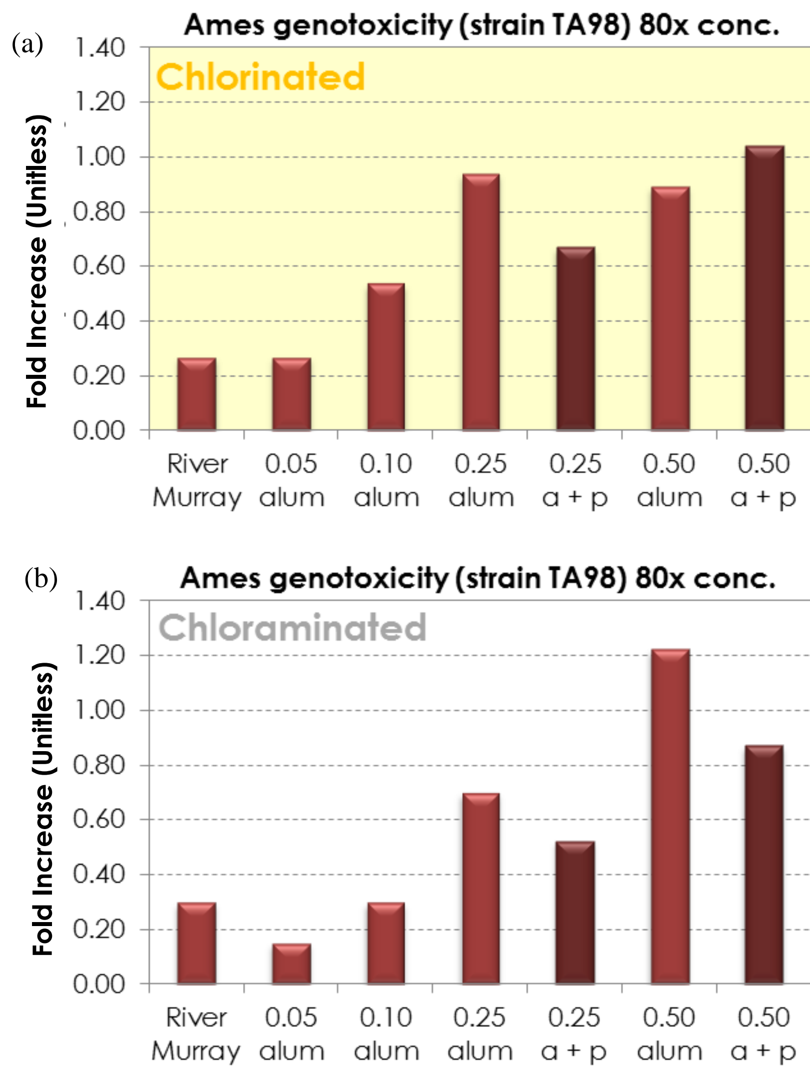


Figure 16: Ames genotoxicity (TA98 strain) for 80x concentrated disinfected carp impacted waters (a) chlorinated and (b) chloraminated. (a+p = alum + PAC)

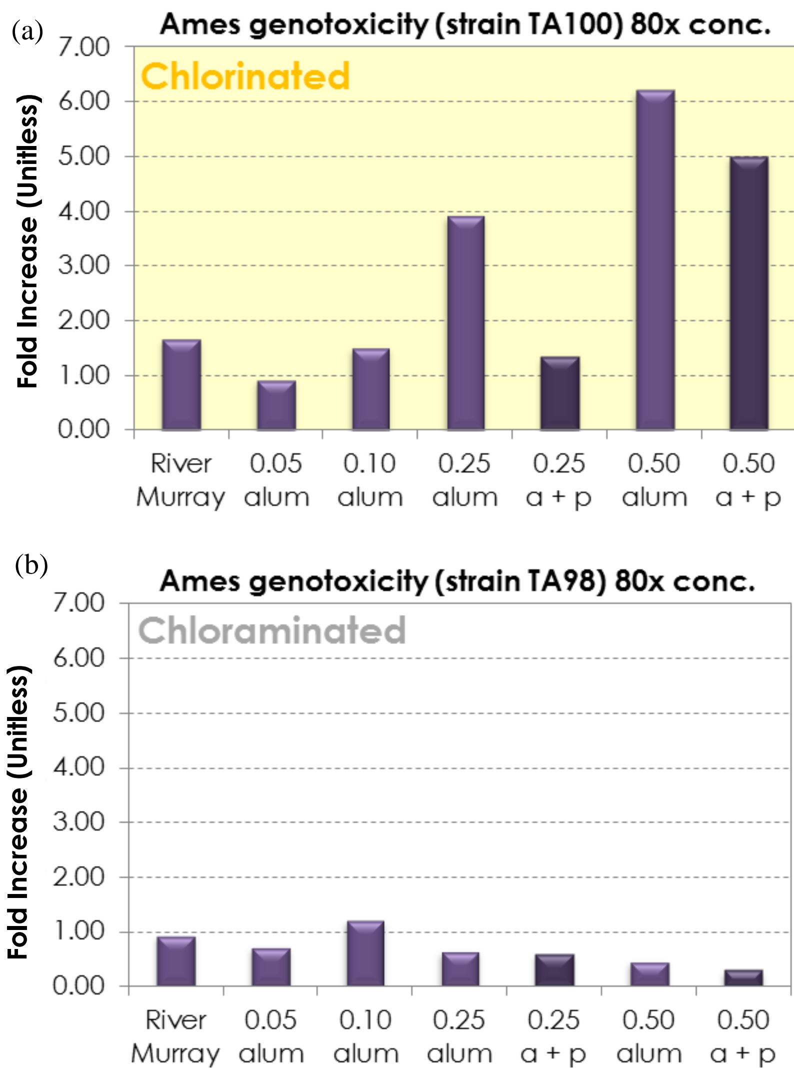


Figure 17: Ames genotoxicity (TA100 strain) for 80x concentrated disinfected carp impacted waters (a) chlorinated and (b) chloraminated. (a+p = alum + PAC)

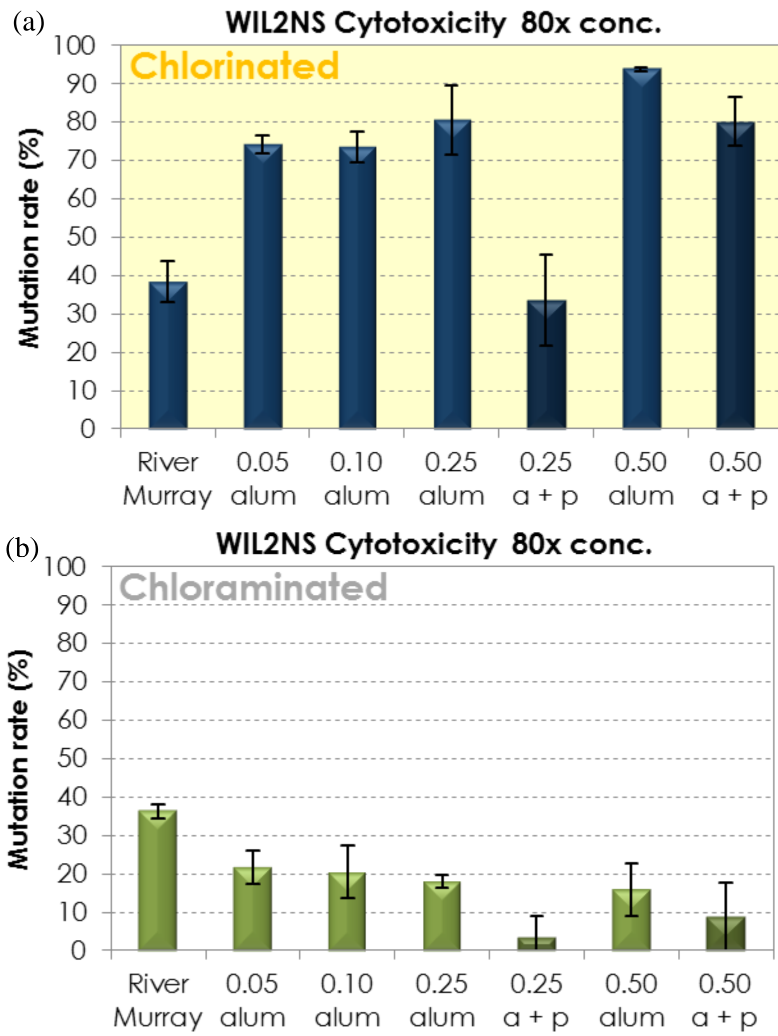


Figure 18: WIL2NS Cytotoxicity for 80x concentrated disinfected carp impacted waters (a) chlorinated and (b) chloraminated. (a+p = alum + PAC)

Conclusion and Recommendations

Two characteristic water quality parameters were determined that indicated the presence of degraded carp biomass and can be potentially used as indicator parameters for the arrival of carp-impacted water at water treatment plants, namely ammonia and peak T_2 fluorescence representing the amino acid tryptophan. Given that fluorescence is a non-destructive spectroscopic parameter, this can be applied as a real-time online sensor using appropriate technology.

Realistic carp densities between 0.05 and 0.10 kg/m^3, roughly equivalent to 200 - 400 kg/ha as identified in Stuart et al. (2019), could be effectively treated by optimising coagulation for DOC removal, with minimal increases in total or regulated DBPs. However, cell bioassays suggest that some unknown but undesirable DBPs could be formed that may increase health risks over long-term exposure.

Higher carp densities between 0.10 and 0.50 kg/m^3 (~ 400 – 2000 kg/ha), could not be treated effectively with coagulation alone and required additional removal of dissolved solutes using PAC. Although a higher proportion of unidentified DBPs were formed, leading to greater cell bioassay responses, regulated DBPs were effectively controlled by this treatment protocol.

Extreme carp densities greater than 1.0 kg/m^3 (over 4000 kg/ha) produce high quantities of organic and inorganic solutes, including ammonia concentrations that would result in water that is untreatable using the technologies available in the majority of Australian drinking water treatment plants. In such a situation, the recommended strategy would be to cease treatment and avoid access until source water quality improves.

This study identifies carp biomass thresholds at which available technology and water treatment capacity can effectively deliver water to given safety standards. While these thresholds lie within predicted carp density loading indicated by biomass modelling, effective reduction of dead carp through effective removal strategies will be an important measure to safeguard against water quality risks.

Project materials developed

Two factsheets (one for industry and one for the general public) and a costing tool for water utilities (excel spreadsheet).

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Appendix A Jar Test data sheets

A1 River Murray (Anstey Hill) source water

Anstey Hill Raw – Alum only

Sample Id	Chemical Additives		Observations					Unfiltered			Filtered	<0.45µm Filtered					
	Alum as $Al_2(SO_4)_3.18H_2O$ (mg/L)	PAC PS1000	Initial Formation Time (min)	*Floc Size (1-7)	Settling Time 80% (min)	*Floc Size (1-7)	Final Settling Time 90% (min)	Turbidity (NTU)	pH (flocculation)	pH (settled)	Turbidity (NTU)	Colour (HU)	% Colour Removed	UV abs (/cm, 254nm)	% UV abs Removed	DOC (mg/L)	% DOC Removed
Raw								46.9		7.3		7		0.065		3.3	
J1	45	-	<1	1	3	5	6	2.8	6.5	6.6	0.14	2	71	0.034	48	2.1	36
J2	48	-	<1	1	4	5	9	2.3	6.4	6.5	0.23	2	71	0.034	48	2.4	27
J3	52	-	<1	1	4	5	9	2.2	6.3	6.4	0.10	1	86	0.032	51	2.1	36
J4	57	-	<1	1	4	5	9	2.1	6.1	6.2	0.13	1	86	0.030	54	1.9	42
J5	63	-	<1	1	4	5	9	2.0	6.0	6.1	0.07	1	86	0.028	57	1.9	42
J6	80	-	<1	1	4	5	9	2.8	5.6	5.7	0.10	1	86	0.026	60	1.9	42

* Floc Sizes: 1= 0.3-0.5mm 2= 0.5-0.75mm 3= 0.75-1.0mm 4= 1.0-1.5mm 5= 1.5-2.25mm 6= 2.25-3.0mm 7= 3.0-4.5mm

A2 Carp Density 0.05 kg/m^3 in River Murray

Carp Juice 0.05 kg/m^3 – Alum only

Sample Id	Chemical Additives		Observations					Unfiltered			Filtered	<0.45µm Filtered					
	Alum as $Al_2(SO_4)_3.18H_2O$ (mg/L)	PAC PS1000	Initial Formation Time (min)	*Floc Size (1-7)	Settling Time 80% (min)	*Floc Size (1-7)	Final Settling Time 90% (min)	Turbidity (NTU)	pH (flocculation)	pH (settled)	Turbidity (NTU)	Colour (HU)	% Colour Removed	UV abs (/cm, 254nm)	% UV abs Removed	DOC (mg/L)	% DOC Removed
Raw								44.6		7.3		8		0.073		4.1	
J1	46	-	<1	1	2	5	4	2.1	6.6	6.7	0.30	3	63	0.039	47	2.3	44
J2	49	-	<1	1	3	5	7	1.87	6.5	6.6	0.17	3	63	0.037	49	2.2	46
J3	54	-	<1	1	3	5	7	1.77	6.4	6.4	0.12	2	75	0.034	53	2.1	49
J4	59	-	<1	1	3	5	7	1.51	6.3	6.3	0.11	2	75	0.034	53	2.1	49
J5	66	-	<1	1	3	5	7	1.50	6.1	6.2	0.09	2	75	0.033	55	2.0	51
J6	84	-	<1	1	3	5	7	2.00	5.7	5.7	0.07	2	75	0.030	59	1.9	54

* Floc Sizes: 1= 0.3-0.5mm 2= 0.5-0.75mm 3= 0.75-1.0mm 4= 1.0-1.5mm 5= 1.5-2.25mm 6= 2.25-3.0mm 7= 3.0-4.5mm

A3 Carp Density 0.10 kg/m^3 in River Murray

Carp Juice 0.10 kg/m^3 – Alum only

Sample Id	Chemical Additives		Observations					Unfiltered			Filtered	<0.45µm Filtered					
	Alum as $Al_2(SO_4)_3.18H_2O$ (mg/L)	PAC PS1000	Initial Formation Time (min)	*Floc Size (1-7)	Settling Time 80% (min)	*Floc Size (1-7)	Final Settling Time 90% (min)	Turbidity (NTU)	pH (flocculation)	pH (settled)	Turbidity (NTU)	Colour (HU)	% Colour Removed	UV abs (/cm, 254nm)	% UV abs Removed	DOC (mg/L)	% DOC Removed
Raw								40.7		7.5		10		0.076		4.6	
J1	44	-	<1	1	3	6	5	1.77	6.7	6.7	0.17	3	70	0.044	42	2.7	41
J2	48	-	<1	1	3	6	5	1.55	6.6	6.7	0.14	2	80	0.042	45	2.5	46
J3	53	-	<1	1	3	6	5	1.44	6.4	6.6	0.20	2	80	0.039	48	2.4	48
J4	58	-	<1	1	3	6	5	1.38	6.3	6.4	0.15	2	80	0.038	50	2.4	48
J5	65	-	<1	1	3	6	5	1.50	6.1	6.2	0.11	2	80	0.036	53	2.3	50
J6	84	-	<1	1	3	6	5	1.45	5.7	5.7	0.19	1	90	0.032	58	2.2	52

* Floc Sizes: 1= 0.3-0.5mm 2= 0.5-0.75mm 3= 0.75-1.0mm 4= 1.0-1.5mm 5= 1.5-2.25mm 6= 2.25-3.0mm 7= 3.0-4.5mm

A4 Carp Density 0.25 kg/m^3 in River Murray

Carp Juice 0.25 kg/m^3 – Alum & Alum with PAC

Sample Id	Chemical Additives		Observations					Unfiltered			Filtered	<0.45µm Filtered					
	Alum as $Al_2(SO_4)_3.18H_2O$ (mg/L)	PAC PS1000	Initial Formation Time (min)	*Floc Size (1-7)	Settling Time 80% (min)	*Floc Size (1-7)	Final Settling Time 90% (min)	Turbidity (NTU)	pH (flocculation)	pH (settled)	Turbidity (NTU)	Colour (HU)	% Colour Removed	UV abs (/cm, 254nm)	% UV abs Removed	DOC (mg/L)	% DOC Removed
Raw								31.8		8.0		16		0.104		6.1	
J1	46	-	<1	1	3	6	5	1.59	6.7	6.9	0.28	5	69	0.059	43	4.0	34
J2	51	-	<1	1	3	6	5	1.12	6.6	6.8	0.21	5	69	0.058	44	3.6	41
J3	56	-	<1	1	3	6	5	0.97	6.5	6.7	0.17	4	75	0.054	48	3.5	43
J4	63	-	<1	1	3	6	5	0.78	6.3	6.6	0.12	4	75	0.051	51	3.6	41
J5	71	-	<1	1	3	6	5	0.69	6.1	6.3	0.13	3	81	0.047	55	3.1	49
J6	94	-	<1	1	4	6	6	1.04	5.4	5.6	0.14	2	88	0.043	59	2.8	54
J7	94	50						n/a	5.6	5.7	0.09	2	88	0.022	79	1.7	72

* Floc Sizes: 1= 0.3-0.5mm 2= 0.5-0.75mm 3= 0.75-1.0mm 4= 1.0-1.5mm 5= 1.5-2.25mm 6= 2.25-3.0mm 7= 3.0-4.5mm

A5 Carp Density 0.50 kg/m^3 in River Murray

Carp Juice 0.50 kg/m^3 – Alum & Alum with PAC

Sample Id	Chemical Additives		Observations					Unfiltered			Filtered	<0.45µm Filtered					
	Alum as $Al_2(SO_4)_3.18H_2O$ (mg/L)	PAC PS1000	Initial Formation Time (min)	*Floc Size (1-7)	Settling Time 80% (min)	*Floc Size (1-7)	Final Settling Time 90% (min)	Turbidity (NTU)	pH (flocculation)	pH (settled)	Turbidity (NTU)	Colour (HU)	% Colour Removed	UV abs (/cm, 254nm)	% UV abs Removed	DOC (mg/L)	% DOC Removed
Raw								13.58		8.9		26		0.165		10.3	
J1	49	-	<1	1	3	6	6	3.93	7.1	6.9	0.72	12	54	0.099	40	5.8	44
J2	56	-	<1	1	3	6	6	3.37	6.9	6.8	0.59	9	65	0.088	47	5.4	48
J3	63	-	<1	1	3	6	6	2.15	6.7	6.6	0.24	8	69	0.081	51	5.0	51
J4	73	-	<1	1	3	6	6	1.67	6.3	6.4	0.20	7	73	0.073	56	4.4	57
J5	84	-	<1	1	3	6	6	1.84	6.0	6.2	0.12	6	77	0.067	59	4.1	60
J6	114	-	<1	1	3	6	6	2.28	5.2	5.3	0.15	4	85	0.059	64	3.8	63
J7	114	50						n/a	5.2	5.3	0.22	2	92	0.035	79	2.7	74

* Floc Sizes: 1= 0.3-0.5mm 2= 0.5-0.75mm 3= 0.75-1.0mm 4= 1.0-1.5mm 5= 1.5-2.25mm 6= 2.25-3.0mm 7= 3.0-4.5mm

A6 Carp Density 1.00 kg/m^3 in River Murray

Carp Juice 1.00 kg/m^3 – Alum & Alum with PAC

Sample Id	Chemical Additives		Observations					Unfiltered			Filtered	<0.45µm Filtered					
	Alum as $Al_2(SO_4)_3.18H_2O$ (mg/L)	PAC PS1000	Initial Formation Time (min)	*Floc Size (1-7)	Settling Time 80% (min)	*Floc Size (1-7)	Final Settling Time 90% (min)	Turbidity (NTU)	pH (flocculation)	pH (settled)	Turbidity (NTU)	Colour (HU)	% Colour Removed	UV abs (/cm, 254nm)	% UV abs Removed	DOC (mg/L)	% DOC Removed
Raw								63.4		7.2		14		0.118		17.0	
J1	64	-	<1	1	3	6	4	2.5	6.8	6.8	0.31	9	36	0.116	2	15.7	8
J2	69	-	<1	1	4	6	6	1.51	6.7	6.7	0.33	8	43	0.112	5	15.2	11
J3	75	-	<1	1	4	6	6	1.39	6.6	6.7	0.18	8	43	0.110	7	15.6	8
J4	82	-	<1	1	4	6	6	1.33	6.6	6.6	0.21	7	50	0.106	10	15.6	8
J5	92	-	<1	1	4	6	6	1.09	6.5	6.5	0.13	6	57	0.102	14	15.8	7
J6	117	-	<1	1	2	7	3	0.95	6.3	6.3	0.13	4	71	0.091	23	15.3	10
J7	117	50						n/a	6.4	6.4	0.22	3	79	0.051	57	14.5	15

* Floc Sizes: 1= 0.3-0.5mm 2= 0.5-0.75mm 3= 0.75-1.0mm 4= 1.0-1.5mm 5= 1.5-2.25mm 6= 2.25-3.0mm 7= 3.0-4.5mm

Appendix B Disinfection

B1 Chlorination

2650

Anstey Hill composite - Control								
	Date: 26/03/2019				Order of Dose:			
	Target Monochloramine: NA				**Sample Volume: 1.5L**			
	Chlorine Dose: 4mg/L				Target pH 7.4			
	Ammonia Dose: NA				**0.2M NaOH: 1.2mL**			
	pH (pre dosing) adjusted							
Date	**Time (days)**	**Residual mg/L**				**pH**	**Temp °C**	**Free NH_3-N (mg/L)**
		Free Cl_2	**NH_2Cl**	**$NHCl_2$**	**Total**			
26/03/2019	0	4	-	-	-	7.6	22.8	
29/03/2019	3	1.55	0.11	0.12	1.78	7.6	21.8	

Carp 0.05 Alum only								
	Date:				Order of Dose:			
	Target Monochloramine:				**Sample Volume:**			
	Chlorine Dose: 4.5mg/L				Target pH 7.4			
	Ammonia Dose:				**0.2M NaOH: 1.0mL**			
	pH (pre dosing) adjusted							
Date	**Time (days)**	**Residual mg/L**				**pH**	**Temp °C**	**Free NH_3-N (mg/L)**
		Free Cl_2	**NH_2Cl**	**$NHCl_2$**	**Total**			
26/03/2019	0	4.5	-	-	-	7.5	23.2	
29/03/2019	3	0.94	0.13	0.10	1.17	7.3	21.3	

Carp 0.10 Alum only								
	Date:				Order of Dose:			
	Target Monochloramine:				**Sample Volume:**			
	Chlorine Dose: 6 mg/L				Target pH 7.4			
	Ammonia Dose:				**0.2M NaOH: 2.2mL**			
	pH (pre dosing) adjusted							
Date	**Time (days)**	**Residual mg/L**				**pH**	**Temp °C**	**Free NH_3-N (mg/L)**
		Free Cl_2	**NH_2Cl**	**$NHCl_2$**	**Total**			
26/03/2019	0	6	-	-	-	7.7	23.0	
29/03/2019	3	0.91	0.17	0.11	1.19	7.4	21.4	

	Carp 0.25 Alum only							
	Date:			Order of Dose:				
	Target Monochloramine:			**Sample Volume:**				
	Chlorine Dose:	**9.7mg/L**		Target pH		7.4		
	Ammonia Dose:			**0.2M NaOH:**		**2.8mL**		
	pH (pre dosing)		**adjusted**					
Date	**Time (days)**	**Residual mg/L**				**pH**	**Temp °C**	**Free NH_3-N (mg/L)**
		Free Cl_2	**NH_2Cl**	**$NHCl_2$**	**Total**			
26/03/2019	0	9.7	-	-	-	7.4	23.1	
29/03/2019	3	0.76	0.15	0.16	1.07	7.1	21.3	

	Carp 0.25 Alum + PAC							
	Date:			Order of Dose:				
	Target Monochloramine:			**Sample Volume:**				
	Chlorine Dose:	**9.0mg/L**		Target pH		7.4		
	Ammonia Dose:			**0.2M NaOH:**		**3.0mL**		
	pH (pre dosing)		**adjusted**					
Date	**Time (days)**	**Residual mg/L**				**pH**	**Temp °C**	**Free NH_3-N (mg/L)**
		Free Cl_2	**NH_2Cl**	**$NHCl_2$**	**Total**			
26/03/2019	0	9	-	-	-	7.5	23.6	
29/03/2019	3	0.73	0.14	-	1.03	7.1	21.3	

	Carp 0.50 Alum only							
	Date:			Order of Dose:				
	Target Monochloramine:			**Sample Volume:**				
	Chlorine Dose:	**18mg/L**		Target pH		7.4		
	Ammonia Dose:			**0.2M NaOH:**		**5.0mL**		
	pH (pre dosing)		**adjusted**					
Date	**Time (days)**	**Residual mg/L**				**pH**	**Temp °C**	**Free NH_3-N (mg/L)**
		Free Cl_2	**NH_2Cl**	**$NHCl_2$**	**Total**			
26/03/2019	0	18	-	-	-	7.2	23.4	
29/03/2019	3	0.77	0.24	0.25	1.26	6.6	21.3	

	Carp 0.50 Alum + PAC							
	Date:			Order of Dose:				
	Target Monochloramine:			**Sample Volume:**				
	Chlorine Dose:	**18mg/L**		Target pH		7.4		
	Ammonia Dose:			**0.2M NaOH:**		**5.0mL**		
	pH (pre dosing)		**adjusted**					
Date	**Time (days)**	**Residual mg/L**				**pH**	**Temp °C**	**Free NH_3-N (mg/L)**
		Free Cl_2	**NH_2Cl**	**$NHCl_2$**	**Total**			
26/03/2019	0	18	-	-	-	7.2	23.4	
29/03/2019	3	1.87	0.16	0.31	2.34	6.7	21.3	

B2 Chloramination

1000

Sample
0.02mg/L

Anstey Hill composite - Control	
Date: 26/03/2019	Order of Dose:
Target Monochloramine: **5 mg/L**	**Sample Volume: 2.5L**
Chlorine Dose: 5 mg/L	Target pH 8.4
Ammonia Dose: 1 mg/L	**0.2M NaOH: 3.5mL**
pH (pre dosing) adjusted	

Date	Time (days)	Residual mg/L				pH	Temp °C	Free NH_3-N (mg/L)
		Free Cl_2	NH_2Cl	$NHCl_2$	Total			
26/03/2019	0	-	5.00	-	-	8.7	23.2	
29/03/2019	3	<0.1	4.13	0.03	4.16	8.4	21.2	

Sample
0.14mg/L

Carp 0.05 Alum only	
Date:	Order of Dose:
Target Monochloramine: **5 mg/L**	**Sample Volume:**
Chlorine Dose: 5 mg/L	Target pH 8.4
Ammonia Dose: 1 mg/L	**0.2M NaOH: 4.0mL**
pH (pre dosing) adjusted	

Date	Time (days)	Residual mg/L				pH	Temp °C	Free NH_3-N (mg/L)
		Free Cl_2	NH_2Cl	$NHCl_2$	Total			
26/03/2019	0	-	5.00	-	-	8.7	23.0	
29/03/2019	3	<0.1	3.94	0.06	4.00	8.3	21.1	

Sample
0.27mg/L

Carp 0.10 Alum only	
Date:	Order of Dose:
Target Monochloramine: **6 mg/L**	**Sample Volume:**
Chlorine Dose: 6 mg/L	Target pH 8.4
Ammonia Dose: 1.2 mg/L	**0.2M NaOH: 4.0mL**
pH (pre dosing) adjusted	

Date	Time (days)	Residual mg/L				pH	Temp °C	Free NH_3-N (mg/L)
		Free Cl_2	NH_2Cl	$NHCl_2$	Total			
26/03/2019	0	-	6.00	-	-	8.7	22.8	
29/03/2019	3	<0.1	3.87	0.05	3.92	7.9	21.1	

Sample 0.52mg/L

Carp 0.25 Alum only								
Date:				Order of Dose:				
Target Monochloramine:			6 mg/L	Sample Volume:				
Chlorine Dose: 6 mg/L				Target pH		8.4		
Ammonia Dose:		1.2 mg/L		**0.2M NaOH:**		3.5mL		
pH (pre dosing)			adjusted					
Date	**Time (days)**	**Residual mg/L**				**pH**	**Temp °C**	**Free NH_3-N (mg/L)**
		Free Cl_2	**NH_2Cl**	**$NHCl_2$**	**Total**			
26/03/2019	0	-	6.00	-	-	8.6	22.8	
29/03/2019	3	<0.1	4.10	0.04	4.14	8.0	21.1	

Sample 0.60mg/L

Carp 0.25 Alum + PAC								
Date:				Order of Dose:				
Target Monochloramine:			6 mg/L	Sample Volume:				
Chlorine Dose: 6 mg/L				Target pH		8.4		
Ammonia Dose:		1.2 mg/L		**0.2M NaOH:**		3.75mL		
pH (pre dosing)			adjusted					
Date	**Time (days)**	**Residual mg/L**				**pH**	**Temp °C**	**Free NH_3-N (mg/L)**
		Free Cl_2	**NH_2Cl**	**$NHCl_2$**	**Total**			
26/03/2019	0	-	6.00	-	-	8.8	22.9	
29/03/2019	3	<0.1	4.26	0.07	4.33	8.1	21.4	

Sample 0.99mg/L

Carp 0.50 Alum only								
Date:				Order of Dose:				
Target Monochloramine:			6 mg/L	Sample Volume:				
Chlorine Dose: 6 mg/L				Target pH		8.4		
Ammonia Dose:		1.2 mg/L		**0.2M NaOH:**		4.05mL		
pH (pre dosing)			adjusted					
Date	**Time (days)**	**Residual mg/L**				**pH**	**Temp °C**	**Free NH_3-N (mg/L)**
		Free Cl_2	**NH_2Cl**	**$NHCl_2$**	**Total**			
26/03/2019	0	-	6.00	-	-	8.8	22.9	
29/03/2019	3	<0.1	3.36	0.02	3.38	8.3	21.4	

Sample 0.97mg/L

Carp 0.50 Alum + PAC								
Date:				Order of Dose:				
Target Monochloramine:			6 mg/L	Sample Volume:				
Chlorine Dose: 6 mg/L				Target pH		8.4		
Ammonia Dose:		1.2 mg/L		**0.2M NaOH:**		3.8mL		
pH (pre dosing)			adjusted					
Date	**Time (days)**	**Residual mg/L**				**pH**	**Temp °C**	**Free NH_3-N (mg/L)**
		Free Cl_2	**NH_2Cl**	**$NHCl_2$**	**Total**			
26/03/2019	0	-	6.00	-	-	8.7	22.9	
29/03/2019	3	<0.1	3.84	0.05	3.89	8.3	21.2	

Appendix C Disinfection By-products

C1 Chlorinated DBPs

Table 7: Chlorinated (Cl_2) DBPs for treated carp impacted waters grouped by class

Carp density (kg/m³) A = alum; P = PAC	0.00 A	0.05 A	0.10 A	0.25 A	0.25 A + P	0.50 A	0.50 A + P
Trihalomethanes							
Bromodichloromethane	29	29	28	30	20	30	26
Bromoform	4	3	3	2	3	<1	1
Chloroform	26	24	26	33	14	51	33
Dibromochloromethane	20	18	19	16	15	10	11
Total THMs (THM4)	**79**	**74**	**76**	**81**	**52**	**91**	**71**
Haloacetic Acids							
Bromoacetic Acid	1	1	1	1	1	2	2
Bromochloroacetic Acid	9	8	8	10	7	11	10
Bromodichloroacetic Acid	10	9	10	13	6	17	15
Chloroacetic Acid	<3	<3	<3	<3	<3	6	<3
Dibromoacetic Acid	4	4	4	3	4	2	3
Dibromochloroacetic Acid	4	3	4	3	2	3	4
Dichloroacetic Acid	10	9	10	15	7	30	20
Tribromoacetic Acid	<1	<1	<1	<1	<1	<1	<1
Trichloroacetic Acid	7	8	8	13	4	29	18
Total HAAs (HAA9)	**45**	**42**	**45**	**58**	**31**	**100**	**72**
Haloketones							
1,1,1-trichloropropan-2-one	1.7	2.8	2.5	4.9	2.2	10.4	6.6
1,1,3-trichloropropan-2-one	<1	<1	<1	<1	<1	<1	<1
1,1-dichloropropan-2-one	<1	<1	<1	<1	<1	<1	<1
1,3-dichloropropan-2-one	<1	<1	<1	<1	<1	<1	<1
Total Haloketones	**4.7**	**5.8**	**5.5**	**7.9**	**5.2**	**13.4**	**9.6**
Haloacetonitriles							
Bromochloroacetonitrile	3.3	4.2	4.5	6	4	7.5	6.1
Dibromoacetonitrile	2.5	2.8	3.1	2.9	2.8	2.2	2.5
Dichloroacetonitrile	1.8	2.8	2.9	5.6	2.8	12.6	7.6
Trichloroacetonitrile	<1	<1	<1	<1	<1	<1	<1
Total Haloacetonitriles	**8.6**	**10.8**	**11.5**	**15.5**	**10.6**	**23.3**	**17.2**
Others							
Chloral Hydrate	5.8	6.3	7.2	9.9	5.6	21.2	12.8
Chloropicrin	<1	<1	<1	<1	<1	<1	<1
Dibromonitromethane	<1	<1	<1	<1	<1	<1	<1
N-Nitrosodimethylamine	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Absorbable Organic Halides	**190**	**185**	**191**	**251**	**144**	**409**	**203**

C1 Chloraminated DBPs

Table 8: Chloraminated (NH_2Cl) DBPs for treated carp impacted waters grouped by class

Carp density (kg/m^3) A = alum; P = PAC	0.00 A	0.05 A	0.10 A	0.25 A	0.25 A + P	0.50 A	0.50 A + P
Trihalomethanes							
Bromodichloromethane	<1	<1	4	<1	<1	2	<1
Bromoform	<1	<1	<1	<1	<1	<1	<1
Chloroform	2	<1	2	2	<1	4	2
Dibromochloromethane	<1	<1	2	<1	<1	<1	<1
Total THMs (THM4)	**5**	**<4**	**8**	**5**	**<4**	**8**	**5**
Haloacetic Acids							
Bromoacetic Acid	<1	<1	<1	<1	<1	<1	<1
Bromochloroacetic Acid	1	1	2	2	1	1	1
Bromodichloroacetic Acid	<1	<1	<1	<1	<1	<1	<1
Chloroacetic Acid	<3	<3	<3	<3	<3	<3	<3
Dibromoacetic Acid	<1	<1	1	<1	1	<1	<1
Dibromochloroacetic Acid	<1	<1	<1	<1	<1	<1	<1
Dichloroacetic Acid	3	3	2	3	2	5	4
Tribromoacetic Acid	<1	<1	<1	<1	<1	<1	<1
Trichloroacetic Acid	<1	<1	<1	<1	<1	<1	<1
Total HAAs (HAA9)	**11**	**11**	**11**	**11**	**11**	**15**	**14**
Haloketones							
1,1,1-trichloropropan-2-one	<1	<1	<1	<1	<1	<1	<1
1,1,3-trichloropropan-2-one	<1	<1	<1	<1	<1	<1	<1
1,1-dichloropropan-2-one	<1	<1	<1	<1	<1	<1	<1
1,3-dichloropropan-2-one	<1	<1	<1	<1	<1	<1	<1
Total Haloketones	**<4**	**<4**	**<4**	**<4**	**<4**	**<4**	**<4**
Haloacetonitriles							
Bromochloroacetonitrile	<1	<1	<1	<1	<1	<1	<1
Dibromoacetonitrile	<1	<1	<1	<1	<1	<1	<1
Dichloroacetonitrile	<1	<1	<1	<1	<1	<1	<1
Trichloroacetonitrile	<1	<1	<1	<1	<1	<1	<1
Total Haloacetonitriles	**<4**	**<4**	**<4**	**<4**	**<4**	**<4**	**<4**
Others							
Chloral Hydrate	<1	<1	<1	<1	<1	<1	<1
Chloropicrin	<1	<1	<1	<1	<1	<1	<1
Dibromonitromethane	<1	<1	<1	<1	<1	<1	<1
N-Nitrosodimethylamine	19	17	30	13	17	20	10
Absorbable Organic Halides	**30**	**45**	**57**	**134**	**43**	**81**	**61**

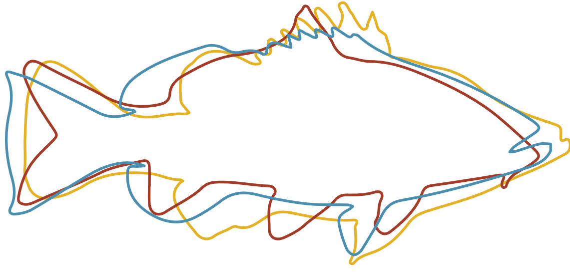
FRDC FINAL REPORT CHECKLIST

Project Title:	NCCP: Risks, costs and water industry response		
Principal Investigators:	R. Fabris, T.Kildea, L. van der Linden, M. Lau, J.Pera		
Project Number:	2017/237		
Description:	This report details the water treatment strategy presented by mass carp mortality, considering a range of potential densities based on the most recent NCCP assessments and recently collected data. Conventional alum coagulation, with and without PAC dosing, together with disinfection (chlorine and monochloramine) was investigated. Membrane fouling potential was also briefly considered. Additionally, the impact on product water quality from the perspective of aesthetics and disinfection by-products (regulated and novel) was explored, resulting in recommendations for optimising treatment through appropriate dose rates of coagulant and activated carbon products.		
Published Date:	18/12/2019	**Year:**	2019
ISBN:	978-1-921732-56-0	**ISSN:**	
Key Words:	Biomass, water quality, treatment		

Please use this checklist to self-assess your report before submitting to FRDC. Checklist should accompany the report.

	Is it included (Y/N)	**Comments**
Foreword (optional)	N	Not required
Acknowledgments	Y	
Abbreviations	N	
Executive Summary	Y	
− **What the report is about**	Y	
− **Background – why project was undertaken**	Y	
− **Aims/objectives – what you wanted to achieve at the beginning**	Y	
− **Methodology – outline how you did the project**	Y	
− **Results/key findings – this should outline what you found or key results**	Y	
− **Implications for relevant stakeholders**	Y	
− **Recommendations**	Y	
Introduction	Y	
Objectives	Y	
Methodology	Y	
Results	Y	
Discussion	N	
Conclusion	Y	
Implications	Y	
Recommendations	Y	
Further development	N	

Extension and Adoption	N	
Project coverage	N	
Glossary	Y	
Project materials developed	Y	
Appendices	Y	



The National Carp Control Plan is managed by the
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