TECHNICAL PAPER 3

NATIONAL CARP CONTROL PLAN

Carp biocontrol and water quality

This suite of documents contains those listed below.

NCCP TECHNICAL PAPERS

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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
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Will carp virus biocontrol be effective?

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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
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Technical Paper 3: Carp biocontrol and water quality

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1.0 About this paper

If Australian governments, after considering relevant evidence and completing mandatory regulatory approvals, eventually choose to implement a biological control program for the pest fish European Carp (*Cyprinus carpio*) (hereafter 'carp') using the virus called Cyprinid herpesvirus 3, large numbers of dead carp would likely result. If some or all of these dead carp were left to decay in waterways, various negative impacts to water quality could potentially ensue. This technical paper explores these potential impacts by providing a synthesis of water-quality research conducted under the National Carp Control Plan (NCCP). The paper covers four key aspects of water quality, consideration of which will be vital in determining future directions for carp biocontrol in Australia. These aspects are:

- dissolved oxygen (DO) responses to the in-situ decomposition of dead carp,
- the potential for nutrients derived from carp decomposition to promote cyanobacterial (harmful algae) blooms,

- the extent to which water treatment plants will be able to cope with water affected by decomposing carp, and additional costs that treating water affected by carp decomposition may entail, and
- the potential for decomposing carp to degrade water quality to the extent that conditions become suitable for the proliferation of dangerous pathogens, including the bacteria that cause botulism.

2.0 Why study water quality?

If Cyprinid herpesvirus 3 (CyHV-3) is deployed in Australian waterways as a biological control agent, major mortalities of common carp are expected as the virus causes outbreaks in targeted sub-populations. Precautionary planning for carp biocontrol needs to acknowledge the possibility that decomposing virus-killed carp in waterbodies could negatively affect water quality, potentially impacting human consumptive and recreational use, livestock, and natural values. Water-quality research under the NCCP aimed to understand the extent to which water quality could be compromised by decomposing carp, and the times and locations at which risk is likely to be greatest. Water-quality research under the program also considered potential impacts on water treatment processes, and how these impacts could be minimised. Indeed, identifying possible mitigation strategies is an important focus of all research projects under this theme. Water quality parameters and characteristics covered by the NCCP research program, and the manner in which they are addressed, are summarised in Table 1.

Table 1: Water quality parameters addressed by NCCP research and planning

Item #	Water quality parameter	How addressed	Quantitative or qualitative treatment?
1	Dissolved oxygen	Purpose-designed modelling study, supported by field data	Quantitative
2	Nutrient enrichment and associated cyanobacterial risk	Purpose-designed modelling study, supported by field data	Quantitative
3	Treatability of water containing decomposing carp	Empirical laboratory experiments	Quantitative
4	Botulism risk	Assessed in broader NCCP risk assessment	Qualitative (literature review)
5	Other secondary infections arising from poor water quality	Assessed in broader NCCP risk assessment	Qualitative (literature review)
6	All of the above	Operational strategies for managing carp carcasses	Qualitative and quantitative (scenario workshops building on NCCP biomass estimation and epidemiological research)

2.1 Why are the selected parameters important?

Conceptually, the parameters outlined in Table 1 divide naturally into two primary classes; 'direct effects' of dead carp on water quality or water treatment processes (items 1–3) (e.g. reductions in DO concentrations), and potential 'secondary impacts' that could arise as a consequence of the direct effects (items 4 and 5). The most obvious example of a secondary impact is the potential for outbreaks of botulism, a serious disease of animals and humans caused by bacteria of the genus *Clostridium*, especially *Clostridium botulinum*. The bacteria that cause botulism require anaerobic (no oxygen)

conditions, and a protein-rich substrate for growth (see review in Beckett et al., 2019). Consequently, major carp mortalities leading to very low DO concentrations and an abundance of decaying fish flesh in waterbodies could establish the preconditions necessary for a botulism outbreak.

Secondary impacts are essentially consequences of direct effects, so it follows that understanding the risks posed by the latter, and developing effective mitigation measures for them, often represents the most effective way to control secondary impacts. The direct and secondary impacts listed in Table 1 are briefly summarised below to provide an overview of the water-quality matters encompassed by the NCCP research program. Readers seeking more detailed explanations of these impacts are directed to Brookes and Hipsey (2019) (DO and cyanobacteria), Beckett et al. (2019) (botulism and secondary infections), and Fabris et al. (2019) (water treatment).

2.2 Introduction to direct effects

2.2.1 Impacts on dissolved oxygen

Aquatic organisms that extract oxygen from the water for respiration and metabolism (i.e. that do not breathe air) require the presence of DO in the water at concentrations (typically expressed as milligrams of oxygen per litre, mg/L) that varies between species. In general, most Australian native fish species cannot tolerate DO levels of less than 3 mg/L for extended periods, and are likely to be stressed when DO drops below 4–5 mg/L (Beckett et al., 2019). The Basin Plan sets targets for river channels and anabranch creeks of ≥50% saturation with a DO concentration of 4.5 mg/L, and these values are widely accepted as critical for Australian aquatic biota (noting that a 2020 review of Basin Plan water-quality targets recommended some refinement of DO targets) (Beckett et al., 2019; RM Consulting Group, 2020). Based on the Basin Plan guidelines, Beckett et al. (2019) suggest that 6 mg/L would provide a useful 'trigger value' for initiation of management intervention during carp mortality events. In this paper, the term 'hypoxia' refers to low DO levels, while 'anoxia' refers to a total absence of DO (i.e. 0 mg/L).

Major fish kills, including CyHV-3-induced carp mortalities, have the potential to reduce DO, or cause dramatic fluctuations in DO levels, because respiration by the abundant bacteria using nutrients from the decaying fish consumes oxygen. If the microbes using the nutrients from decaying carp are predominantly cyanobacteria (blue-green algae), then the pattern that emerges may best be characterised as large daily fluctuations in DO, rather than sustained suppression (Brookes and Hipsey, 2019). Such fluctuations arise because cyanobacteria photosynthesise, so aquatic habitats in which these organisms are abundant can experience elevated DO during the day, when oxygen is produced as a by-product of cyanobacterial photosynthesis, and low DO at night, when cyanobacteria continue to respire (i.e. use up oxygen) but do not produce oxygen (Brookes and Hipsey, 2019).

2.2.2 Nutrient enrichment and associated cyanobacterial risk

Nutrients, especially phosphorus (as phosphate, PO_4) and nitrogen (as nitrate, NO_3, and ammonium NH_4), are liberated from dead fish decaying in aquatic habitats. These nutrients fuel the growth of many algal and microbial species. In a river with flowing water, and consequent mixing through the water column, these nutrients are likely to be used by beneficial microorganisms that facilitate nutrient cycling. In rivers with no, or low, flow, these nutrients may be used by cyanobacteria, including species that produce toxins harmful to humans and animals. Thus, there is a risk that nutrients derived from carp kills could fuel major cyanobacterial blooms. These blooms could in turn have negative 'feedback effects' on water quality. In particular, bloom collapse and decay creates an additional biological oxygen demand, exacerbating problems with hypoxia and anoxia (Brookes and Hipsey, 2019).

Cyanobacteria are most likely to proliferate in aquatic habitats that are stratified (i.e. where the water column divides into a distinct warmer, oxygenated surface layer and a colder, oxygen-poor deeper layer). Stratification is in turn largely a product of low-flow conditions, as waterbodies are typically only able to stratify when flows are too low to cause mixing through the water column. Thus, development of cyanobacterial blooms requires the co-occurrence of high nutrient levels, warm temperatures, and low- or no-flow hydrological conditions (Brookes and Hipsey, 2019).

In addition to the potential role of nutrients from dead carp in fuelling cyanobacterial blooms, ammonia in its un-ionised form (NH_3) is directly toxic to aquatic organisms (although ionised ammonia can also be toxic under some conditions) (Australian Government, Australian & New Zealand Guidelines for Fresh & Marine Water Quality, 2019). The proportion of total ammonia that is in the un-ionised (i.e. toxic) form depends on temperature and pH (Australian Government, Australian & New Zealand Guidelines for Fresh & Marine Water Quality, 2019). For example, at 20°C and pH 8.5, un-ionised ammonia contributes approximately 11% to the total ammonia concentration, whereas at the same temperature and pH 6, it contributes approximately 0.04% (CCREM, 1987, cited in Australian and New Zealand Guidelines for Fresh and Marine Water Quality, 2019). Susceptibility to ammonia toxicity also varies among and within various taxa (Hickey and Vickers, 1994; Richardson, 1997). Because the proportions of ionised and un-ionised ammonia present in any situation depend upon temperature, pH, and numerous other factors, water quality research in the NCCP reports total ammonium (NH_4), and sets the tolerable limit as 0.5 mg/L (Brookes and Hipsey, 2019).

2.2.3 Water treatability

Prior to any potential future CyHV-3 deployment, water treatment plant operators need to understand whether expected dead carp loadings are likely to be of a magnitude that compromises their capacity to treat water such that it is safe and aesthetically acceptable (i.e. free from unpleasant smells, tastes, and colours). Project aims for the water-treatment research co-funded by the NCCP in partnership with state and regional water authorities (Fabris et al., 2019) were to determine:

- whether small amounts of residual carp breakdown product could impair product water quality in the absence of enhanced or optimised water treatment (i.e. if no additional water treatment activities were conducted),
- whether additional water treatment chemicals will be required to maintain water health and aesthetic characteristics,
- whether there is a threshold carp density (and associated concentration of carp decay products) at which the production of drinking-water quality to acceptable standards cannot be maintained, and
- if carp-derived disinfection by-products (i.e. chemicals produced during the water disinfection process) have the potential to cause adverse health impacts.

2.3 Introduction to secondary impacts

2.3.1 Botulism outbreaks

Decomposition of carp in aquatic habitats could result in nutrient- and protein-enriched and/or hypoxic or anoxic conditions suitable for the proliferation of various pathogenic microbes. Botulism risk is discussed in greatest detail, because fish kills (regardless of cause) are theoretically well-suited to initiating botulism outbreaks by fulfilling the two basic preconditions for *Clostridium botulinum* growth and toxin production.

3.0 Interdependencies in the NCCP research program and how these affect understanding of water-quality impacts

As with most aspects of the NCCP research program, the various projects assessing water quality rely on data and knowledge from other projects within the program to formulate their projections. Understanding these interdependencies is crucial to clearly understanding the manner in which uncertainty and measurement error propagate through the various projects in the program, and hence to understanding the confidence with which outcomes of virus release can be predicted.

All NCCP projects with a primary focus on water quality depend on the NCCP carp biomass estimation and epidemiological modelling projects. These dependencies arise because biomass estimates indicate total carp biomass (in kg/ha) present in particular habitat types throughout the species' Australian distribution, while epidemiological modelling indicates the proportion of that biomass likely to be killed by the virus, and the biophysical and ecological conditions under which kills are likely to occur. Because uncertainty is inevitable in both biomass estimation and epidemiological modelling, the water quality modelling projects have all investigated scenarios involving considerably higher carp biomasses (and larger kills) than are expected to understand 'worst case' (from a water-quality perspective) scenarios.

4.0 Interpreting NCCP water quality research: the environmental context for impacts

In many Australian inland and coastal aquatic ecosystems, the hydrological, geomorphological, and biological processes that regulate DO levels and nutrient inputs have undergone considerable alteration relative to pre-European conditions (see Technical Paper 1 for details). In particular, DO dynamics within many Murray-Darling Basin (MDB) catchments have been influenced by flow regulation leading to accumulation of plant material on floodplains and subsequent deoxygenation ('blackwater events') when floodplains are inundated (Stuart and Jones, 2006). Similarly, factors such as flow regulation and extreme climate and weather events can promote prolonged and spatially extensive cyanobacterial blooms (Lal and Hargreaves, 2020; Grentell et al., 2022). Thus, the information presented here should be interpreted in the context that any impacts resulting from carp decomposition take place against a complex backdrop of other water-quality issues. Indeed, the presence of high live-carp densities in a waterway may itself be linked to reduced water quality including increased risk of cyanobacterial blooms.

A striking example of the need to consider the pre-existing environmental conditions upon which decaying carp impacts would be superimposed (without effective carcass management) is provided by the ephemeral dryland rivers of the northern MDB (Zhai et al., 2022). During dry times, these rivers shrink to disconnected waterholes, the more permanent of which provide drought refuges for native fish and other aquatic organisms, including those of high conservation concern (Zhai et al., 2022). While representing the only available aquatic habitats during dry times, these refugial holes are difficult places for many native species to inhabit. Throughout most of their depth profile, refugial waterholes typically present DO levels of 2 mg/L or less, which is at or below the extreme tolerances of most native fish species (Zhai et al., 2022). Water-temperature regimes in these environments are similarly inhospitable to native fish. Climate change is projected to further decrease the suitability of these habitats for native fish (Zhai et al., 2022). Carp have much broader DO and temperature tolerances than most Australian native fishes, and can tolerate these conditions with relative ease (Zhai et al., 2022). Whether native fish populations inhabiting these waterholes have evolved physiological adaptations to improve their tolerance to conditions in these waterholes is unclear.

Decision making on carp biocontrol needs to account for these complex histories of DO and nutrient input variability. In locations where low DO levels and potential for cyanobacterial blooms already cause problems, the potential for virus-induced carp mortalities to exacerbate these issues must be considered. These considerations are pertinent to the dryland refugial waterholes described previously, where even minor increases in biological oxygen demand as a result of carp decomposition could lead to native fish deaths, potentially leaving carp as the only surviving fish species in these environments. Conversely, in many other environments, the absolute values for DO sag (decline in dissolved oxygen levels through time) and nutrient input reported by NCCP research are, with some exceptions, within the range of currently observed variability, implying that any additional oxygen demand and nutrient input will not extend outside the range of values already observed in many systems (Brookes and Hipsey, 2019). Exceptions to this generalisation are discussed in section 5.1.2.

5.0 Water quality project summaries

5.1 Dissolved oxygen and cyanobacterial risk

These two projects, combined to form NCCP research project 9 (Brookes and Hipsey, 2019), used the same modelling platform and case-study sites, and are therefore discussed together. As with other key projects in the NCCP research program, water quality modelling used a 'case-study' approach, in which study sites with qualities representing some of the diversity found across carp's Australian distribution were selected for detailed simulation. For DO and cyanobacterial modelling, locations were selected to encompass diverse hydrologies and geomorphologies broadly representative of those occurring across carp's Australian distribution (Brookes and Hipsey, 2019). This case-study approach was necessary because the modelling reported here is both comprehensive and detailed, making its extension to carp's entire Australian distribution prohibitively time-consuming and costly. If required, the models can be extended to additional sites. The four modelled locations, with rationale for their selection, are listed in Table 2.

Table 2: Overview of sites selected for anoxia/hypoxia and cyanobacterial risk following major carp mortalities resulting from biocontrol using Cyprinid herpesvirus 3. Adapted from Brookes and Hipsey (2019)

Site	State	Rationale for selection
Lock 1 to Swan Reach (Lower Murray River)	SA	An important river channel reach with extensive connections to shallow wetlands with periodic connectivity. Downstream site in the Murray-Darling Basin, likely to experience high dead carp loadings during biocontrol operations.
Tailem Bend to Murray Bridge (Lower Murray River)	SA	As above, including several sites of concern to water treatment plant offtakes.
Chowilla	SA	A geomorphologically and hydrologically complex system with regulated flows and locks likely to exhibit complex patterns of carp accumulation. Chowilla has high carp densities, and high environmental values.
Lower Lakes	SA	Shallow lake/wetland system of regional significance. Existing data and model calibration make this site a safe test-case. Potential for accumulation in Lake Albert or shallow areas around barrages.
Moonie River (portion only)	Qld	Inland river system in northern Murray-Darling Basin, subject to seasonal reductions in river pool connectivity and warm temperatures.

The model platform is the three-dimensional coupled model TUFLOW-FV (hydrodynamics) and AED-2 (water quality). This model platform has been developed and refined for over a decade, and is designed for modelling hydrodynamic, sediment transport, and water quality processes in aquatic and marine environments (see https://www.tuflow.com/Tuflow%20FV.aspx for details and Bruce et al. (2014) for an example). The platform can model stratification (Brookes and Hipsey, 2019).

The overall modelling approach for understanding DO and cyanobacterial risk is shown in Figure 1. First, the ecohydrological models simulate the fundamental processes by which the study ecosystems function. While the models capture several different aspects or parameters of ecosystem function, DO and temperature were the primary focus. Second, a carp mortality model adds dead carp to the system and simulates the effects of their decay on the parameters of interest. Both the ecohydrological model and the carp mortality model were validated by comparing model outputs with either field monitoring (ecohydrology) or experimental data.

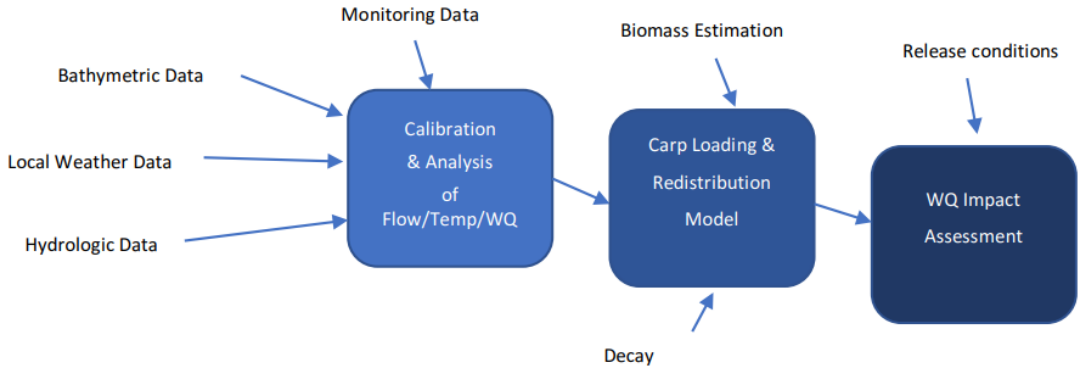

Figure 1: Conceptual representation of the water-quality modelling approach used in NCCP research. WQ = water quality. Source: (Brookes and Hipsey, 2019)

The approach taken to understand cyanobacterial risk involved first modelling the flux of nutrients from decomposing carp (derived from experiments), then combining this information with the other variables that lead to cyanobacterial bloom formation. Both DO and nutrient flux modelling use linked models that enable exploration of water flow patterns (hydrodynamic model) and the various biological and biochemical/geochemical processes that influence and regulate the variables of interest (biogeochemical model). Additionally, a 'carp particle model' mimics the manner in which dead carp are added to the modelled system, and their accumulation and movement (by wind and water currents at various scales) following addition.

The carp particle model uses two basic approaches; the 'homogenised carp' (HC) approach which assumes that dead carp are distributed evenly throughout the modelled system, and the more realistic 'decaying carp particles' (DCP) approach in which dead carp are added to, and move through, the system in a manner that reflects likely mortality patterns following a CyHV-3 outbreak and subsequent transport and accumulation of dead carp through wind and water movement. Additions of dead carp to the system occur in amounts reflecting results of the NCCP carp biomass estimates and epidemiological modelling, with higher carp densities (i.e. 2–5 times greater than those expected) also modelled to define worst-case (from a water-quality perspective) scenarios. In the DCP approach, a

carp 'particle' doesn't necessarily represent an individual fish, but rather a representative set of fish, with a defined mass (Brookes and Hipsey, 2019).
Fundamentally, DO dynamics represent a balance between the processes that deplete oxygen, and those that replenish it, such as river flows, wind, and wave action (Brookes and Hipsey, 2019). Thus, areas subject to effective re-aeration can, on average, tolerate higher dead carp loadings before DO is depleted to levels that endanger aquatic life than can areas where re-aeration through natural processes is limited (Brookes and Hipsey, 2019).

An important distinction also needs to be drawn between DO and nutrient impacts following carp mortalities. Regardless of how low DO levels may drop, they are eventually reset by various natural processes over time periods that vary depending upon the severity of the initial deoxygenation and the intensity of the processes driving re-oxygenation. In contrast, nutrients are more likely to remain in the environment, potentially causing legacy issues, such as ongoing cyanobacterial blooms, into the future (Brookes and Hipsey, 2019).

5.1.1 Metrics for reporting dissolved oxygen impacts, nutrient levels, and cyanobacterial risk

The impacts of in-situ carp carcass decomposition on DO were assessed and reported by calculating oxygen sag using a mathematical formula. A similar approach was used for nutrients, with the mean (average) increase in nutrient concentrations calculated over the carp mortality and decay period. Each of the modelled ecosystems comprise a complex network of existing factors influencing DO and nutrient dynamics, and the research accounts for these histories by reporting both variables (DO and nutrients) as the extent to which carp mortality and decomposition has increased risks. For NH_4, the frequency with which the tolerable limit of 0.5 mg/L was reached was also computed as an indicator of the potential for ammonia toxicity (Brookes and Hipsey, 2019). Modelling uses a model 'mesh', which covers the modelled ecosystems with a net-like spatial arrangement of polygons. Carp decomposition and its effects on the various water quality parameters are modelled within each of these polygons, providing a fine spatial resolution (Brookes and Hipsey, 2019).

In contrast to oxygen and nutrient dynamics, which can be reported as stand-alone variables, the formation of cyanobacterial blooms is a complex process, requiring the coincidence of hydrological, biogeochemical, and meteorological factors favourable for algal growth. Therefore, rather than attempting to use a single measure of algal risk, a metric called the HAB Score Index (HSI) was used. The acronym 'HAB' signifies 'harmful algal bloom'. The formula for calculating HSI incorporates temperature, light levels, water velocity (i.e. flow), the degree of vertical stratification, and the nutrient levels available to fuel cyanobacterial growth. As for both DO and nutrients, HSI is reported as 'change in HSI' (ΔHSI), providing a measure of the way in which carp decomposition has increased risks relative to the conditions prevailing prior to carp deaths (Brookes and Hipsey, 2019). Mathematical formulas for deriving all metrics described above are reported in full by Brookes and Hipsey (2019).

5.1.2 Key results for dissolved oxygen, nutrient, and cyanobacterial modelling

The overall picture to emerge from DO and cyanobacterial modelling is one in which risk is focussed in particular locations and habitat types, such as the refugial waterholes described in 4.0. These 'pockets' of relatively higher risk lie within a broader matrix in which hypoxia/anoxia, cyanobacterial, and ammonia toxicity risk are within the range of variability currently observed for these parameters. That is, throughout most of the four case study locations, the risks posed by in-situ decomposition of carp carcasses at the densities indicated by the NCCP biomass estimation project (Stuart et al., 2019) do not pose unmanageable risks to DO, nutrient levels, and cyanobacterial blooms.

Nonetheless, results indicating a generally low level of risk associated with negative DO and cyanobacterial outcomes must be carefully qualified and contextualised, as modelling did reveal some important higher-risk areas for oxygen depletion, potential ammonia toxicity, and cyanobacterial risk. Similarly, some uncertainties and knowledge gaps remain, rendering careful interpretation of results imperative. One of the most important general conclusions to emerge from the research was that risk is not evenly distributed among or within the various case study sites. Shallower habitats that are not directly linked to flows in main river channels are particularly susceptible to DO sag, elevated nutrient levels, increased ammonia toxicity risk, and cyanobacterial blooms, largely reflecting limited hydrodynamic flushing in these locations. Risks of negative water-quality outcomes are particularly high if locations with the characteristics described above also feature carp biomass of 300 kg/ha or more (Brookes and Hipsey, 2019). These results reinforce the basic tenet that water quality outcomes following carp kills will reflect local relationships between biomass loading and hydrodynamic flushing (Brookes and Hipsey, 2019). Elevated cyanobacterial risks in these locations reflect the increased likelihood of thermal stratification typical of low flow conditions, exacerbated by high nutrient availability from decomposing carp.

Of the four case-study site, Chowilla emerged as the highest risk area for water quality impacts, including hypoxia and anoxia, cyanobacterial risk, and ammonia accumulation (with associated risk of ammonia toxicity). The higher risks modelled for Chowilla relative to the other case-study sites reflect the geomorphology, hydrology, and environmental history of this unique area. The Chowilla case-study site features numerous shallow wetland and lake habitats that do not directly experience flows from the main river channel, have a high carp biomass, and a history of low DO events. Superimposing the in-situ decomposition of large numbers of carp onto this suite of characteristics results in increased risk.

Another risk identified by the modelling is the potential for nutrients from decaying carp to become sequestered in benthic sediments, where they could potentially fuel cyanobacterial blooms in the future. The extent to which such 'legacy nutrients' are likely to be problematic is unclear; Australian aquatic ecosystems already harbour sediment nutrient loadings of various magnitudes, so those derived from carp may, like many of the variables addressed in the modelling, simply fall within the range of currently observed variability. Nonetheless, planning for post-release clean-up should, wherever possible include manipulation of flows to encourage oceanic export of nutrients.

Modelling also incorporated dead carp biomasses up to five times higher than those reported by the NCCP carp biomass estimation project. Modelling these higher biomasses provides an understanding of the water-quality impacts associated with 'worst-case' scenarios, and allows for uncertainty in biomass estimation and the recognised potential for carp populations to fluctuate markedly through time in response to environmental conditions. When biomass levels two to five times higher than those predicted by the NCCP biomass estimation project were modelled, very high PO_4 and NH_4 accumulations emerged in some sites.

Model outputs for the Lower Lakes case study site also warrant discussion, as these results illustrate the general spatial patterning with which water quality issues are likely to emerge. Additionally, the Lower Lakes have some particular features requiring careful consideration in the context of carp biocontrol (see Table 2). The primary sites of hypoxia risk in the Lower Lakes are shallow areas around the lake margins, and in tributaries (Brookes and Hipsey, 2019). Although illustrated particularly clearly by the Lower Lakes' geomorphology, this pattern of focused water quality impacts in shallow areas occur throughout the case-study sites. Accumulation of dead carp in such locations, through wind or water currents, could potentially exacerbate these impacts by creating focussed areas of high biomass. Nonetheless, while the Lower Lakes are generally shallow, reaeration by wind action at the

water's surface largely prevents serious DO depletion and the emergence of hypoxic or anoxic conditions, even at higher biomass loadings (Brookes and Hipsey, 2019).

5.2 Water treatment

The capacity of water-treatment plants to maintain drinking-water quality standards in line with the Australian Drinking Water Guidelines following carp mortalities is an important consideration for determining feasibility of carp biocontrol using CyHV-3. Research in this area (Fabris et al., 2019) addressed the following questions:

- Is there are a dead carp density threshold above which carp decomposition products impair standard water treatment protocols?
- What quantity of additional water-treatment chemicals will be required to maintain the health and aesthetic (taste and odour) properties of drinking water supplies following carp mortalities?
- At what threshold density of dead carp does water treatment become non-viable (i.e. use of additional chemicals and/or treatment processes cannot produce water that meets relevant standards)?
- Does treating water containing decomposing carp produce by-products that could harm human health?

All information cited in this section is drawn from Fabris et al. (2019), except where other citations are given. To avoid repetitive citations of this source, in-text references have not been used, but this attribution applies throughout.

Water treatment research under the NCCP used volumetric measures of dead carp density (kg/m^3), whereas other key NCCP projects (e.g. carp biomass estimation and epidemiological modelling) used an areal measure (kg/ha). These differences reflect project contexts; water treatment research required precise quantification of carp decomposition products, which is provided by the volumetric approach. The biomass and epidemiological projects, in contrast, were concerned with approximate carp abundance in varied natural habitat across large geographic extents. Incorporating depth into these estimates to provide a volumetric measurement of carp abundance would have complicated analyses immensely. Table 3 provides approximate conversion values between volumetric and areal measurements, but it should be noted that this conversion is indicative only and could vary considerably with water depth.

Table 3: Converting volumetric (kg/m^3) carp biomass to areal (kg/ha). Table redrawn from Fabris (2019).

Volumetric (kg/m^3) measurement	**Approximate areal (kg/ha) measurement**
0.05	200
0.10	400
0.5	2000
1.0	4000

Water treatment involves taking water from rivers or other water supplies and treating it using the addition of various chemicals and filtration procedures so that it (i) is aesthetically acceptable (i.e. clarity, colour, taste, and odour) and (ii) contains no disease-causing microorganisms. The processes involved in step (i) are called water treatment, while those in step (ii) are called disinfection. Disinfection is fundamental to public health and cannot be compromised. Aesthetic considerations are important for public perception of drinking-water quality, and hence for community confidence in the ability of water-treatment plants and processes to safeguard public health.

Disinfection processes ultimately aim to create a 'residual' of disinfectant in the water distribution network. A residual is a portion of the disinfectant chemical that remains in the water pipes and storage tanks once the initial treatment is complete, ensuring that harmful microbes cannot grow in the stored water. Some disinfection techniques used in Australia do not involve adding chemicals to the water, and so cannot produce a residual. Examples include UV radiation and ozone treatment. When these processes are used, a second disinfection step is added that does create a residual.

Whenever a chemical strong enough to kill harmful microbes is added to water (regardless of the presence or otherwise of dead carp or other contaminants), disinfection by-products (DBPs) form in the disinfected water. More than 600 DBPs have been characterised, with many more remaining unidentified (Li and Mitch, 2018). Some DBPs can be harmful to human health, particularly with long-term exposure. Water treatment authorities thus face a challenge of minimising DBP formation in accordance with accepted guidelines, while ensuring that treated water remains free of harmful organisms. The most effective approach for achieving this goal is to remove as much of the 'precursor' material from the water as possible during the initial treatment phase. Removing precursors during the treatment phase reduces the total amount of this material available to react with disinfectant chemicals—potentially creating harmful DBPs—during the disinfection phase. In the context of carp biocontrol, water treatment plant managers needed to

(i) identify the general character of the DBPs produced by disinfecting water containing decaying carp at varying densities,
(ii) assess the potential of DBPs to threaten human health, and
(iii) evaluate treatment and disinfection options that could minimise DBP formation.

Determining dead carp densities at which disinfection is no longer possible was also an important component of this work.

The research revealed that standard, or slightly modified, but readily applicable, water-treatment protocols can treat water supplies containing dead carp at densities of 0.05–0.10 kg/m^3. NCCP biomass estimates and epidemiological modelling indicate that these densities are typical of those occurring across much of the species' Australian distribution. At these densities, water treatment using optimised alum coagulation alone resulted in water quality equivalent to that obtained by treating standard Murray River water (i.e. without any dead carp loading). Additionally, the research identified a property (called 'aromatic protein-like fluorescence') that has potential as a monitoring tool for detecting carp-affected water at plant inlets, triggering management procedures. Overall, carp densities likely to be encountered across much of the control area are unlikely to threaten treated water quality and should be operationally manageable at the treatment plant scale.

Nonetheless, dead carp densities exceeding 0.05–0.10 kg/m^3 are possible in some areas following CyHV-3 release. NCCP carp biomass estimates identified some areas with carp biomass of up to 550 kg/ha, while carp-population modelling demonstrates that successive flood years could result in substantial increases again above this. Dead carp could also be moved around by wind or water currents, creating small areas of concentrated biomass. Understanding the treatability and disinfection properties of water containing high carp biomasses therefore remains important for assessing biocontrol feasibility.

Once dead carp density exceeded 0.25 kg/m^3, additional water-treatment procedures became necessary to produce water of equivalent quality to that obtained by treating Murray River water without dead carp. At dead carp densities between 0.25 and 0.50 kg/m^3, an additional treatment using powdered activated carbon (PAC) produced acceptable water quality. This procedure is available to

most water treatment plants and is already routinely used to remove tastes and odours associated with algal blooms. At dead carp densities above 1.0 kg/m^3, the water treatment procedures available to most plants were no longer effective and the water was non-viable for drinking water production. A carp density of 1.0 kg/m^3 (i.e. approximately 4000 kg/ha) is, however, extreme, and unlikely to eventuate in a biocontrol scenario.

Disinfection trials indicated that carp densities within the ranges identified by NCCP biomass estimation can be effectively disinfected using existing technologies. As with the treatment trials, the 1.0 kg/m^3 carp density was very difficult to disinfect. Water containing dead carp at this density would be non-viable for water treatment, and treatment operations would need to cease until carp had cleared. Extreme difficulties in disinfecting water from the 1.0 kg/m^3 treatment were identified early in the trial, so the remainder of the testing focussed on water from carp densities of 0.5 kg/m^3 (i.e. approximately 2000 kg/ha) and below.

The final disinfection parameter requiring consideration is the formation of DBPs at varying carp densities. Regulated DBPs (i.e. those routinely monitored in Australian water treatment plants) were well below Australian Drinking Water Guidelines for all the dead carp densities used in disinfection trials (i.e. 0.5 kg/m^3 and below). However, disinfecting water containing dead carp also produced other DBPs that are not routinely monitored in water treatment plants. These unregulated DBPs are called 'unidentified halogenated DBPs', denoted by the acronym 'uAOX'. As dead carp concentrations increased, uAOX comprised a greater proportion of the total DBPs present. That is, as more dead carp are added to the water, novel uAOX that are not routinely monitored in water treatment replace the standard DBPs for which water treatment plants routinely monitor.

At this juncture, an important explanatory note is required for understanding the procedures used to investigate DBPs. The approach used to test DBP toxicity to living organisms involved cell bioassays in which mammalian and bacterial cell cultures were exposed to DBPs resulting from the various dead carp concentrations. Cell cultures were then monitored for development of potentially harmful changes. The cell bioassay approach to assessing DBP toxicity is currently a research methodology only, and is not used for formal DBP hazard assessment in any Australian jurisdictions. This situation will, however, probably change, with cell bioassays likely to play an increasingly important role in formal DBP hazard assessment. The cell bioassay approach is useful because it bypasses the need for analytical testing of individual DBPs (many of which remain uncharacterised), instead simply indicating toxicity from the full mix of DBPs occurring in a given disinfection scenario. Results from cell bioassays for DBP effects are interpreted as relative to a set of predetermined baseline conditions, so absolute values from the testing are not relevant in themselves.

In the cell bioassays used to identify potentially toxic DBPs arising from disinfection of water containing dead carp at various concentrations, none of the water samples were toxic in their standard, unconcentrated form (i.e. as water that would come out of the tap). To produce measurable results and enable comparison of DBPs resulting from different carp loadings, the treated and disinfected water samples were concentrated 80 times before conducting the cell bioassays. This approach is consistent with the guidelines for contaminants, including DBPs, in drinking water. These guidelines are set several orders of magnitude lower than is required to produce effects in laboratory trials (e.g. using rodents) to minimise lifetime exposure risks.

Cell bioassays using the concentrated water samples revealed that those produced using chlorine as a disinfectant were generally more toxic than were samples produced using chloramine (an alternative disinfectant containing both chlorine and ammonia). Furthermore, toxicity in the chlorinated samples tended to increase with increasing carp concentration. At a carp loading of 0.25 kg/m^3, toxicity could

be reduced by adding PAC to the water. However, at a carp density of 0.50 kg/m^3, PAC addition was unable to reduce toxicity.

Results indicating formation of toxic DBPs at high carp densities appear concerning. However, these results were produced using highly concentrated samples, and untreatable impacts were only apparent at a high carp loading of 0.50 kg/m^3, which equates to approximately 2000 kg/ha. Even at these extreme densities, health impacts would only be likely if high carp loadings persisted for extended periods. Addressing the effects of short-term exposure to the novel DBPs generated at high carp densities was beyond the project's scope.

5.3 Botulism outbreaks

Information presented in this section is drawn from a review conducted for the NCCP by Beckett et al. (2019), as part of the broader NCCP risk assessment project. To avoid repetitive citations of this source, in-text references have not been used, but this attribution applies throughout the section

Botulism is a serious illness caused by bacterial toxins that attack nerve tissue. The toxins that cause botulism are produced by several bacteria from the genus *Clostridium*, notably *C. botulinum*. Botulinum toxin is classified into seven primary strains, denoted as strains A–G. Mosaic strains also exist that combine characteristics of two primary strains. The strains most relevant to this discussion are types C, D, C–D mosaic, and E.

Growth and toxin production by *C. botulinum* require two basic preconditions; an environment devoid of oxygen ('anaerobic') and a protein source. When environmental conditions are not suitable for active growth, the bacteria form dormant, extremely robust spores. Clostridial spores can circulate in the environment (including in the digestive tracts of animals and birds, and in aquatic habitats) for decades without causing any harm.

When conditions become suitable, the spores germinate (becoming 'vegetative'), and toxin production ensues. In some areas and circumstances, such as wetlands inhabited by colonial waterbirds, botulism outbreaks can become self-sustaining, as each decaying bird carcass provides an anerobic environment and protein source for bacterial growth. Eventually, however, environmental conditions become unsuitable for bacterial growth, the bacteria returns to the spore phase, and the outbreak fades away. The spores, however, remain in the environment, and can resume vegetative growth when suitable conditions return. Once vegetative growth and toxin production have ceased, preformed botulinum toxin may remain in the environment for periods ranging from days to months, depending upon environmental conditions. In general, the toxin is deactivated more quickly when exposed to sunlight, aeration, and drying conditions.

The various strains of botulinum toxin present different risk profiles in the context of carp biocontrol. Types C, D, and C–D mosaic cause disease in birds and cattle, and do not affect human beings. Type E, in contrast, affects fish and birds, and is also very dangerous to humans. Toxicity to humans meant that type E required consideration in the NCCP risk assessment, but there is considerable doubt as to whether type E occurs in Australia, and its distribution and prevalence if it does. No type E outbreaks have been reported in either humans or other animals in Australia, yet no surveys have been conducted to systematically search for the *C. botulinum* serotypes that produce type E toxin. If type E does occur within Australia, it is probably rare and patchily distributed. Overall, types C, D, and C–D mosaic are the strains most likely to emerge if carp kills create conditions propitious for botulism outbreaks.

Given the life history summarised above, botulism requires consideration in the context of carp biocontrol because major carp kills have the potential to establish the preconditions for an outbreak.

Most obviously, numerous decaying carp in waterbodies could remove oxygen from the water and provide a protein source for bacterial growth. More subtly, cyanobacterial blooms potentially resulting from carp-derived nutrients could also produce conditions conducive to a botulism outbreak as they die and decay, while carp 'legacy nutrients' remaining in aquatic sediments after major kills and in situ decomposition could theoretically elevate future botulism risk levels.

In the context of continental-scale carp biocontrol, botulism risk is inherently difficult to predict. While botulism outbreaks have occurred in both livestock and wild birds in Australia, the occurrence of suitable conditions does not mean that an outbreak is inevitable. The reasons why outbreaks may fail to occur despite apparently suitable environmental conditions are unclear, but probably reflect the probabilistic (chance-based) nature of the phenomenon, wherein a critical mass of toxin is required to initiate an outbreak (Beckett et al., 2019).

The context-dependent and probabilistic nature of botulism outbreaks made meaningful experimental investigation within the NCCP research program difficult, and of dubious utility. Therefore, botulism has been addressed as part of a broader risk assessment (NCCP research project 15). As part of this assessment, a literature review was conducted, which included discussions with microbiologists specialising in clostridial bacteria. A risk assessment process was then applied to the review results to formally assess botulism risk.

The risk assessment concluded that major carp mortalities could possibly trigger either multiple outbreaks of type C or C–D botulism, or single, large-scale outbreak. This statement reflects a balanced weighting of biological knowledge about botulism generally, and Australian field experience with the disease. On one hand, large fish kills (regardless of whether they originate with viral disease or some other mechanism), have the capacity to create the preconditions for a botulism outbreak under some circumstances. NCCP research modelling oxygen and nutrient dynamics following carp mortalities indicates serious oxygen depletion (i.e. one of the important preconditions for a botulism outbreak) is only likely in shallow, off-channel habitats with high carp densities. These, unfortunately, are the kinds of environments in which a botulism outbreak could occur. Balancing these risk factors is considerable observed experience indicating that botulism outbreaks are relatively rare in Australian freshwater habitats, even under apparently suitable conditions. The peer-reviewed scientific literature contains no information on any possible relationships between fish kills and botulism in Australia, and the only documented case (from a lagoon in northern New South Wales) is reported in a media news story that contains few scientific details. Government agency staff who have responded to fish kills have not reported any secondary botulism outbreaks. Nonetheless, the possibility that major carp kills could precipitate botulism outbreaks remains, must be considered in decision-making and planning for carp biocontrol.

The only strategy available for mitigating botulism risk following major carp kills is carcass removal. Removing carp carcasses from areas that could be at risk of botulism outbreaks would both prevent the onset of anoxic conditions and deprive the bacteria of the nutrients required for growth. There are, however, several challenges associated with effective carcass removal. Perhaps most notably, physical access to many areas where high carp biomass may occur is likely to be difficult as a result of their remote location and/or swampy, snag-ridden terrain. Additionally, ongoing surveillance of risk areas to detect outbreaks of CyHV-3-induced disease will be necessary.

5.4 Secondary bacterial infections

Decomposing carp carcasses will provide a substrate for bacteria and other microorganisms. The NCCP risk assessment project (Beckett et al., 2019) considered the risk that these microbial

communities could include those harmful to humans, livestock, and terrestrial and aquatic native animals. As with botulism outbreaks, there is very little published information investigating waterborne pathogens associated with fish kills, despite numerous health advisories based on ensuring that carcasses of fish and other animals do not contaminate drinking water.

Beckett et al. (2019) concluded that, in some settings, humans, livestock, and native animals could possibly be exposed to harmful waterborne microorganisms that proliferate in and around decaying carp. As with botulism, this risk is highest in areas with low or no water flow. Additionally, risks associated with harmful bacteria increase at temperatures above 20°C. Permissive temperatures for CyHV-3-induced disease mean that major carp kills caused by the virus will almost invariably occur at temperatures above 20 °C. As with botulism risk, removal of carp carcasses, particular where they occur at high densities, is the only plausible approach to treating this risk.

6.0 Conclusions

Water-quality research under the NCCP has investigated the potential impacts of carp decomposition on various water-quality parameters. Water-quality considerations associated with carp biocontrol are interlinked, in that changes to some primary parameters, such as DO and nutrient levels, have the potential to trigger secondary impacts such as proliferation of harmful bacteria.

In general, risks to water quality posed by the dead carp densities predicted through NCCP research appear manageable. In particular, anoxia/hypoxia, nutrient enrichment, and elevated cyanobacterial risk are unlikely to be widespread or prolonged at projected dead carp densities, particularly in areas where river flows and other aerating processes operate. Similarly, water treatment and disinfection processes remain feasible and effective at all but extremely high dead carp densities.

Nonetheless, some risks remain. At carp densities above approximately 300 kg/ha, habitats such as shallow, off-channel wetlands that are not subject to re-aeration through river flow, wind, or wave action can experience extended periods of hypoxia or anoxia, nutrient enrichment, and elevated risk of harmful algal blooms. Similarly, at very high dead carp densities (i.e. approximately 2000 kg/ha), water treatment is no longer feasible, and DBPs that could be harmful to human health with extended exposure begin to appear in treated water. The potential, if any, for 'legacy' nutrients sequestered in sediments to fuel algal blooms in future is unclear.

Large numbers of carp decomposing in waterbodies could establish the preconditions for a botulism outbreak, or proliferation of other, potentially harmful bacteria. However, neither botulism nor other serious bacterial diseases have been widely reported in Australian following fish kills. Botulism outbreaks are highly probabilistic events, and are consequently difficult to predict. Managing carp carcasses to prevent the onset of anoxic, protein-rich conditions represents the most practical risk mitigation measure for both botulism and other bacterial infections. Given these considerations, there is a clear need for strategic and effective management of carp carcasses during biocontrol operations.

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