Fish kills in lakes; implications to water quality and ecology

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Certificate of Original Authorship

I, Joe Pera declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Life Sciences at the University of Technology Sydney. This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This document has not been submitted for qualifications at any other academic institution. This research is supported by the Australian Government Research Training Program.

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Preface

This thesis consists of six chapters. Chapters 2 to 5 have been written as individual articles that have either been published or are in preparation for submission to peer reviewed scientific journals. These papers are included close to their published or submitted form, and as a result, there is some repetition. To prevent duplication, a single reference list has been provided at the end of this thesis.

This thesis is a compilation of my original work, with guidance from my academic and industry supervisors. I formed the idea of this research, carried out the majority of the data collection and analysis, and wrote the manuscripts. Publications details from this thesis are provided below, and co-author contributions are specified.

Chapter 2: Pera J. B., Davie, A. W., Rohlfs, A.-M., & Mitrovic, S. M. 2021. Simulating the potential effects of a carp virus fish kill on water quality and phytoplankton in lentic environments. Marine and Freshwater Research. 73(2) 178-192 https://doi.org/10.1071/MF20368

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Other publications produced during my candidature but not forming part of this thesis:

Stocks, J. R., Rodger, M., **Pera, J.,** Gilligan, D. 2019. Monitoring aquatic plants: An evaluation of hydroacoustic, on-site digitising and airborne remote sensing techniques. Knowl. Manag. Aquat. Ecosyst., 420 (2019) 27 https://doi.org/10.1051/kmae/2019016

Davie, A. W., **Pera J**. B. 2021. The Fish Health Risk Indicator: linking water quality and river flow data with fish health to improve our predictive capacity around fish death events. Marine and Freshwater Research 73(2) 193-199 https://doi.org/10.1071/MF20360

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Rohlfs, A.-M., Davie, A., **Pera, J.,** 2021. Alternative cyanobacteria management approaches. Water e-Journal Vol 6 No 2 https://doi.org/10.21139/wej.2021.009

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List of Abbreviations

CPUE	Catch per unit effort
CyHV-3	Cyprinid herpesvirus 3
DIDSON	Dual frequency IDentification SONar
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DTM	DIDSON transect method
MBD	Murray Darling Basin
MIB	2-Methylisoborneol
MOA	Macroscopic organic aggregates
NCCP	National Carp Control Plan
OTU	Operational taxonomic unit
RNA	Ribonucleic acid
TN	Total nitrogen
TOC	Total organic carbon
TP	Total

Ethics statement

This study involved the use of animals. Approval for this research was granted by the NSW Department of Primary Industries Animal Care and Ethics Committee research authority (ACEC 16/05), (ACEC 17/05) and scientific collection permit P01/0059(A). The research was conducted in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (8th Edition 2013), the NSW Animal Research Act (1985) and Regulations (2010).

Abstract

Common carp *Cyprinus carpio* are well established within many of Australia's rivers and lakes and are now dominant parts of aquatic ecosystems. Reducing the biomass of this species is integral in returning waterways to pre-European conditions. Removal of carp from waterways may benefit the aquatic ecosystems but based on the area covered and the resources needed, traditional removal methods are not feasible. Biological control of common carp using a virus would permit widespread coverage with minimal resource use. The virus kills up to 80% of common carp drastically reducing its biomass in the water bodies, but the fate of these carcasses within the lake remains poorly understood. This thesis aimed to better understand the fate and impact of decaying fish carcasses in lakes. This included the influence of this nutrient pulse on water quality and microbes using manipulative mesocosm studies in lakes and field monitoring of fish biomass and carcass movement and scavenging in south-east Australia.

The mesocosm studies with common carp carcasses showed a rapid drop in dissolved oxygen concentrations in all treatments to levels that would not sustain fish or invertebrates. The addition of dead carp biomass led to changes microbial assemblage structure, which shifted from a community comprising typical lake bacteria, to a eutrophic signature community, including higher relative abundance of environmental copiotrophic bacteria. Following the microbial shift phytoplankton began to dominate in all treatments and correlated positively with increasing carcass biomass as did total nitrogen, total phosphorus and chlorophyll a. Several bacterial and algal species correlated with the odour causing compounds geosmin and 2-Methylisoborneol (MIB) in dead carp treatments.

Field monitoring of dead carp fate at different depths revealed that once the carcass had sunk to the lake bottom interactions with large scavengers was limited to 5 m, and beyond that depth microbes were the main driver of decomposition. Depth of the carcass in the lake was significant in determining if it would resurface, with carcasses less likely to float from microbial bloating with increased pressure at greater depths. Field surveys of biomass showed that lake depth was a factor in determining the biomass of common carp. Large lakes with increased volume and low biomass of carp would have reduced water quality impacts and an increased number of carcasses

not returning to the surface due to fish sinking below levels where pressure overrides microbial bloating of the carcass. Small shallow lakes had higher carp biomass and would be at greater risk of catastrophic water quality changes if the virus was implemented.

This thesis contributes to a more comprehensive understanding of fish kills and water quality and ecological responses from the input of decaying carp derived nutrients. The fate and impact of fish carcasses will be influenced by the depth profile and size of lakes. The results highlight areas of concern from a virus driven carp biocontrol management program and suggests some control strategies to minimise potential impacts.

Chapter 1: General Introduction

Australian rivers and lakes have been negatively impacted by a range of anthropogenic stressors. These include river regulation and water extraction, increased nutrients and eutrophication, climate change as well as introduced aquatic species (Mac Nally et al., 2011, Prosser et al., 2021, Mosley et al., 2023). The most significant introduced aquatic species by biomass in Australia is the freshwater fish the common carp *Cyprinus carpio* and its distribution covers the full extent of the Murray-Darling Basin (Stuart and Jones, 2002, Stuart et al., 2021). Management of the impact of introduced impact carp has been limited to physical removal and screening and in some areas only, as both are cost prohibitive for the whole basin. The discovery in 1998 of a new herpes virus which was specific to common carp allowed Australian authorities to investigate the use of this virus as a biological control (McColl and Crane, 2013), however, the water quality and ecological risks of possible mass fish deaths need to be considered (Davie and Pera, 2021, Sheldon et al., 2022).

1.1 Fish in Australia; native and introduced

Australia's freshwater fish species composition and distribution is in part due to desert and semi-arid conditions found over much of the country (Allen, 2003). The lowest annual rainfall in proportion to landmass and least surface runoff have also shaped Australian freshwater ecosystems. This combined with Australia's separation from Gondwana 99 million years ago have contributed to the species endemism patterns that were likely established in the distant past as early as the Miocene (23.03 to 5.332 million years ago) (Unmack, 2001, Allen, 2003). This long isolation has meant Australian fish have adapted to very specific conditions presented by Australia's climate and geography (Unmack, 2001). Fish species diversity in Australia is historically low, with only 302 species recorded compared to North America, South America, China and Africa which have 600, 2000, 600 and 1400 species, respectively (Allen, 2003).

Australian freshwater ecosystems are vulnerable to stressors such as eutrophication, land-use change, changes in temperature and introduced species (Angeler et al., 2014). In the past 200

years since European colonisation there have been over 20 species of fish introduced into Australian freshwaters (Allen, 2003, Lintermans, 2023). Acclimatisation societies encouraged the introduction of non-native species mainly from Europe as they believed that would enrich the waterways, with examples including salmonids, cyprinids and perch (McDowall, 1980). In the last 50 years popular aquarium species have also been released into streams by irresponsible hobbyists (Allen, 2003).

Large scale damming of many of the rivers on the eastern part of Australia for irrigation and drinking water have made lakes more common than prior to European settlement and lakes and reservoirs now make up a sizeable part of Australian freshwater ecosystems. This has potentially increased habitat for non-native species that are not well adapted to deal with Australian conditions such as drought (Greenhalgh, 1999).

Once introduced, fish species enter waterways and expand in biomass and they can affect ecosystems in various ways including:

- Modify the behaviour of native species by dominating through being more aggressive than native species, introducing new selection pressures as they now have to avoid a new predator, distributional effects on native species by decreasing their abundance through predation and by displacing them from preferred habitats through competitive exclusion (Cucherousset and Olden, 2011).

- Species extinction such as with the introduction of Nile Perch to Lake Victoria (Africa). This has caused one the largest vertebrate extinctions (Kitchell et al., 1997).

- Modification of biochemical cycles such as modified nutrient regimes by increasing nitrogen and phosphorus availability in waterways via excretion, promoting algal growth and contributing to eutrophication (Cucherousset and Olden, 2011).

- Introduction of diseases in Australia, such as the viral disease epizootic haematopoietic necrosis with the introduction of Redfin Perch *Perca fluvaitilis* which is fatal to native Macquarie Perch *Macquaria australasica* (McDowall, 1980).

- Habitat alteration by engineering species. A number of case studies have reported that non-native fish species such as common carp can act as engineering species. Carp modifies aquatic vegetation (submerged macrophytes) directly through uprooting or eating and indirectly via bioturbation and excretion of nutrients, changing the trophic status of water from clear to turbid (Matsuzaki et al., 2009).

- Specialist species are increasingly showing to be declining and experiencing higher extinction risk than relative to generalist species (Devictor et al., 2010). Thus, the introduction of a generalist species such as carp with high tolerances to water quality variation, allows them to survive in highly variable, modified and changing environments. Through its morphological, behavioural and physiological adaptions it can engineer change in ecosystems and has the potential to dominate Australian ecosystems for millennia.

1.2 Carp in Australia

Common carp are one of the most commercially important and widely cultivated freshwater fish, contributing to 11% of the total world freshwater aquaculture production (FAO, 2012). Common Carp were introduced to central Europe from Asia about 2000 years ago and are one of the oldest farmed fish (Greenhalgh, 1999). They were introduced into Australia in the 19th Century as part of a push to bring animals and plants from Europe and to make Australia seem more like the United Kingdom. There were no major environmental problems until the 1960s when a strain adapted for European aquaculture, known as the Boolara strain, escaped from farm dams into the Murray-Darling River, the population exploded during major floods in the mid 1970s. This triggered a major population increase over the following decades and carp can now make up to 90% of the animal biomass in many lakes and rivers (Shearer and Mulley, 1978, Smith, 2005). The rapid expansion was assisted by the Boolara strain being more invasive in nature. Interbreeding

of the Boolara carp with the other two strain of carp already in the Murray (Prospect and Yanco strains) resulted in increased fitness of the cross-bred offspring which increased survivability. Large floods in the Murray– Darling Basin in 1974/5 and again in 1993 also helped the rapid spread of carp throughout the basin. A combination of other events such as deliberate and accidental release contributed to the expansion (Shearer and Mulley, 1978, Smith, 2005). Carp have also spread to the eastern draining catchments such as the Hawkesbury-Nepean and Shoalhaven.

Carp are considered the fourth major invasive invertebrate in Australia and are the dominant species in many systems where they are introduced (Barwick, 2016). They have been considered a major contributor to the deterioration of water quality, bank erosion, and habitat destruction around the world and within Australia's major rivers and lakes (Vilizzi and Tarkan, 2015). They are listed as a noxious species in New South Wales, Victoria and Queensland, and a declared exotic species in South Australia and a controlled fish in Tasmania (VFA, 2010, DPI, 2014, DAF, 2022, NRE, 2023, PIR, 2023). Their importation is prohibited by the Commonwealth government (BICON, 2023).

1.3 Carp as Ecosystem Engineers

Invasive species can alter ecosystems via a number of pathways. Catastrophic ecological impacts are often completed by ecosystem engineering (Matsuzaki et al., 2009). Common carp have been identified as one of the main causes of loss of biodiversity and water clarity in shallow lakes and ponds worldwide (Zambrano et al., 2001). Common carp can dominate rivers, wetlands and lakes because of their long life spans and large size (Baldry, 2000). They reduce water quality through their feeding activities by physically disturbing sediments and recycling nutrients. These activities can result in an increase in chlorophyll a, nutrient concentrations and turbidity, and a decrease in macrophytes (Chumchal et al., 2005). By carp reducing submerged macrophyte abundance it changes the role macrophytes have in freshwater ecosystems. Their role is diminished in the amount they influence water clarity and nutrient dynamics and the diversity of the physical habitat (Matsuzaki et al., 2009).

The number of carp in a system rather than just their presence is a key factor in their ability to engineer and alter ecosystems (King et al., 1997, Huser et al., 2016). This combined with their differing feeding approaches compared to Australian native fish approach as well as their tolerance to differing water quality means they can survive the changes in water quality that they help create. Biomass changes of omnivorous fish such as carp in lakes can change the nutrient pathways of the water body. It has been found that in shallow eutrophic lakes, water transparency is inversely related to the biomass of omnivorous cyprinids as they increase turbidity through bioturbation and soft plant removal (Tátrai et al., 2003).

1.4 Carp and Algae

Algae play an important role in freshwater ecosystems. They are the primary producers which supply energy to the bottom of the food web and their composition and biomass will depend on environmental factors such as flow, light and nutrient availability (Bellinger and Sigee, 2015). Depending on the prevailing conditions such as calm weather and warm surface waters algal blooms can occur (Mitrovic et al., 2011). This can lead to blooms largely comprised of cyanobacteria such as *Microcystis aeruginosa* and *Dolichospermum circinale* which can produce toxins such as hepatotoxic microcystins or neurotoxic saxitoxins. Carp have a competitive advantage in dealing with cyanobacteria as they are agastic (have no stomach) and their digestive tract is neutral or basic (Carbis et al., 1997, Billard, 1999). This limits the absorption of microcystins, which requires an acidic environment. The carp's digestive tract rather than being a point of break down for cyanobacteria, may actually be a source of nutrients for the cyanobacteria. Viable cyanobacteria have been found in the gut passage of the filter-feeding fish Atlantic menhaden, *Brevoortia tyrannus* (Friedland et al., 2005) and Cyprinids such as silver carp *Hypophthalmichthys molitrix* (Gavel et al., 2004). After digestion of *Microcystis* colonies by silver carp it was shown that larger colonies (>1000 cells) were disintegrated to smaller colonies of up to 50-100 cells/colony. These smaller colonies remained viable and actively growing, seven days after excretion by silver carp (Gavel et al., 2004). Additionally, Kolmakov and Gladyshev (Kolmakov and Gladyshev, 2003) found that the dominant phytoplankton species in a reservoir

near the township of Krasnoyarsk in Russia, *M. aeruginosa,* showed a significant increase of growth after passage through the intestine of crucian carp *Carassius auratus*.

The sediments of still water ecosystems are known to be a store of resting stages (cysts) of cyanobacteria and other algae (Bellinger and Sigee, 2015). *Microcystis* is regarded as a typical planktonic alga, but may also occur as granular masses on lake bottoms and over winter on lake sediments rather than in the water column (Bellinger and Sigee, 2015). Inflows can bring turbid water, which potentially can smother the lake sediments. Thus, the importance of the bioturbation processes in releasing algal colonies below the surface sediments can in turn potentially increase the numbers of resting cyanobacterial colonies recruited due to bioturbation of bottom sediments. The feeding habits of benthivorous fish, predominantly in the shallow littoral zone during the summer period (Persson and Nilsson, 2007), can be considered as one of the important factors contributing to recruitment of cyanobacterial colonies. Therefore, carp with their predominant benthic feeding are constantly turning over the sediments and releasing algal colonies into the water column.

1.5 Carp and Turbidity

Benthic fish such as carp play an important role in linking the benthic and the pelagic parts of an ecosystem. This is performed by disturbing the sediments during feeding. Separating the food by a winnowing process in the buccal cavity then discharging sediment particles via the gill rakers causes bioturbation (Sibbing, 1988, Koehn et al., 2000). Bioturbation can be defined as the reworking and mixing of sediment at the sediment–water interface (Adámek and Maršálek, 2013). This resuspension of the particles affects the ecosystem in two main ways. The first is in reducing light penetration through the water column. The reduction of light penetration affects aquatic plant composition and visual predators by reduced visibility. The second is the resuspension of nutrients from the sediments into the pelagic zone (Huser et al., 2016).

1.6 Carp and Nutrient Dynamics

Fish are important in nutrient cycling in freshwater ecosystems (Schaus et al., 1997). The main mechanism is excretory processes that supply nutrients (nitrogen and phosphorus) at rates comparable to major external nutrient sources. In addition, fish exert strong impacts on the species composition of primary producers by effecting the nutrient supply rates and ratios (Vanni et al., 2006). In comparison to most Australian native fish, carp per unit area are relatively large. The maximum size as recorded in fisheries literature is 760 mm and 8.5 kg (Stuart and Jones, 2002) with an average 1-3 kg. More recent findings suggest that the average carp size is now 4-5 kg (DPI, 2014). Several extremely large carp have been caught in Hawkesbury-Nepean system one at 900 mm and weighing 18 kg (Griffith et al., 2014) and in Sydney's Centennial Park at a record of 24 kg. Due to the fact carp are a large freshwater fish means they are particularly important for potentially stimulating new primary production by increasing nutrients into new habitats by translocation (Vanni et al., 2006). Nutrient fluxes can also be affected by the impact carp have on prey and macrophytes. Carpenter (Carpenter et al., 1992) suggest that changes in trophic structure that derive from trophic cascades can be viewed as changes in the phosphorus cycle driven by fishes.

Nitrogen (N) and phosphorus (P) cycling by freshwater fish has been estimated to constitute a major part of the internal nutrient load and can exceed other inputs and nutrient demand by algal species (Morgan and Hicks, 2013). As external P loading to lakes decreases, benthivorous fish become an even more important P source to lakes (Hendrixson et al., 2007). In a small 35.1 ha stratified eutrophic lake in central Minnesota, USA, when they reduced carp biomass from 300 to 40 kg/ha total phosphorus reduced, water clarity and aquatic vegetation density increased (Bajer and Sorensen, 2013, Huser et al., 2016). This suggested that the increase in sediment mixing depth caused by carp increased the amount of mobile P potentially available for release by 55–92%.

There is a general trend that carp nutrient excretion rates increase as body mass increases (King et al., 1997, Morgan and Hicks, 2013). Larger carp can also mobilise phosphorus through sediment suspension as well as excretion, whereas smaller carp tend to mobilise phosphorus only through excretion (Driver et al., 2005). Only 25–30% of the nitrogen (N) and phosphorus (P) in food is typically retained in carp, the remainder being lost to the environment (Jahan et al., 2003). The increase in N and P from carp can alter phytoplankton community composition favouring cyanobacteria (Schaus et al., 1997). This suggests management of N and P can be manipulated by altering the biomass of fish. Population changes in cyprinids have altered the internal P loading of lakes and thus changed the size and composition of the algal community (Boros et al., 2009). Depending on conditions, this can lead to unwanted cyanobacterial blooms, and in particular toxic blooms (Mitrovic et al., 2011). These conditions can lead to oxygen depletion (hypoxia) and in turn death for fish unable to tolerate low oxygen levels.

1.7 Carp and Zooplankton

The feeding preferences and diet of carp change throughout its life. Larval carp are visually orientated predators feeding on zooplankton such as rotifers, copepods and cladocerans (Smith, 2005). The diet of small carp (<30 cm) includes chironomids (their preferred prey item), benthic macroinvertebrates (Hume et al., 1983 , Vilizzi and Tarkan, 2015) and crustaceans (Khan, 2003). Adult carp generally prefer benthic feeding habitats, feeding on benthic macroinvertebrates. When plankton and benthic macroinvertebrates are available in the system they will feed on benthic macroinvertebrates. In the absence of benthic macroinvertebrates, their feeding niche can shift to the water column and carp can spend 85% of their time feeding principally on zooplankton (Rahman et al., 2010). When cladocerans and other zooplankton disappear, phytoplanktonic algae proliferate as they are no longer being actively consumed (Billard, 1999). Thus carp can alter lake function by feeding predominantly on micro-crustacea, causing a top-down trophic cascade (Khan, 2003).

1.8 Carp and Other Fish Species

Carp mature at one year for males and at around two years for females and at only 125-150 mm in length (McDowall, 1980). Fecundity is very high compared to native fish of an equivalent length with a female carp of 6 kg having up to 1.5 million eggs. Carp spawn only when the water temperature exceeds 17-18 °C (Greenhalgh, 1999). Large females may breed more than once in a season and have been recorded breeding up to three times in a season in Australia. This reproductive strategy gives carp a competitive advantage over native fish in two ways. Firstly, with their high fecundity they produce many more eggs than natives. A native silver perch *Bidyanus bidyanus* with similar feeding patterns will shed around 300,000 eggs per season compared to a 6 kg female carp with 3 million (spawning twice in a season). That is 10 times more juveniles competing for the same zooplankton food source. Secondly, because carp spawn earlier, their juveniles are at a more developed stage and are capable of feeding on the semi-buoyant fertilised eggs of the native fish. Silver perch populations in the Murray River have declined by 93% from 1940 to 1990 (McDowall, 1980). Most native fish species tend to be specialists whereas carp have a diverse diet and feeding methods. They can feed on bottom dwelling invertebrates, worms, snails, mussels and crustaceans (Greenhalgh, 1999). When food in the water column is unavailable, they will feed on soft plants and detritus (McDowall, 1980). This allows carp to switch diet depending on what resources are available. Another competitive advantage over natives is due to their size as they have very few predators. Carp make up a large part of the fish biomass in NSW waters, about 87% of the biomass in the Murrumbidgee catchment and at last 50% in the lower Murray (DPI, 2010). This domination magnifies the impact of juvenile recruitment and the fight for food resources.

1.9 Carp and Macrophytes

Aquatic macrophytes have an important role in structuring communities in aquatic environments. Plants provide physical structure, increase habitat complexity and diversity and influence the numbers of organisms like invertebrates, fish and water birds (Thomaz and Cunha, 2010). Structural complexity also significantly affects the size structure of animal populations

and assemblages. In general, more complex habitats support higher densities and a greater diversity of small-bodied animals (Thomaz et al., 2008). This allows animals to become specialised to the conditions offered by small curved or geometrical shapes of macrophytes in the environment mosaic and makes it possible for a level of diversity not found among groups of larger organisms (Brown, 1981).

Carp have been credited with significant structural damage to macrophytes and wetlands in Australia and overseas (Williams et al., 2002, Hertam, 2010) partly because they contribute more to water quality variation such as increases in turbidity. Carp interfere with the recruitment, growth and diversity of wetland macrophytes (Williams et al., 2002). Swirepik (1999) found that carp significantly reduced vegetative regrowth of *Potamogeton tricarinatus* in large ponds and that this led to increased reliance on seed-bank regeneration. Carp significantly affect species abundance and diversity of macrophytes (Miller and Crowl, 2006). By decreasing the diversity of macrophytes they also decrease the diversity of small fish and macroinvertebrates associated with them. With reduced habitat, larger predatory species lose habitat for their juveniles and therefore have reduced recruitment. Lower macrophyte densities also allow for increased predation pressure from birds on fish. Bajer and Sullivan (2009) found that a carp biomass of <30 kg/ha had no discernible effects on vegetative cover (which exceeded 90%) or waterfowl (which exceeded 150,000 individuals during fall censuses). An increase to 100 kg/ha was associated with a 50% decrease in both vegetative cover and waterfowl. Carp abundance in the Bogan River, NSW was estimated at 609 kg/ha (Smith, 2005) and in Sydney Model Yacht pond (Bicentennial Park) 1200 kg/ha and Malluga Passive Park pond (Bankstown) and at 650 kg/ha (Pera, 2007, Pera, 2008). This suggests that many of the rivers and lakes in Australia have carp biomass volumes that are at or above the biomass needed to significantly impact on macrophytes and thus impact on the ecosystem.

1.10 Current Status of Carp and Management

Carp have spread to all states and territories except for the Northern Territory and have the ability to dominate total catch numbers and biomass in a range of catchments from highly

regulated lowland rivers of the Murray River to the cooler, higher-altitude areas around Cooma in the Snowy Mountains (Smith, 2005, DPI, 2010). They can tolerate poor water quality including low oxygen levels, high turbidity, moderate salinities (up to 12.5 ppt at 16-21°C) and high levels of toxicants (Hart et al., 1991). Carp can be found within all major and minor habitats including main channels, floodplains, lakes, swamps, billabongs, irrigation channels, undercut banks and among macrophytes and woody debris. Their environmental tolerances have allowed them to survive and flourish in relatively pristine streams through to highly degraded waterways. Carp are bottom feeders, sucking up sediments with their mouths and expelling indigestible material through their gills, but they can also feed on planktonic crustaceans and rise to the surface to take hatching midges as well as eating soft aquatic plants (Billard, 1999, Greenhalgh, 1999). Their ability to tolerate a wide range of habitat, water quality, flow dynamics along with their high fecundity rate has enhanced their role as ecosystem engineers (Matsuzaki et al., 2009).

With carp having severe impacts on Australian freshwater ecosystems, a Carp Control Group (CCG) was established in 1999 to look at future directions for carp research. An investigation of options for controlling carp was undertaken and looked at fishing competitions, commercial fishing, poisoning, exclusion devices, daughterless gene technology and a kio herpes virus (also known as cyprinid herpesvirus 3 (CyHV-3)) (DPI, 2010).

Some of the major recommendations and consequences were:

- Commercial harvesting - This approach had little effect on established populations of carp, due to their high fecundity. Increased food resources that are available to those carp that escape allowed very rapid repopulation by the survivors (DPI, 2010).

- Daughterless carp - The approach is to control carp by genetically interfering with sex ratios and biasing towards male-only offspring. If successful over many decades there would be a gradual reduction in the number of female carp in wild populations, with a reduction in overall numbers of carp (DPI, 2010).

- Immunocontraception - This approach to reduce carp fertility is at a very early stage of development, and still requires more work before being considered for implementation (DPI, 2010).

- Environmental restoration - This involves restoring the health of rivers and in the process making them less attractive to carp and more appropriate for native fish. The reintroduction of carnivorous native fish such as Murray Cod and Golden Perch, would contribute to a reduction in carp numbers by predation on young carp (DPI, 2010).

- Exclusion Devices - Carp in Australia have predictable seasonal spawning movements and display distinctive jumping behaviours that lend them to trapping and screening technologies (DPI, 2010). These were investigated and trialed including cage and lifting infrastructure developed for optimised wetland carp separation. It was trailed at Lake Bonney in South Australia, a 1700 ha shallow freshwater lake fed from the Murray River through the Chambers Creek Wetlands (IA CRC 2016). Although successful at removing carp, the traps fouled quickly and trapped non target species including bony bream *Nematalosa erebi* and turtles.

- Chemical controls - The large amount of ecological work done on carp in the wild shows that carp release and respond to chemical cues, including pheromones and environmental cues (Elkins et al., 2009). The Invasive Animal CRC is investigating the isolation and evaluation of these controls on carp (DPI, 2010).

- Biological control with infectious agents - While spring viraemia (SVCV) of carp virus has been considered a potential candidate in the past, there are documented limitations with it (Leong, 2008). For the virus to have a high mortality rate, carp need to be in poor condition to be affected. Also being a RNA virus, SVCV could adapt to new hosts (Crane and Eaton, 1996). For that reason, attention in Australia has more recently focused on the Cyprinid herpesvis-3 (CyHV-3) as a biological control (Stuart et al., 2021).

Most options above were resource intensive and had large implementation and ongoing costs. In 2013 the Commonwealth Scientific and Industrial Research Organisation (CSIRO) and the Australian Animal Health Laboratory (AAHL) released a report into the susceptibility of carp and the sensitivity of non-target species to the Cyprinid herpesvis-3 (CyHV-3) virus. It found the virus has the potential to be used for the biological control of carp, and non-target species were unaffected and did not carry the virus (McColl and Crane, 2013).

1.11 Cyprinid Herpesvirus 3 (CyHV-3)

Cyprinid herpesvirus 3 (CyHV-3) previously known as the Kio herpesvirus is a member of the order Herpesvirales and newly designated family Alloherpesviridae. Alloherpesviridae viruses can infect fish and amphibians (Michel et al., 2010). The viruses in the family Herpesviridae are based upon the presence of a linear, double-stranded DNA (Waltzek et al., 2005). The first outbreaks of CyHV-3 connected with high mortality of common carp were reported in 1998 in Israel and in the United States (Pokorova et al., 2005). Since then, several cases have been confirmed all over the world. Mass deaths caused by CyHV-3 infection of common carp in Lake Biwa, Japan resulted in a cleanup of an estimated 100,000 dead carp but an estimated 200,000 to 300,000 more carp died but were not collected from the lake (Matsui et al., 2008).

In the mid-2000s, work began on developing an integrated carp control program (DPI, 2010), including an investigation of CyHV-3, as a potential biological control agent. The virus is specific to carp and other cyprinids such as goldfish would not be affected and would not act as carriers. CyHV-3 was assessed by the Australian Animal Health Laboratory for its potency as a control agent. Determining the host specificity of a viral biological control agent and understanding the mortality in the carp target species was critical in approving the virus. Equally as important was its effect on non-target species (Mcoll and Crane 2013). Thirteen non-target native species of fish were tested in susceptibility trials and these included rainbow trout, galaxiids, silver perch, golden perch, Murray cod, eels and catfish. Birds, mammals, amphibians and reptiles have also been tested including chickens, mice, frogs, turtles and water dragons as representatives of their

class. The virus showed no effect on the tested animals (CSIRO 2016). The virus is still active in Israel in aquaculture where workers actively swim with infected fish to remove them without any ill effects on them. In Singapore if there is an outbreak detected in aquaculture ponds the fish are harvested and sent to market for human consumption (Barwick, 2016). The testing results in Australia and overseas experiences have made CSIRO confident the virus would not affect humans.

1.12 Other Cyprinivirus

The CyHV-3 is one of four viruses assigned to the genus Cyprinivirus. The other three are the cyprinid herpesvirus 1 (CyHV-1), cyprinid herpesvirus 2 (CyHV-2) and the Anguillid herpesvirus 1 (AngHV-1) (Lepa et al 2012). The cyprinid herpesvirus 1 (CyHV-1) or the fish pox virus is the oldest known disease in fish, and was recorded in the Middle Ages (Davison 2012). The virus has two phases of infection, an acute, generally lethal, systemic disease in young carp and a recurring, generally nonlethal, proliferative skin disease linked to periods of lower water temperature among survivors or fish exposed at older ages. CyHV-1-like lesions are seen in Australia especially in koi carp. The suspicion is that the disease is present, although there appear to be no formal reports of its presence (Mcoll and Crane 2013).

The cyprinid herpesvirus 2 (CyHV-2) is the agent of a disease affecting two species of the Carassius genus, goldfish (*C. auratus*) and Prussian Carp (*C. gibelio, L*.). Described for the first time in Japan in 1992, it has now spread worldwide and has been associated with serious fish losses in Australia, Taiwan, China, Italy, England, Czech Republic and the United States (Boitard et al 2016). In 2009 a screening test for CyHV-2 was developed by Aquatic Animal Health Subprogram. This was in part due to the discovery of goldfish in Western Australia with lesions and virus particles described as similar to those found in goldfish affected by goldfish haematopoietic necrosis virus (CyHV-2) (Stephens et al., 2004). It is the only known reporting of CyHV-2 in Australia. Due to the unknown status of CyHV-1 or CyHV-2 within Australia the Invasive Animal Cooperative Research Centre is investigating any possible cross reactions due to other related viruses that may already be present with CyHV-3 (CRC, 2012).

The cyprinid herpesvirus 3 (CyHV-3) is an emerging agent that causes fatal disease in common carp and its subspecies koi and mirror carp. It emerged in the late 1990s and this highly contagious pathogen has caused severe financial losses in common and koi carp culture industries worldwide (Michel et al., 2010). The virus shares morphological features with the carp pox herpesvirus (Cyprinid herpesvirus 1, CyHV-1) and of goldfish (Cyprinid herpesvirus 2, CyHV-2), but differs in display symptoms, host range, protein alteration in order to evade a host immune response, growth characteristics and type of structural changes in host cells that are caused by the viral invasion (Waltzek et al., 2005). The International Committee on Taxonomy of Viruses noted that the large genome of CyHV-3 is larger than any known herpesvirus apart from the other cyprinid herpesviruses (McColl and Crane, 2013). This length plus the fact it is a double stranded DNA virus means it is more stable and less likely to adapt to new species like RNA viruses such as the spring viraemia (SVCV)(Crane and Eaton, 1996).

Infected fish suffer from loss of appetite, and exhibit erratic swimming behaviour prior to death. The most consistent sign of the disease is discolouration and increased respiratory frequency. The virus remains infective in water for at least 4 hours. The virus replicates in the diseased gills and is shed into the water and transferred through the white blood cells to the kidney where it induces severe kidney failure (Pokorova et al., 2005). Its effectiveness in killing varies from 70-100% of the infected hosts in all age groups (CRC, 2012).

1.13 Water Quality and Ecosystem Effects after Mass Fish Mortality

Large fish kills can occur naturally as part of ecosystem nutrient recycling and this is a part of the life cycle of many salmonid fish (Nagasaka et al., 2006). The adult fish return from the ocean to rivers to spawn and die after spawning. They play a major role in the return of nutrients into highlands both from a direct release of nutrients from decaying fish in the upper reaches of rivers and from animals such as bears leaving carcasses on land, which is a source for terrestrial plants (Bilby et al., 2003). These pulse derived nutrients are common in ecosystems where the lifecycle of the fish involves the death of the adults after spawning (Wipfli et al., 1998). The pulse-derived

nutrients and trace elements are critical in determining the biomass of biofilm and the numbers of macroinvertebrates, a major food source for larger vertebrates. Wipfli et al. (1998) found streams in Alaska that were carcass-enriched had up to 15 times higher biofilm ash-free dry mass (AFDM) and total macroinvertebrates were between 8 and 25 times higher. This feedback mechanism was crucial for sustaining aquatic ecosystem productivity and salmonid populations.

Fish are generally perceived as nutrient sinks because large amounts of internal lake phosphorus and nitrogen can be bound in fish tissue and tissue derived nutrients are only released when the fish dies. Unlike salmonids, carp are long lived and death is usually from predation, disease, old age or environmental factors. Thus, in ecosystems where carp are present, pulse derived nutrients are uncommon and the extent and types of changes are generally not well understood. Most fish kills and mass mortality in these ecosystems are the result of an environmental change such as pollution, disease or drought. These changes are not seasonal and thus ecosystems are generally not able to cope with them in the short term. Schoenebeck et al. (2012) found that in severe winters, fish inhabiting shallow, glacial lakes of the Prairie Pothole Region would experience hypoxia and die. The high biomass of dead fish was responsible for an increase in total N and subsequently chlorophyll-a increased the year following the winterkill. These fish-derived nutrients contributed to nutrient availability within systems and increased the biomass of the phytoplankton community. During the winter of 2007–2008, hypoxia-induced winterkills resulted in the mass mortality of common carp populations of 750 kg/ha in eutrophic glacial lakes in South Dakota, USA (Weber and Brown, 2016). This carrion driven pulse of nutrients leads to higher nutrient concentrations, pH, turbidity, chlorophyll-a, zooplankton and benthic invertebrates and less periphyton compared to no carrion lakes. The biomass of carp at 750 kg/ha is within the range found within lakes in the Sydney region.

Winter induced hypoxia from lakes freezing such as in northern regions of Europe and North America is unlikely in Australia. The most likely causes would be eutrophic driven hypoxia and black water from post drought inflows (Sheldon et al., 2022, Durrant-Whyte, 2023). A virus driven fish kill of a single species that makes up 70% to 90% of the biomass could result in low dissolved

oxygen levels from two sources. The first as the carrion breaks down and bacterial respiration dominates and consumes oxygen from the water and the second with increased nutrients causing a spike in algal growth could cause oxygen depletion during the night-time respiratory phase of photosynthesis.

Kushlan (Kushlan, 1974) monitored a small pond (0.115 ha) during the dry season in southern Florida, USA. The fish died from a concentration of fish biomass due to the drying wetland and a gradual depletion of dissolved oxygen. Water quality changed dramatically over the next 30 days following the fish kill. Most measured parameters increased such as dissolved inorganic phosphate to from baseline levels of near zero to 40 mg/l. Turbidity increased from 20 to nearly 200 NTU and iron and manganese levels rose from 0 to 0.18 and 1.0 mg/l respectively. The high metals results coincided with the dissolved oxygen depletion. Phytoplankton increased rapidly with a threefold increase in cyanobacteria and a 200-fold increase in Chlorophyta (green algae). Phytoplankton increased within 5 to 9 days of the fish kill and coincided with the peak nutrient results. Most water quality parameters returned to pre fish kill levels after 30 days. This study is of interest as the timing of this kill would be similar to the CyHV-3 release in Australia occurring in the warmer months and highlights the need for studies on fish kill effects in Australia.

1.14 Research Gaps

Investigation of CyHV-3 as a potential biological control agent had been supported by Fisheries agencies and the Australian government (Gilligan et al., 2010, McColl and Crane, 2013). The research focused on the transability of the virus to non-target species but there were no suggestions to look into the responses of a virus induced fish kill on water quality and ecology, and address the key knowledge gap of understanding of ecological responses to differences in carcass biomass and duration of decay. To fill this knowledge gap, a multi-stepped approach of further research is required to examine aspects of the ecological response to this large fish based nutrient source. Firstly, how the fish based nutrient source effects oxygen, nutrients and microbes in lakes across a range of carcass biomass. Secondly, fate of the carcass within the lake and interactions with scavengers and the movement of this nutrient input with the lake

ecosystem. Finally, does the size of a lake change the movement of this biological input to influence the fate of the nutrients and hence the ecological response. By studying these water quality and ecological responses will further our understanding of impacts and threats from a large-scale virus induced fishkill. Previous research has focused heavily on monitoring natural fish kills (Kushlan, 1974, Aralar et al., 2001). However, few have attempted to clearly understand the role of varying biomass of carp carcasses play in influencing water quality and ecological response. Emerging evidence suggests the addition of fish carcasses to water bodies would induce algal blooms and other ecological responses such as scavenging (Boros et al., 2015, Boros et al., 2020). Combining these areas of research will be crucial in increasing our understanding of how a virus induced fish kill of a single species such as carp, which makes up to 80% of the fish biomass in rivers and lakes influences water quality, primary production and scavenging in Australian lakes.

1.15 Thesis aims and overview

The overarching objective of this thesis is to examine the effects of a fish kill from the release of CyHV-3. The separate studies within this thesis examine a range of scientific and management questions concerning various issues from a common carp fish kill in lakes. The specific aims and hypotheses are summarised below;

Chapter 2: Simulating the potential effects of a carp virus fish kill on water quality and phytoplankton in lentic environments?

This chapter aimed to identify changes in water quality from decaying carp in mesocosm experiments simulating post virus release scenarios. The main question was, will dead carp alter oxygen levels within the water body and to what extent, and how will nutrient and phytoplankton levels change at different biomass levels of dead carp.

Aim 1: To assess the impact of dead and decaying carp on water quality in the short-term immediately after release of the virus.

Hypothesis 1: The water quality changes as a result of decaying of carp from the simulated release of the virus should show an increase in nutrients, reduced dissolved oxygen and alter the concentration, community assemblage and diversity of phytoplankton towards toxic cyanobacteria algal species.

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Chapter 3: The impact of decaying fish on microbial communities in lakes.

This study aimed to identify and compare the changes in microbial communities with the introduction of dead carp at different biomass levels within lake based mesocosms.

Aim 1: To assess the impact of dead and decaying carp on microbes in the short-term immediately after release of the virus.

Hypothesis 1: Decaying carp from the simulated release of the virus should show a change in the community assemblage and diversity of microbes towards heterotrophic species which prefer nutrient rich anoxic environments and an increase in significant taste and odour compounds such as MIB and geosmin will occur.

Chapter 4: The rise and fall of dead common carp; carcass movement through depth in lakes and interaction with scavengers

This study examined the time a dead carp will spend on the sediment at the bottom of a lake at different depths. It also investigated what depths fish species are found, and the likelihood of those species being potential scavengers. It will also cover interactions with scavengers and dead carp carcasses.

Hypothesis 1: Depth at which a carcass is placed will alter the likelihood of floating due to pressure differences.

Hypothesis 2: Depth at which a carcass is placed will change interactions with scavengers due to the presence or absence of scavengers due to depth.

Chapter 5: Estimating Fish/Carp biomass in lakes and reservoirs: Evaluation of DIDSON transects as a rapid biomass estimation method.

This study employed a range of survey methods to determine biomass of common carp in lakes to determine if there is any variability in biomass of common carp based on the size or the depth of the lake.

Hypothesis 1: Deeper lakes will have less carp per hectare than shallow reservoirs.

Hypothesis 2: Lakes with a smaller surface area will have less carp per hectare

Chapter 6: General Discussion

This chapter provides a general synthesis of the results of this thesis and suggests management recommendations and future studies based on these findings.

Chapter 2: Simulating the potential effects of a carp virus fish kill on water quality and phytoplankton in lentic environments.

Statement of contribution to co-authored published paper

This chapter includes the co-authored peer-reviewed paper with bibliographic details, including authors, as follows:

PERA, J. B., DAVIE, A. W., ROHLFS, A.-M. & MITROVIC, S. M. 2021. Simulating the potential effects of a carp virus fish kill on water quality and phytoplankton in lentic environments, Marine and Freshwater Research, 73(2) 178-192 https://doi.org/10.1071/MF20368

My contribution to the paper included data pre-processing, analysis and interpretation, and manuscript writing.

Signed: Production Note: Signature removed prior to publication. 17/02/2024

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2.1 Abstract

Decaying fish play an important role in delivering nutrients into rivers and lakes but can create water quality issues. The Cyprinid herpesvirus-3 (CyHV-3) release in Australia with an associated mass common carp mortality may have serious effects on water quality in lakes. To evaluate the impact of a virus induced fish kill, different biomasses of dead common carp 250 to 6000 kg ha^{-1} were placed into 2000 L mesocosms within Prospect Reservoir, Australia for up to 40 days. Decaying carp created anoxic conditions within all treatments except the 250 kg ha^{-1} which dropped to 30% oxygen saturation. Higher biomass of carp led to longer periods of anoxia. Total nitrogen increased from 0.25 to 1.5-30 mg L^{-1} while total phosphorus rose from 0.01 to 0.05-5.0 mg L^{-1} in treatments. Chlorophyll a level increased from <5 µg L^{-1} to between 100-1000 µg L^{-1} in treatments. Mean nutrient levels (TN and TP), chlorophyll-a and phytoplankton biovolume all showed a significant ($P<0.05$) linear relationship with carp biomass. This relationship can be used to support quantification of water quality risk from a known biomass of carp. Our experiment suggests that carp mortality may significantly reduce water quality in shallow lakes where biomass is above moderate levels (250 kg ha^{-1}).

2.2 Introduction

Fish are nutrient sinks in freshwater ecosystems, as fish biomass contains large amounts of internal phosphorus and nitrogen which are only released through decomposition of the fish tissue (Sereda et al., 2008). Mass fish mortality events can therefore trigger large, rapid fluxes of energy and nutrients into the water column, where they are available for uptake by other organisms (Wipfli et al., 1998, Nagasaka et al., 2006). In some systems, mass fish mortality is a recurring phenomenon, such as the migration of salmonid fish in North American rivers. However, in other cases, mass fish mortality is unpredictable and uncommon, and its effects on the surrounding ecosystem are rarely documented in the literature.

Common carp (*Cyprinus carpio*) were first introduced into Australia in the 19th century and can now constitute up to 90% of the fish biomass in lakes and rivers (DPI, 2010). They have been identified as a priority pest species (Shearer and Mulley, 1978, Smith, 2005, DPI, 2010, PestSmart, 2018, Stuart et al., 2021) and are considered ecosystem engineers (Crooks, 2002, Parkos Iii et al., 2003, Matsuzaki et al., 2009), by stirring up silt which increases turbidity (King et al., 1997, Zambrano et al., 2001, Weber and Brown, 2009, Vilizzi and Tarkan, 2015), promotion of algal blooms (Kolmakov and Gladyshev, 2003, Adámek and Maršálek, 2013, Huser et al., 2016), and loss of aquatic vegetation (Swirepik, 1999, Williams et al., 2002, Miller and Crowl, 2006, Bajer and Sorensen, 2015). Hence, strategies are required to manage the issue. In Australia, an engineered mass fish kill has been proposed in the form of the Cyprinid herpesvirus-3 (CyHV-3) release, which is intended as a biological control agent for invasive carp (DPI, 2010, McColl and Crane, 2013). The virus has a mortality rate that can reach 80-100% with the peak of mortality between 8-12 days post infection, so is expected to cause rapid mass fish kills once it is released (Adamek et al., 2013).

Widespread carp mortality following the virus release may cause significant water quality impacts in lakes and reservoirs across Australia (Marshall et al., 2018, Staight, 2018). High biomass fish kills can lead to oxygen depletion in water bodies (Kushlan, 1974, Weber and Brown, 2016). Other water quality changes such as increases in dissolved nutrients can occur after death and decay (Bilby et al., 2003, Boros et al., 2015). These increased nutrients may contribute to secondary ecological responses such as phytoplankton blooms (Nõges, 2009, Schoenebeck et al., 2012,

Weber and Brown, 2016) where phosphorus or nitrogen is limiting phytoplankton growth (Abell et al., 2010, Kolzau et al., 2014, Mueller and Mitrovic, 2015, O'Donnell et al., 2017). Cyprinid tissue has a nitrogen to phosphorus ratio of 16:1 which is similar to ratios considered to favour some cyanobacteria (Schindler, 1977, Smith, 1983, Sterner and George, 2000). If environmental conditions are conducive, this may also result in a harmful toxic cyanobacterial bloom causing drinking water and recreational use issues (Falconer, 1999).

There are several knowledge gaps that make it difficult to predict and manage the potential impacts of mass carp mortality to water quality across Australian lakes and reservoirs where carp are present (Koehn, 2004). While most studies document the size of the fish kill with estimates on numbers killed (Matsui et al., 2008, Minamoto et al., 2009, Hickey, 2018) and the economic impact (Sunarto et al., 2005), there is little literature on the actual water quality implications from fish kills (Kushlan, 1974, Boros et al., 2015, Weber and Brown, 2016). This is particularly important given recent mass fish kill events in Australian rivers such as the Lower Darling (Vertessy et al., 2019). Research into water quality impacts of fish kill events generally only monitor after the event (Uchii et al., 2009), are spatially limited and rarely include estimates of fish biomass (Aralar et al., 2001, Johnston et al., 2004). There are few studies that examine dissolved oxygen concentration as a function of decaying fish, or that compare water quality effects across a range of different fish biomass levels (Brooker et al., 1977, Weber and Brown, 2016, Sergeant et al., 2017).

To evaluate the potential impact of a virus induced fish kill on water quality, a range of biomass levels of dead carp (250 to 6000 kg ha^{-1}) were introduced into 2000 L mesocosms in Prospect Reservoir, New South Wales, Australia. Prospect Reservoir was selected as it is an important part of the drinking water supply network for Sydney, and at the time of the experiment had an estimated carp population of between 13-400 kg ha^{-1} (Hicks et al., 2006, Sorensen et al., 2015, Stuart, 2019). It is hypothesised that increased biomass of dead carp in mesocosms (simulating the virus release) would lead to decreased dissolved oxygen, increased nitrogen and phosphorus levels and increased phytoplankton biomass. Further, it is hypothesised that the magnitude of the response would be proportional to the biomass of carp.

2.3 Materials and methods

Study Location

The study was conducted in a bay on the north eastern side of Prospect Reservoir, situated in western Sydney, NSW, Australia. (-33.813600, 150.905706) (Figure 1). Prospect Reservoir is the first earth fill embankment dam built in Australia. Completed in 1888, it measures 26 m high and 2.2 km long and has a storage volume of 2,892×103 m3 (Beasley, 1998). As part of the Upper Nepean Scheme, it supplied Sydney with water collected from the Illawarra Plateau. Today, the reservoir remains an integral part of Sydney's drinking water supply when water demand is high or when other parts of the water supply system are unavailable during maintenance.

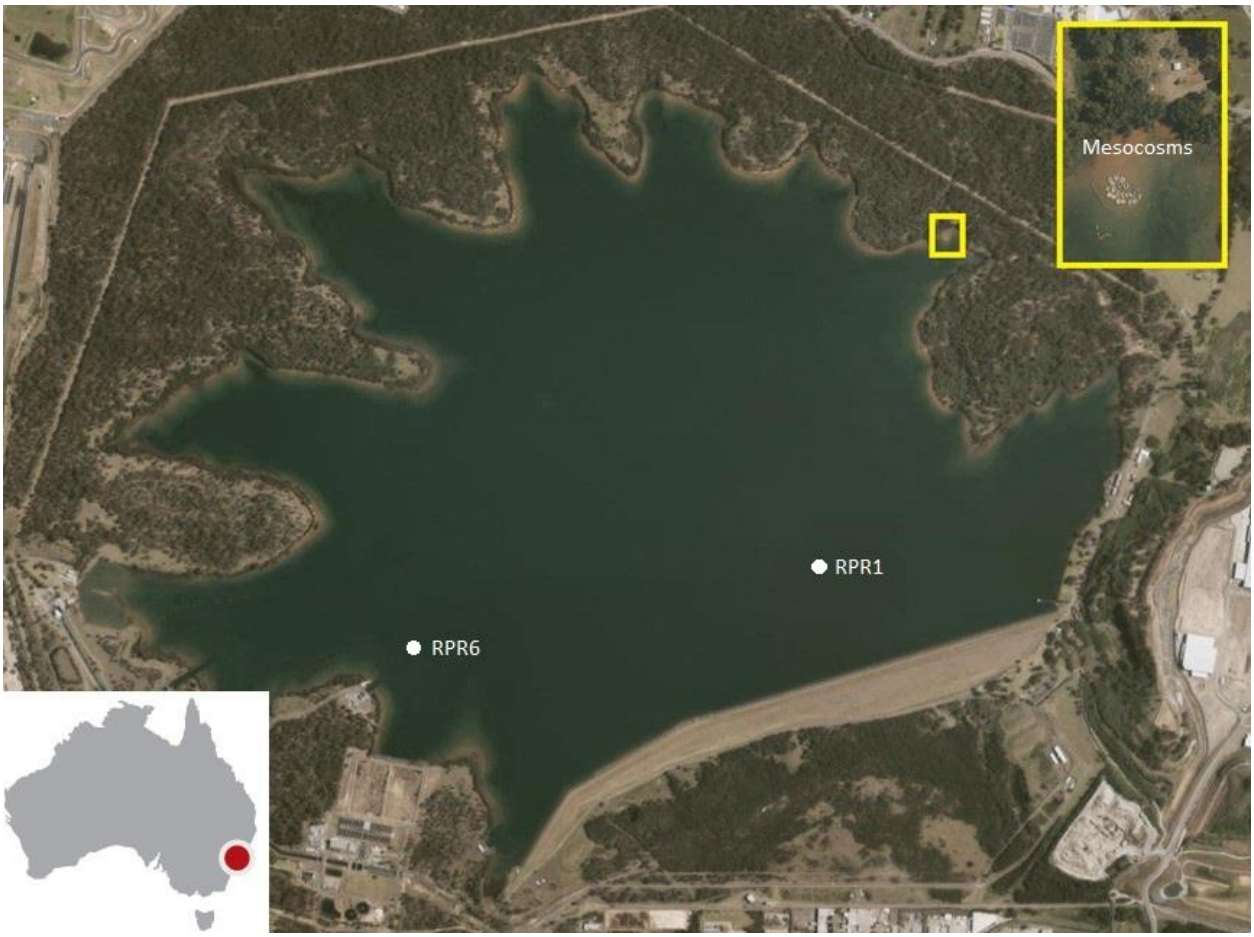

Figure 1. Location of the mesocosm experiment within Prospect Reservoir, Australia.

Experimental Design

To assess the effect of decaying carp biomass on water quality, two experiments encompassing low and high biomass loadings were performed. The first experiment was performed between 28 November 2016 and 5 January 2017 hereafter referred to as the "high biomass experiment" and another between 13 March and 27 March 2017 hereafter referred to as the "low biomass experiment". The second experiment was shorter in time due to increases in water level in the reservoir which risked overtopping the mesocosms.

Dead carp were placed into 2000 L mesocosms and four replicates were used for each treatment level. Only whole fish were used to simulate an intact fish following virus mortality. Large carp (550>250 mm) were sourced from the Prospect Creek catchment, while smaller carp came from a commercial koi carp farm. Treatments used in the low biomass experiment equated to 0 (control), 250, 500, 1000 kg ha^{-1} of dead carp and for the high biomass experiment 0 (control) 1200, 2300, 6000 kg ha^{-1} of dead carp. The biomass estimates were based upon previous surveys of wild populations (Hicks et al., 2006, Wanner et al., 2009, Bajer and Sorensen, 2013, Tempero, 2013) and survey data from lakes within the Sydney Basin (Stuart et al., 2021).

Mesocosm Design

Mesocosms consisted of a cylindrical outer shell made from rolled steel (COLORBOND®) 2.5 m in diameter and 1.2 m in height (Figure 2). The outer shell was designed to withstand wave action generated by southerly winds across the lake and had an open top and bottom to allow air and water to circulate around the inner bladder. The inner bladder of the mesocosm was made of PVC and 2.44 m in diameter and 0.45 m in height. Inner bladders were filled with ~2000 L of lake water prior to the experiment commencing. The PVC bladder had an inflatable ring (~0.1 m high) which kept the inner part of the mesocosm at the level of the storage, stopped splashing of lake water into the mesocosms and kept them neutrally buoyant with the lake for the duration of the study. The water inside the mesocosms was generally well mixed due to gentle agitation brought about by wind and wave action.

Mesocosms were clustered together and placed in a relatively flat and shallow bay, with water depth between 0.6-0.8 m. The outer part of each mesocosm was secured to the lakebed using

steel posts and wire. Each mesocosm experienced the same light and ambient lake temperatures for the duration of the experiments. To prevent birds fouling the water inside each mesocosm or eating the decaying carp, bird netting was used to exclude them from the mesocosms.

Sampling procedures and analysis

Daily measurements of temperature, dissolved oxygen, conductivity, pH and turbidity were measured with a calibrated YSI EXO multiparameter sonde. Two mesocosms from each treatment level had a MS5 Sonde probe logging at ten-minute intervals for the length of each experiment. There were also two YSI EXO multiparameter probes placed in two of the treatments logging temperature, conductivity, dissolved oxygen, pH and turbidity at ten-minute intervals.

Prior to taking daily physicochemical measurements, or when water quality samples were collected, each mesocosm was mixed by stirring to homogenise. Samples collected from each mesocosm for water quality used a 10 L stainless-steel bucket then decanted into smaller bottles for specific analytes provided by a commercial laboratory for nitrogen (APHA 4500 NORG/903), phosphorus (APHA 4500 P-F), organic carbon (APHA 5310B) and Sydney Water Laboratory (SWL) for chlorophyll (APHA 10200 H), algal species and biomass (APHA 10200 I). All tests were performed by National Association of Testing Authorities (NATA) accredited laboratories ALS and SWL. All equipment was thoroughly rinsed with distilled water and each treatment level had its own stirrer and stainless-steel bucket to avoid cross contamination.

Samples for nutrients were collected on days 0, 2, 9, 14, 17 and 19 for low biomass treatments and on days 0, 4, 7,11, 14, 21, 40 for high biomass treatments. Samples were analysed in the laboratory using standard methods.

Samples for chlorophyll and algal counts were collected on days 0, 9, 17 for low biomass treatments and on days 0, 7,14, 21, 40 for high biomass treatments. Additional routine monitoring samples from two lake sites, one situated in the deepest section of the lake RPR1 and another on the southern side of the lake RPR6, had the same analytes collected throughout both experiments. Samples for total and dissolved carbon and filterable nitrogen and phosphorus were collected on days 0, 9 and 17 for low biomass treatments.

Statistical analysis

All data were tested for heteroscedacity and normality and, where necessary, transformed using a Log x+1 transformation before analysis. The nutrient, chlorophyll and algal biomass was compared to that of the control using a 2-way ANOVA with treatment and days as factors. A Bonferroni post hoc test for pairwise differences was used. Analyses were considered significant at the probability level of 0.05. The relationship between carp biomass concentration and nutrients, Chlorophyll-a and algal biomass was analysed by linear correlation. Where a significant correlation was found, the relationship was defined using linear regression after first checking residuals plots to ensure the data met the relevant assumptions. Error bars plotted on graphs represent standard error of the mean (SEM). GraphPad Prism 5 was used to perform 2-way ANOVAs and the linear regression and create graphs.

Figure 2. Installed mesocosms at Prospect Reservoir.

2.4 Results

During the high biomass experiment water temperature in the mesocosms and the lake varied between 22.5 and 26.6 °C and for the low biomass experiment between 20.4 and 21.4 °C (Table 1 and 2). Initial pH values in the lake and all treatments were ~7.5 for both experiments. Following slight declines, pH in the mid–level treatments of 1200 kg ha^{-1} and 2300 kg ha^{-1} rose to above 10 by day 11, remained at that level for 11 days and were significantly higher ($P<0.001$) from controls (Table 1). In lower biomass treatments, pH rose above 9 by day 7 and remained above 9 for 8 days. Other parameters are summarised in Table 1 and 2.

Dissolved oxygen

Initial dissolved oxygen (DO) levels were between 95 -100% saturation. For both experiments DO levels began to drop after day two in the treatments (Figure 4 & 5). DO declined more rapidly with increasing fish biomass, with most treatments reaching anoxic conditions within four days. The exception was the 250 kg ha^{-1} treatment which dropped to approximately 30% saturation at day five (Figure 4). These low DO levels continued for between 4-5 days in the 500 kg ha^{-1} and 1000 kg ha^{-1} treatments, lasted for seven days in the 1250 kg ha^{-1} and 2000 kg ha^{-1} treatments and up to 14 days for the 6000 kg ha^{-1} treatment. After two weeks DO levels in the 1250 kg ha^{-1} and 2500 kg ha^{-1} treatments began fluctuating on a diel cycle from 100%-300% saturation. Similarly, the 500 and 1000 kg ha^{-1} treatments had diel fluctuations from 35 - 200% saturation.

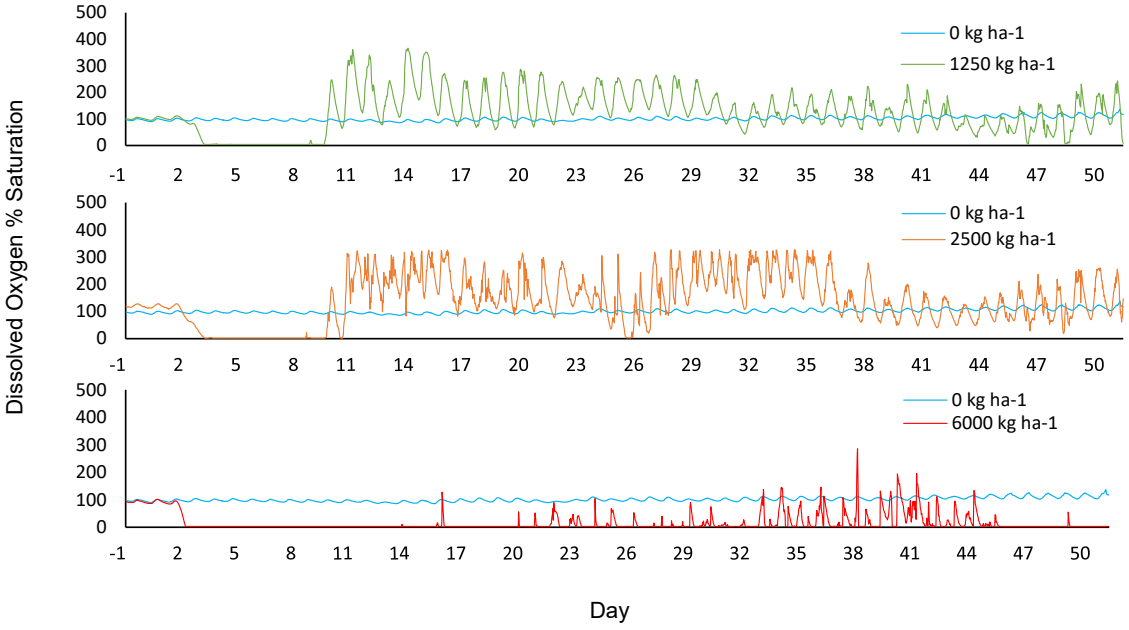

Figure 3. Dissolved oxygen levels of each of the high biomass treatments compared to the control of 0 kg ha^{-1} during the mesocosm experiments. Initial readings were recorded the day prior to application of fish biomass treatments (Day -1).

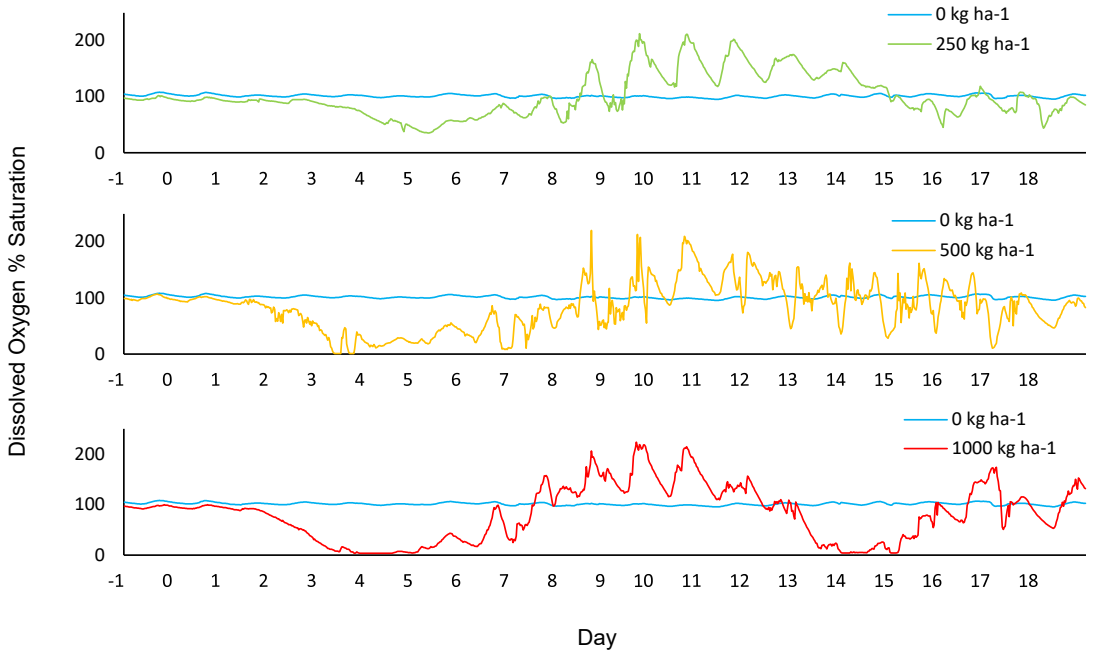

Figure 4. Dissolved oxygen levels of each of the low biomass treatments compared to the control of 0 kg ha^{-1} during the mesocosm experiments. Initial readings were recorded the day prior to application of fish biomass treatments (Day -1).

Nutrients

Nitrogen and phosphorus concentrations followed a similar pattern and were significantly higher ($P<0.001$) in the treatments than the control by day 4 in the high biomass experiment and by day 7 in the low biomass experiment (Figure 5). There was no significant difference between the controls and the lake samples throughout the experiment ($P>0.05$). In the high biomass experiment, nutrients peaked at day 14, whilst in the low biomass experiment, they peaked at day 9 (Figure 5). These concentrations stayed well above control levels for the remainder of each experiment. During the low biomass experiment dissolved fractions of N, P and organic carbon made up 60-90% of the nitrogen, phosphorus and carbon within the mesocosms (Table 2).

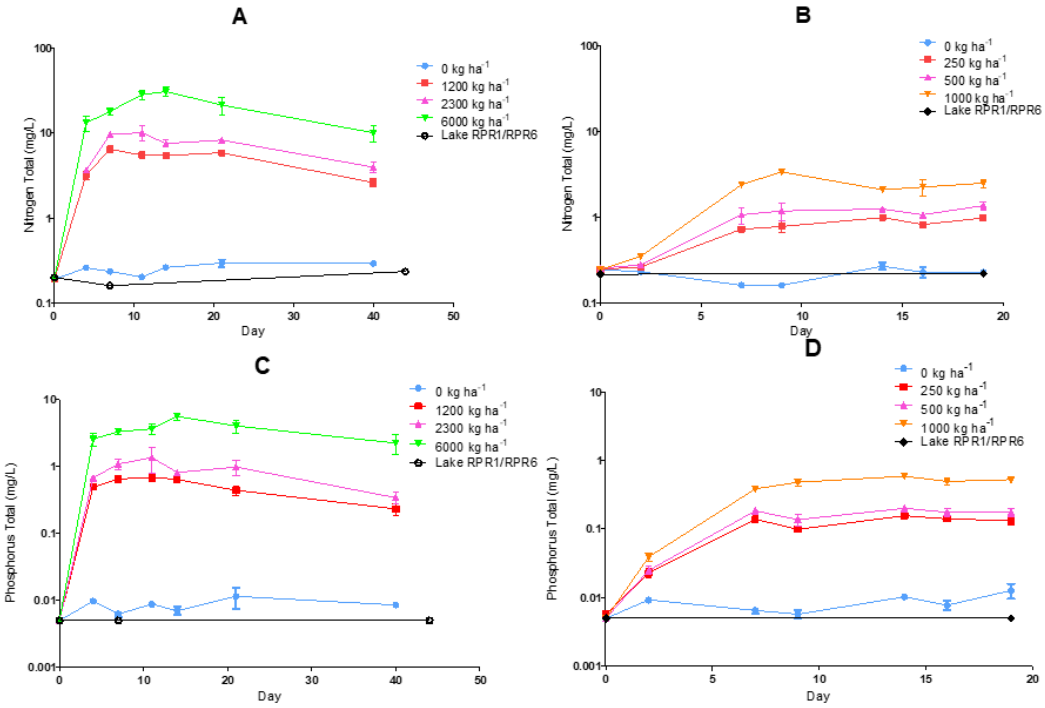

Figure 5. Nutrient levels during the two experiments. (A) Total nitrogen high biomass experiment (B) Total nitrogen low biomass experiment (C) Total phosphorus high biomass experiment (D) Total phosphorus low biomass experiment. Error bars ± standard error mean, n=4 (lake n=2)

The concentration of total nitrogen and total phosphorus on day 14 both had a positive linear relationship with carp biomass (Figure 6). By day 14 total phosphorus was related to carp biomass

with the relationship Y=0.0006303X -0.05432 with r^2=0.84. Total nitrogen was also related to carp biomass with the linear regression relationship Y=0.004738X - 0.3986 and r^2=0.91).

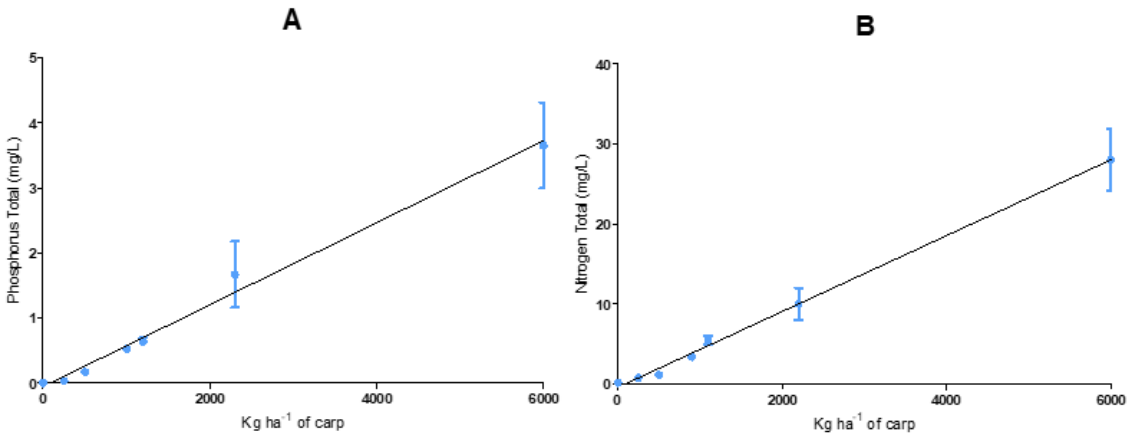

Figure 6: Linear relationship between carp biomass with mean (A) total phosphorus (B) total nitrogen on day 14

Phytoplankton Biomass and biovolume

Chlorophyll-a was significantly higher (P<0.001) in all treatments with the exemption of the 6000 kg ha^{-1} than the control (0 kg ha^{-1}) on day 7. Chlorophylla concentrations increased with increasing fish biomass, peaking in the second week of the experiments. Chlorophyll-a concentrations remained significantly higher than the control and lake sites at day 40 in the two highest biomass treatments of the high biomass experiment (Figure 7). By day 40 the 1200 kg ha^{-1} treatment had dropped to typical lake sites but was still significantly higher than the control (P<0.01). The concentration of chlorophyll a in the control treatment and lake sites were not significantly different (P>0.05) for the duration of both experiments.

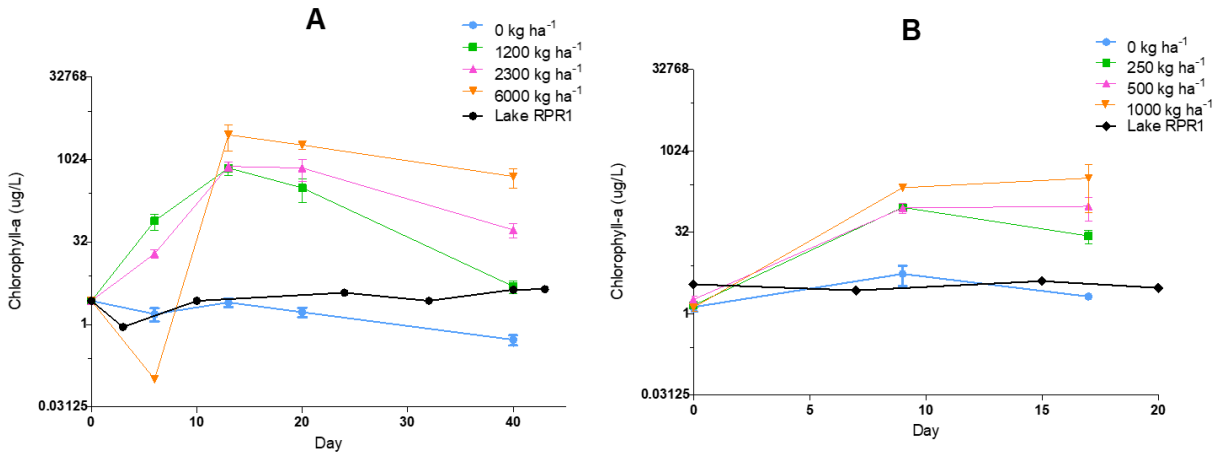

Figure 7. Chlorophyll-a concentration in the (A) high carp biomass experiment and (B) low carp biomass experiment. Error bars ± standard error mean, n=4 (lake site n=1)

In the high biomass experiment algal biovolume was significantly higher ($P<0.001$) by day 7 in all treatments except the 6000 kg ha^{-1} compared to the control. By day 14 all treatments were significantly higher than controls ($P<0.001$). All treatments peaked at day 20 and declined by day 40 except the 6000 kg ha^{-1} treatment which peaked on day 40 (Figure 8). In the low biomass experiment, peak algal biomass was reached at day 9. Control treatments and lake sites were not significantly different ($P>0.05$) in algal biomass in both experiments.

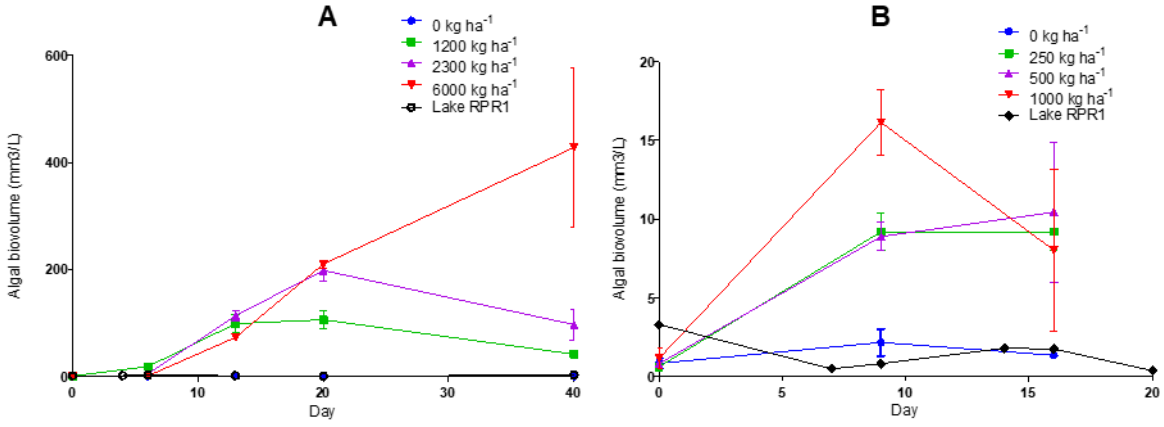

Figure 8. Algal biomass in the (A) high carp biomass experiment and (B) low carp biomass experiment. Error bars ± standard error mean, n=4 (lake n=1)

The increase in chlorophyll-a biomass and algal biovolume were also positively correlated with carp biomass (chlorophyll-a r^2=0.7469 and algal biomass r^2=0.9052) (Figure 9) by day 20 for the high biomass experiment. The low biomass data was not used as the experiment only ran for 17 days. The linear regression model for chlorophyll-a was Y=0.3161X-25.89 and for algal biovolume was Y=0.07029X+14.86. This suggests that predictions of algal biomass and biovolume response based on known biomass of carp within water bodies at densities between 1200-6000 kg ha^{-1} at 20 days post a fish kill event.

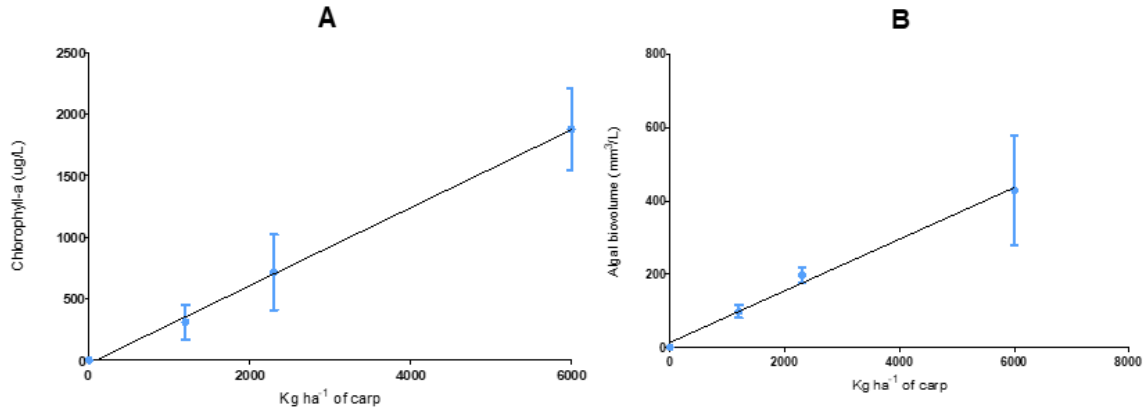

Figure 9: Linear relationship between carp biomass and (A) chlorophyll-a (B) algal biomass

Algal community structure

Algal community composition in the high biomass experiment mesocosms changed from a mixture of groups including Bacillariophyceae, Dinoflagellata, Cyanophyta and Chlorophyta to a community dominated by Chlorophyta and to a lesser extent Euglenophyta and the Cryptophyta. By day 7 all treatments were dominated by Chlorophyta while the control had dinoflagellates making up 50% of the biomass. Chlorophyta dominated in treatments and controls by day 40, with green picoplankton making up 70% of the biomass (Figure 10). The low biomass experiment mesocosms were initially composed of a mixture of algae with Bacilaariophyta, Cyanophyta and Dinophyta being the most dominant. By day 7 all treatments were dominated by Chlorophyta while the control had dinoflagellates as the most dominant (Figure 11). On day 14 Chlorophyta increased its dominance in treatments as in the control and this was more so in the higher biomass treatments.

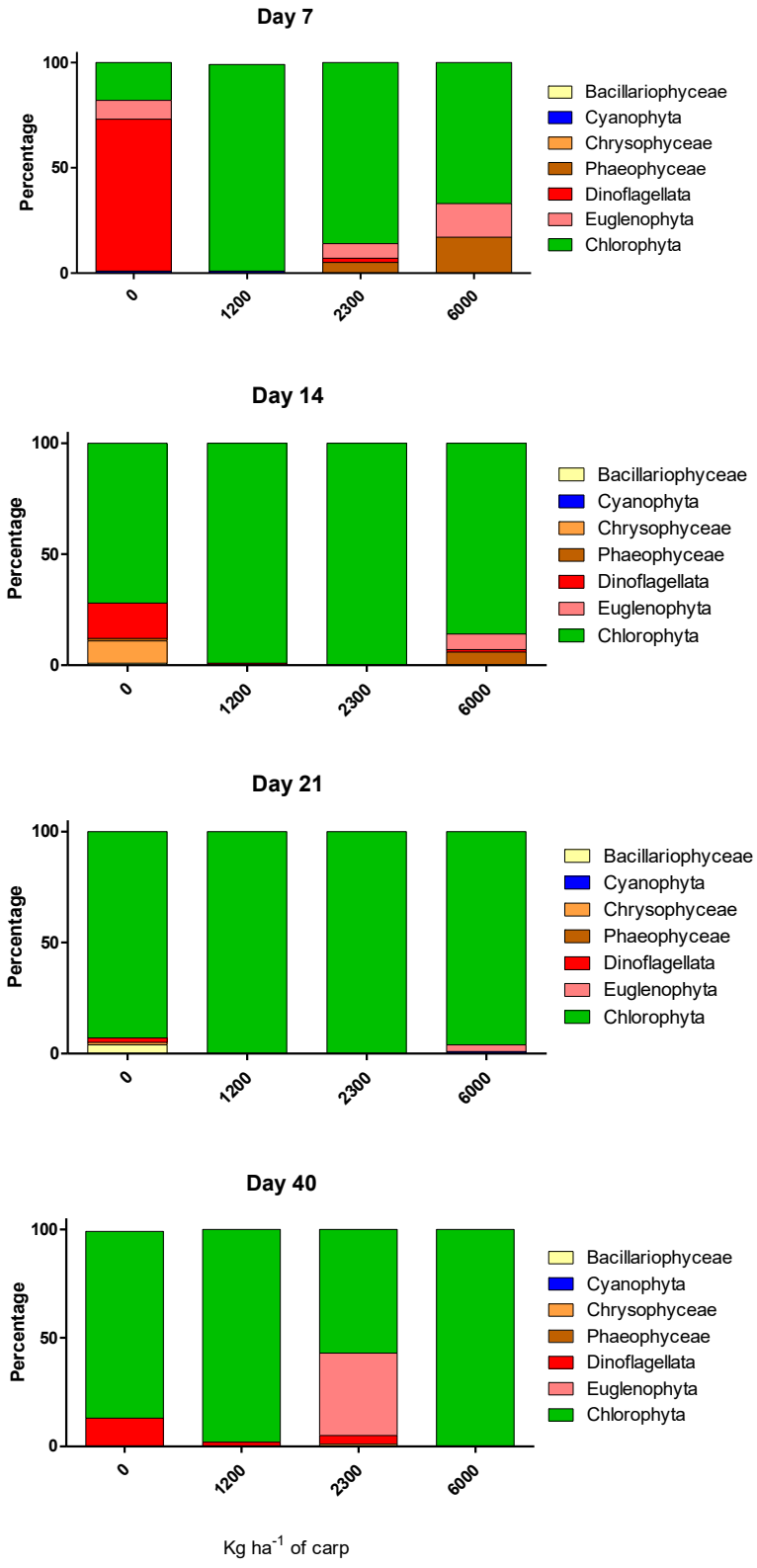

Figure 10. Relative biomass of phytoplankton major groups in the high biomass experiment.

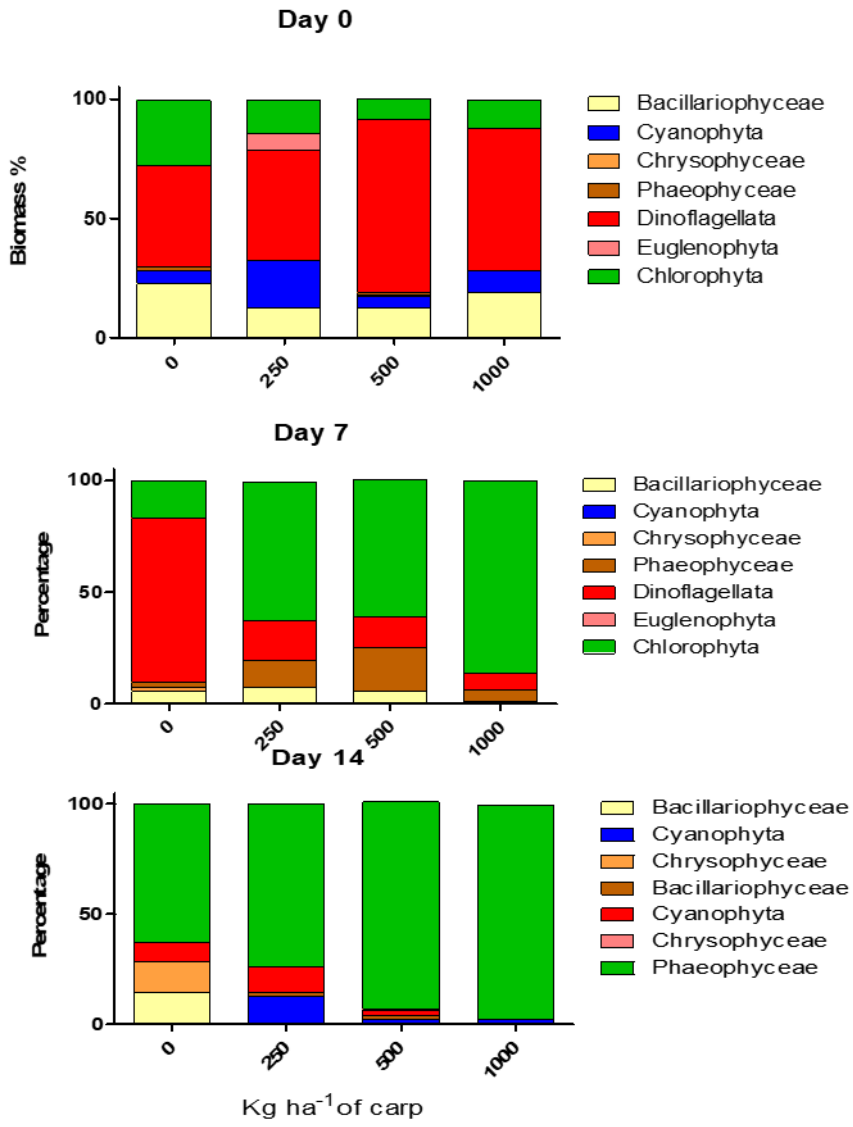

Figure 11. Relative biomass of phytoplankton major groups in the low biomass experiment.

2.5 Discussion

The lake mesocosm study found that the addition of dead carp at levels typically found in freshwater environments 250-1000 kg ha^{-1} resulted in significant nutrient release to the water column and had a long lasting and significant impact on water quality at all biomass levels tested. The impact on water quality was more pronounced and ecosystem recovery was slower with

increasing biomass of dead carp. This study further shows that changes in water quality following fish kills, such as after the release of the carp virus, may have a significant effect on algal community ecology. In the face of scientific concern regarding the proposed release of the cyprinid herpesvirus-3 (Lighten and van Oosterhout, 2017, Marshall et al., 2018), this study provides valuable information to assist in predicting and managing the potential ecosystem effects of decaying carp in aquatic environments. This study examined the impact of carp/unit area, not per unit volume in deep lakes or reservoirs. The mesocosms represented a shallow lake with a relatively large surface area. To utilise results from this experiment in predicting outcomes in other lakes, a dilution factor will be needed. This is to account for differences in surface area to volumes ratios in other lakes. The greater the surface area to volume ratio the more dilution is needed. In the case of Prospect reservoir, the dilution factor would be 30. This was calculated by the determining the carp per unit area and then adjust the difference between the volume/area ratio of the mesocosm compared to the volume/area ratio of Prospect reservoir. Shallow lakes such as the ones in Centennial Park in Sydney Australia would have a dilution factor close to 1. In general, the shallower the lake the larger the potential impact, due to carp biomass being calculated on surface area and not volume.

DO changes and ecological effects

Dissolved oxygen declined rapidly within two to three days in all treatments except the lowest treatment of 250 kg ha^{-1} which dropped to approximately 30% saturation by day 5. This is typical of anerobic microbial decomposition of organic matter (Kristensen et al., 1995). By day 9 in the high biomass experiment, oxygen levels in the 1250 kg ha^{-1} and 2300 kg ha^{-1} treatments rose to 200-300% saturation and persisted for the remainder of the experiment. Oxygen supersaturation coincided with increases in chlorophyll and algal biovolume, and was probably influenced by photosynthesis of the high phytoplankton biomass (Marshall and Falconer, 1973). (DO levels remained low in the highest treatment of 6000 kg ha^{-1} suggesting that the highest biomass of dead fish was still actively decomposing at the end of the 50-day experiment and respiration dominated (Ayers, 2010).

Prolonged anoxia in lakes or reservoirs with a large surface area and small volume would have serious consequences for the ecosystems. Anoxic conditions lasting longer than an hour could

potentially cause direct mortality of non-targeted aquatic species such as native fish (Beamish, 1964, Dean and Richardson, 1999, Riedel et al., 2013) and invertebrates (Conley et al., 2009, Galic et al., 2019). Water bodies can contain a high biomass of other species, such as the freshwater yabby *Cherax destructor,* between 500 and 1000 kg ha^{-1} (Mills and McCloud, 1983, Sang et al., 2011, McCormack, 2014) . These non-target species would be vulnerable to an anoxic event triggered by a carp fish kill. Compounding effects on water quality and potentially resulting in unintended mortality of threatened or recreationally valuable non-target species. This would follow the pattern of a typical black water event by increasing on the organic matter loading during the fish kill (Morgan and Hicks, 2013, Dutton et al., 2018)

Nutrient changes and ecological effects

All carp biomass treatments showed an increase in total phosphorus and total nitrogen concentrations (Figure 5). The treatments all had TN and TP concentrations well above the controls and the lake for the duration of both experiments. In the high biomass experiment this was for 40 days and in the low biomass experiment this was 14 days. Dissolved nutrients were only measured in the low biomass experiment and made up between 70-90% of the total C, N and P concentrations (Table 2).

There is little literature on the drivers and consequences of nutrient release from major fish kill events outside of salmonoid spawning events in upper reaches (Johnston et al 2004). It has been shown that decomposition of fish following large mortality events can induce brief algal blooms in ecosystems (Boros et al., 2015). However, it is well known how anthropogenic nutrient inputs can lead to eutrophication of water bodies (Smith et al., 1999, Heisler et al., 2008), leading to algal blooms and a reduction in algal diversity (Proulx et al., 1996, Ptacnik et al., 2008, Elser et al., 2009). The nutrient concentrations observed in this experiment are likely to lead to increased algal growth and blooms if environmental conditions are conducive, such as adequate light, temperature and euphotic depth to mixing depth ratio (Mitrovic et al., 2011). Freshwater phytoplankton blooms are known to be phosphorus limited (Schindler, 1977), nitrogen limited or sometimes co-limited by both (Mueller and Mitrovic, 2015). Further, the low mass ratios of N:P found in this study (5:1 to 10:1) may favour toxic cyanobacterial dominance in lakes (Pick and Lean, 1987, Orihel et al., 2017) particularly where there is light limitation (Havens et al., 2003).

An increase in toxic cyanobacteria can impact drinking water supplies due to potent cyanotoxins harmful to humans (Dokulil et al., 2000).

The available nutrients led to a phytoplankton bloom starting between day 7 and day 12 for all treatments. Interestingly a decrease in chlorophyll-a and algal biomass was observed only in the highest 6000 kg ha^{-1} treatment at day 7, after which it increased at day 14. This temporary reduction in algal biomass may be due to high bacterial growth outcompeting the phytoplankton for the nutrients as seen when high concentrations of DOC are added to river ecosystems (Carney et al., 2016). The blooms persisted for the length of both experiments (40 days in high biomass experiment and 14 days for the low biomass experiment). The presence of Euglenophyta is common in water with a high organic content (Çelik and Ongun, 2007) and increased with increasing carp biomass in this study. This represents a possible mesocosm effect (Schindler, 1998) of algal dominance possibly due to the shallowness of the mesocosms and less mixing than in the lake or no zooplankton grazing (Felisberto et al., 2011, Sitta et al., 2018). Despite the increased nutrient loads in both experiments, there was no significant increase in cyanobacteria biomass in fact there was a reduction. There is also the risk that if these algal blooms die rapidly it could lead to further hypoxic or anoxic conditions (Chislock et al., 2013, Ansari and Gill, 2014).

There were very different climatic conditions between the high and low biomass densities experiments, and this may have been a contributor to major differences in algal biomass beyond day 7 (Assemany et al., 2015). No rain fell during the high biomass experiment Nov/Dec 2016 but 170mm of rain fell during the first 12 days of the low biomass experiment. This combined with 27% lower mean daily solar exposure meant the low biomass experiment had been diluted and had less solar exposure than the high biomass experiment. These factors may have contributed to lower chlorophyll-a and biomass levels during the low biomass experiment.

Implications for water treatment

With such high levels of nitrogen, one concern is the amount of free ammonia available which is often found in water of low dissolved oxygen environments (Bhateria and Jain, 2016). During decomposition of organic matter, heterotrophic bacteria can also decompose proteins, releasing ammonia (Mühlenbruch et al., 2018, Martikainen, 2022). In the aquatic environment, toxicity of

ammonia increases with pH, because ammonia enters organisms as NH_3. This proportion of NH_3 increases with water pH (Randall and Tsui, 2002) and our experimental results showed pH reaching 10. The level of free ammonia in raw water is also critical for water treatment as it determines the chlorine dose required for disinfection (Twort et al., 2006). The higher the level of free ammonia the higher the dosage of chlorine required due to chloramines produced which are less effective at disinfection than chlorine (Twort et al., 2006). Increased disinfection doses can also lead to carcinogenic by products such as trihalomethanes and n- nitrosodimethylamine (Villanueva et al., 2015, Li et al., 2019). The high pH could lead to other water treatment issues as optimal pH range for coagulation is 6 to 7 when using alum and 5.5 to 6.5 when using iron. For high alkalinity water, excessive amounts of coagulant may be needed to lower the pH to the optimal pH range (Iaspub.epa.gov., 2018).

The regression analysis showed a strong relationship between carp biomass and water quality impacts (Figure 6). Knowledge of the carp biomass within a reservoir, lake or river section will help determine the likely water quality impacts. As changes to the water quality happened fairly rapidly, early intervention or clean-up is critical from a management perspective. An example of this is the fish kills which occurred near the township of Menindee, NSW, Australia on the Darling River. Three successive fish kills occurred between December 2018 and January 2019. The dead fish not removed increased the carbon load creating a feedback loop which contributed to subsequent fish deaths (Vertessy et al., 2019). If dead fish are removed from the water body within two to three days of them dying will reduce the impact as the fish carcasses are generally still floating and bacterial activity is still low (Figure 3 & 4). If a virus was released to kill carp, a clean-up response based on knowledge of fish biomass should be implemented.

2.6 Conclusion

The introduction of dead fish (common carp) into a water body resulted in the prevalence of an anoxic environment which persisted between 1 to 12 days. This long-term anoxic environment could have adverse effects to ecological communities and may cause a secondary kill of non-targeted fauna such as native fish. Anoxia was followed by an increase in nutrients which increased relative to carp biomass and led to an increase in algal biomass. The scale and the length of the water quality impact increased with the biomass (kg ha^{-1}) of dead fish. It is suggested

that timing of the removal of dead fish is critical to maintaining good water quality. Fish removed within the first 2-3 days of mortality should have considerably less impact on water quality when compared to fish not removed.

Table 1. High biomass summary statistics for W/T: water temperature; COND: conductivity; pH and Turb: turbidity. Mean 4 standard error (s.e.) are shown for each observation (n: 4) for which median values are shown.

	Control			1200 kg ha^{-1}			2300 kg ha^{-1}			6000 kg ha^{-1}		
Day	0	7	14	0	7	14	0	7	14	0	7	14
WT (OC)	25.8±0.1	26.0±0.1	23.6±0.1	25.4±0.2	26.2±0.2	21.9±0.1	25.9±0.1	23.8±0.1	21.0±0.1	25.9±0.1	24.1±0.1	21.9±0.1
Cond(uScm)	194.8±0.9	209.3±1.8	198.1±1.6	193.4±0.9	225.5±3.6	204.5±0.4	194.7±1.3	259.7±3.4	218.3±8.2	195.3±1.7	365.6±24.8	337.0±23.2
pH	7.6±0.0	6.4±0.1	7.4±0.0	7.7±0.0	7.9±0.5	9.9±0.1	7.6±0.0	7.0±0.1	10.3±0.2	7.6±0.0	7.5±0.2	8.7±0.2
Turb (NTU)	2.6±0.6	1.0±0.7	0.6±0.2	4.6±1.3	7.1±0.8	17.8±3.3	5.6±2.3	15.7±4.2	32.5±10.6	1.9±0.5	34.2±5.0	44.9±10.3

Table 2. Low biomass summary statistics for W/T: water temperature; COND: conductivity; pH ; Turb: turbidity ; TN : total nitrogen ; DN : dissolved nitrogen ; TP : total phosphorus ; DP : dissolved phosphorus ; TOC : total organic carbon ; DOC : dissolved organic carbon. Mean 4 standard error (s.e.) are shown for each observation (n: 4) for which median values are shown.

Mar-17	Control			250 kg ha^{-1}			500 kg ha^{-1}			1000 kg ha^{-1}		
Day	0	7	14	0	7	14	0	7	14	0	7	14
WT (OC)	21.4±0.3	20.4±0.0	22.1±0.0	21.0±0.3	20.5±0.1	22.1±0.1	21.0±0.3	20.6±0.0	22.1±0.0	21.5±0.3	20.5±0.1	22.2±0.1
Cond (uS cm)	199.7±2.2	143.6±0.9	143.9±0.8	201.5±2.3	139.8±2.0	137.7±2.3	196.7±2.0	147.7±3.1	145.7±6.7	201.4±2.6	156.9±1.3	157.2±8.8
pH	7.5±0.1	7.2±0.0	7.6±0.0	7.3±0.0	9.3±0.2	9.0±0.1	7.5±0.0	9.4±0.1	9.7±0.2	7.5±0.0	9.1±0.1	9.3±0.4
Turb (NTU)	1.3±0.6	1.9±0.2	3.0±0.2	2.1±0.5	3.7±1.3	10.8±4.2	0.6±0.2	2.2±0.4	7.8±1.2	0.5±0.2	4.2±1.1	15.71±3.8
TN	0.2±0.0	0.2±0.0	0.3±0.0	0.2±0.0	0.7±0.0	1.0±0.1	0.3±0.0	1.1±0.2	1.2±0.1	0.2±0.0	2.4±0.1	2.1±0.0
DN	0.2±0.0	0.1±0.0	0.2±0.0	0.2±0.0	0.5±0.0	0.5±0.0	0.2±0.0	0.7±0.1	0.6±0.1	0.2±0.0	1.6±0.1	1.4±0.1
TP	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.0	0.2±0.0	0.0±0.0	0.2±0.0	0.2±0.0	0.0±0.0	0.4±0.0	0.6±0.0
DP	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.3±0.0	0.2±0.0
TOC	4.5±0.0	3.8±0.1	4.0±0.1	4.7±0.1	5.4±0.2	10.2±0.1	4.6±0.1	5.4±0.1	12.1±0.4	4.5±0.1	10.1±1.6	15.2±0.3
DOC	4.5±0.0	3.6±0.1	3.9±0.0	5.0±0.1	4.9±0.4	9.5±0.8	4.4±0.1	5.7±0.3	9.9±0.5	4.5±0.1	6.8±0.8	13.7±0.6

Table 3. High biomass F-values, degrees of freedom and P values from 2 way ANOVA analysis of water chemistry and phytoplankton response variables.

* Denotes non-homogenous dispersion (p<0.05) and a lowered significance level of 0.01. Bold denotes significant tests.

	Treatment			Day			Treatment*Day		
	F	df	*P*	F	df	*P*	F	df	*P*
Univariate tests									
Chlorophyll-a	340.1	3	**0.0001**	191.7	4	**0.0001**	40.66	12	**0.0001**
Algal biomass	221.4	3	**0.0001**	209.5	4	**0.0001**	32.97	12	**0.0001**
Total nitrogen	225.9	3	**0.0001**	262.9	6	**0.0001**	36.45	18	**0.0001**
Total phosphorus	75.60	3	**0.0001**	47.94	6	**0.0001**	12.20	18	**0.0001**
Conductivity	21.83	3	**0.0001**	63.22	16	**0.0001**	19.45	48	**0.0001**
pH	191.2	3	**0.0001**	171.8	16	**0.0001**	29.94	48	**0.0001**
Dissolved Oxygen		3			16			48	

Table 4. Low biomass F-values, degrees of freedom and P values from 2 way ANOVA analysis of water chemistry and phytoplankton response variables.

* Denotes non-homogenous dispersion (p<0.05) and a lowered significance level of 0.01. Bold denotes significant tests.

	Treatment			Day			Treatment*Day		
	F	df	*P*	F	df	*P*	F	df	*P*
Univariate tests									
Chlorophyll-a	31.81	3	**0.0004**	80.59	2	**0.0001**	6.456	6	**0.0001**
Algal biomass	8.591	3	**0.0001**	39.65	2	**0.0001**	3.275	6	**0.0001**
Total nitrogen	368.8	3	**0.0001**	75.83	6	**0.0001**	16.24	18	**0.0001**
Dissolved Nitrogen	415.4	3	**0.0001**	120.7	6	**0.0001**	28.57	18	**0.0001**
Total phosphorus	458.0	3	**0.0001**	103.6	6	**0.0001**	26.69	18	**0.0001**
Dissolved phosphorus	64.68	3	**0.0001**	53.01	6	**0.0001**	20.82	18	**0.0001**
Total Organic carbon	86.09	3	**0.0001**	142.1	2	**0.0001**	27.07	6	**0.0001**
Dissolved Organic Carbon	50.18	3	**0.0001**	161.8	3	**0.0001**	26.59	12	**0.0001**
pH	165.3	3	**0.0001**	333.4	8	**0.0001**	32.09	24	**0.0001**
Conductivity	8.591	3	**0.0002**	142.6	8	**0.0001**	3.354	24	**0.0001**
Dissolved Oxygen									

Chapter 3: The impact of decaying carp on microbial communities in lakes: results from mesocosm experiments

Statement of contribution to co-authored chapter and paper to be submitted to Aquatic Microbial Ecology

This chapter includes the co-authored details, as follows:

My contribution to the paper included data pre-processing, analysis and interpretation, and manuscript writing.

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3.1 Abstract

A Cyprinid herpesvirus-3 (CyHV-3) release in Australia, resulting in an associated mass common carp mortality and decomposition event could have serious implications to water quality and public health in rivers and lakes. To assess the impact of a virus induced fish kill on the microbial community, common carp carcasses from 250 to 6000 kg ha^{-1} were placed into 2000 L mesocosms within Prospect Reservoir, Australia for up to 30 days. The addition of dead carp biomass led to significant changes in microbial assemblage structure, which shifted from a community comprising typical lake bacteria, such as *Limnobacter*, *Limnohabitans* and the *hgcl_clade*, to a community characteristic of eutrophic conditions, including higher relative abundance of copiotrophic bacteria, such as *Flavobacterium*, *Roseococous* and *Rhodobacter*. Significant increases in signature fish gut bacteria such as *Clostridium, Fusobacterium and Bacteroides*, were also observed, along with increases in potentially pathogenic genera such as *Vibrionaceae, Campylobacter, Arcobacter* and *Shewanella*. The cyanobacteria *Pseudanaebena* and *Merismopedia* were significantly correlated with the odour causing compounds Geosmin and MIB in dead carp treatments. These results confirm that a mass carp mortality event following release of the Cyprinid herpesvirus-3 will significantly change the microbiology of freshwater ecosystems, indicating that clean-up of dead fish following a virus release is critical to maintaining water quality.

3.2 Introduction

Microorganisms underpin many ecosystems and their abundance (Lynch and Neufeld, 2015), diversity and distribution play an important role in determining the type of environment that will be established (Yachi and Loreau, 1999, Salam, 2019) and drive chemical and energy cycles in lotic environments (Shade et al., 2012, Carney et al., 2015). Bacteria are important in decomposition of organic carbon (Koehler et al., 2012) and the carbon source is crucial in the makeup of the Bacterial Community Composition (BCC) (Muylaert et al., 2005).

In Australia the introduced fish species, the common carp can comprise up to 80% of the fish biomass in rivers and lakes (Shearer and Mulley, 1978, Koehn, 2004, Huser et al., 2016, Stuart et al., 2021). This species is considered a pest (National Carp Control Plan, 2021) due to its potential to alter the environment as ecosystem engineers and propensity to become the dominant species (King et al., 1997, Parkos Iii et al., 2003, Adámek and Maršálek, 2013, Lorenzo and Ali, 2015). Over the last 20 years efforts have been made to try to control or eradicate the species from Australian waterways (Koehn et al., 2000, DPI, 2010). Among these, the National Carp Control Plan has completed a feasibility study of using the Cyprinid herpesvirus 3 (CyHV-3) as a biological control agent for introduced common carp in Australia. CyHV-3 is a species-specific DNA virus causing a viral disease in common carp (Waltzek et al., 2005, Rakus et al., 2013). In unintended releases, this virus has been responsible for large mortalities of common carp (Sunarto et al., 2005, Thresher et al., 2018) with over 100,000 killed in Lake Biwa in Japan (Minamoto et al., 2009).

A large fish kill occurring following the release of CyHV 3 will likely lead to the release of large amounts of organic and inorganic matter from decomposing fish, leading to potentially substantial but undefined consequences, including algal blooms and other water quality issues (Killberg-Thoreson et al., 2014, Pera et al., 2021). The resulting input of nutrients from the decaying carp could also fuel a substantial microbiological change in the lake ecology (Capo et al., 2017). Changes in the bacterial community can also result in shifting the biochemical cycling and the potential growth of pathogenic species (Bush et al., 2015, Guo et al., 2022) and the production of taste and odour compounds (Benner et al., 2004) which can all cause problems for drinking water supply (Young et al., 1996). Little is known about the impacts of carp and other types of fish kills on the resident bacterial community

within lakes and the potential water quality risks associated. Changes in the bacterial community can result in the production of taste and odour compounds (Benner et al., 2004) which in turn can cause problems for drinking water (Young et al., 1996).

This study used mesocosm experiments to examine the potential impact of a virus induced fish kill on the bacterial community and associated taste and odour issues. Bacterial community composition was measured over a range of different biomass levels of dead carp (1200 to 6000 kg ha^{-1}). It is hypothesised that increased biomass of dead carp in mesocosms would change the microbial composition, subsequently reducing oxygen levels and potentially producing significant taste and odour compounds such as MIB and Geosmin.

3.3 Methods

Experimental Design

To assess changes in BCC from decaying carp biomass, dead carp were placed into mesocosms in Prospect Reservoir, New South Wales, Australia (Figure 12) and four replicates were used for each treatment level. Treatments used in the study equated to biomass of 0 (control treatment), 1200 (low treatment), 2300 (medium treatment) and 6000 (high treatment) kg ha^{-1} of dead carp tare. There was an additional treatment of 6000 kg ha^{-1} (Removed treatment) where the carcasses of the carp was removed after 8 days to simulate a clean-up of dead carp from a lake. Biomass estimates were established from previous surveys of populations in rivers and lakes (Hicks et al., 2006, Bajer and Sorensen, 2012, Tempero, 2013, Stuart et al., 2021). High biomass levels occur naturally between 670 to 1160 kg ha^{-1}, with 2200 kg ha^{-1} found in the Sydney catchment and up to 3144 kg ha^{-1} in many areas of the Murray Darling Basin (MDB) (Department of Environment and Planning, 2017). Pre-spawning migration of common carp could see potentially 90% of the lake biomass concentrated in small shallow areas of the lake (Hicks et al., 2012, Sorensen et al., 2015). Whole fish were used to simulate an intact fish following virus mortality.

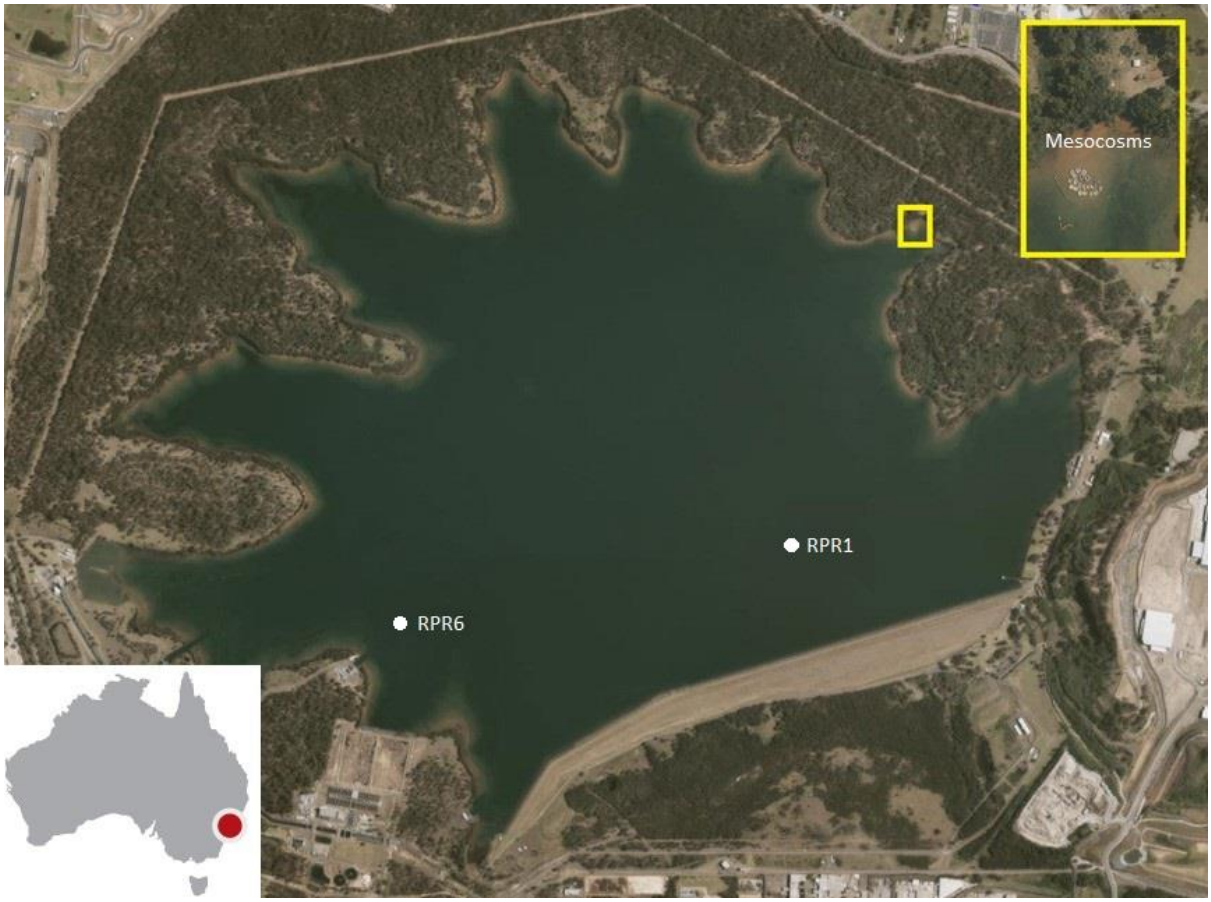

Figure 12: Location of the mesocosm experiment within Prospect Reservoir, Australia.

Mesocosm Design

Mesocosms consisted of a cylindrical outer shell made from rolled steel with an inner PVC bladder filled with ~2000 L of lake water prior to the experiment commencing as per Chapter 2 and (Pera et al., 2021).

Sampling procedures and analysis for plate counts and taste and odour compounds

Daily measurements of temperature, conductivity, dissolved oxygen, pH and turbidity were measured in all mesocosms with a YSI EXO multiparameter sonde. Two mesocosms from each treatment level had a MS5 Sonde probe logging at ten-minute intervals for the length of each experiment. There were also two YSI EXO multiparameter probes placed in two of the treatments logging temperature, conductivity, dissolved oxygen, pH and turbidity at ten-minute intervals as per Chapter 2 and (Pera et al., 2021).

Prior to taking samples, each mesocosm was mixed by stirring to homogenise. Samples were collected using a 10 L stainless-steel bucket and were decanted into smaller bottles for

specific analysis in the laboratory. All equipment was thoroughly rinsed with distilled water and each treatment level had its own stirrer and stainless-steel bucket to avoid any cross contamination (Pera et al., 2021). Samples for odour compounds 2-Methylisoborneol (MIB) and Geosmin were collected on days on days 7, 14, 21, 28. Samples for Microbial DNA was collected on days on days 7, 14 and 21. Additional routine monitoring samples from lake site RPR1 had the same analytes collected for Geosmin throughout the experiment.

Samples collected were decanted into smaller bottles for specific analytes provided by a commercial laboratory for MIB and Geosmin (EP-115) were measured using rapid trace analysis at Australian Laboratory Services (ALS). Total coliforms and *E. coli* were determined by enumeration by enzyme substrate method MI28 (Edberg et al., 1990) at the Sydney Water Laboratory (SWL). Samples for bacterial DNA analysis (2 L volume) were filtered at University of Technology Sydney laboratory

Bacterial community analysis

Microbial DNA was extracted from 0.22 µm membrane filters using the Powerwater DNA isolation Kit (MoBio Laboratories, Inc) in accordance with the manufacturer's instructions. DNA quantity and purity was evaluated using a Nanodrop-1000 Spectrophotometer. Patterns in bacterial community diversity and composition were characterised using 16S rRNA amplicon sequencing. The V1–V3 variable region of the 16S rRNA gene was amplified using the primers 27f 5'-AGRGTTTGATCMTGGCTCAG-3' and 519r 5'-GTNTTACNGCGGCKGCTG-3'. Sequencing was performed using the Illumina MiSeq platform following the manufacturer's guidelines.

16S rRNA gene sequences were analysed using the QIIME pipeline (Caporaso et al., 2010, Kuczynski et al., 2011). Briefly, paired-end DNA sequences were joined and de novo Operational Taxonomic Units (OTUs) were defined at 97% sequence identity using UCLUST (Edgar et al., 2011). Chimeric sequences were detected using ChimeraSlayer (Haas et al., 2011) and filtered from the dataset. Taxonomy was assigned against the SILVA v128 database using the BLAST option in QIIME. Sequences were rarefied to the same depth to remove the effect of sampling effort upon analysis. All chloroplast OTU's were filtered out of the bacterial dataset.

The alpha-diversity of the bacterial community was measured and compared between samples by applying the Chao1 algorithm (Chao, 1987) to rarified OTU sequence reads. The Chao1 diversity metric was chosen to measure alpha-diversity because it is well suited to calculating diversity in microbial amplicon sequence datasets that inherently contain a large proportion of rare taxa (with zero and one counts).

Principal Components Analysis (PCoA) (Davis and Sampson, 1986) was used inside the Paleontological Statistics 3.18 (PAST) software (Hammer et al., 2001) to explore similarity/dissimilarity in the composition of bacterial communities between mesocosm treatments and sample time points. The implementation of PCoA in PAST follows the eigenanalysis algorithm provided in (Legendre and Legendre, 1998). The ordinations (OTU relative abundance values) were given as site scores. Prior to analysis, OTU relative abundance values were square-root transformed to minimise the effect of outliers while maintaining proportionality.

Variance in bacterial community assemblages between mesocosm treatments and sample time points were tested using permutational analysis of variance (PERMANOVA) (Anderson, 2001). Where significant variation was detected across treatments and time points, Bonferroni-corrected p-values were used post-hoc to identify which treatments and/or time points were significantly different. Furthermore, similarity percentage analysis (SIMPER) (Clarke, 1993), using the Bray-Curtis similarity measure was employed to assess which bacterial taxa were primarily responsible for an observed difference between mesocosm treatments.

Exploration of statistically significant covariance between bacterial OTUs, algal species (using algal count data) and taste and odour compounds, MIB and geosmin was conducted using Extended Local Similarity Analysis (eLSA) (Xia et al., 2011). eLSA outputs were used for subsequent construction of microbial networks. Only pairwise interactions that occurred across 10% or more of the time points were included, and each remaining interaction was subjected to 1000 permutations to calculate statistical significance (p value) and the false detection rate (q value). Interactions where $p > 0.001$ and $q > 0.01$ were excluded to reduce the risk of Type I and Type II errors. Resultant data defined all statistically significant covariance events. eLSA data was ultimately visualised using the edge-weighted spring

embedded function in Cytoscape 3.4.0 software package, using the Pearson's Correlation value of pairwise correlations to define the darkness in colour of edges connecting nodes.

3.4 Results

Dissolved oxygen

Dissolved oxygen (DO) levels began to drop after day two in all treatments. The higher biomass treatments led to greater reductions, with most treatments dropping to close to zero (<4% saturation) within three days. Low DO levels continued for seven days in the 1250 and 2000 kg ha^{-1} treatments and continued to the end of the experiment (32 days) for the 6000 kg ha^{-1} treatment. After two weeks DO levels in the 1250 and 2500 kg ha^{-1} treatments fluctuated diurnally from 100-300% saturation. When fish carcasses in the 6000 kg ha^{-1}treatments were removed after 8 days, DO levels increased within two days and had similar daily fluctuations to the 1250 and 2500 kg/ha treatments, reaching 300% during the day but dropping to between 0 and 40% at night (Figure 13).

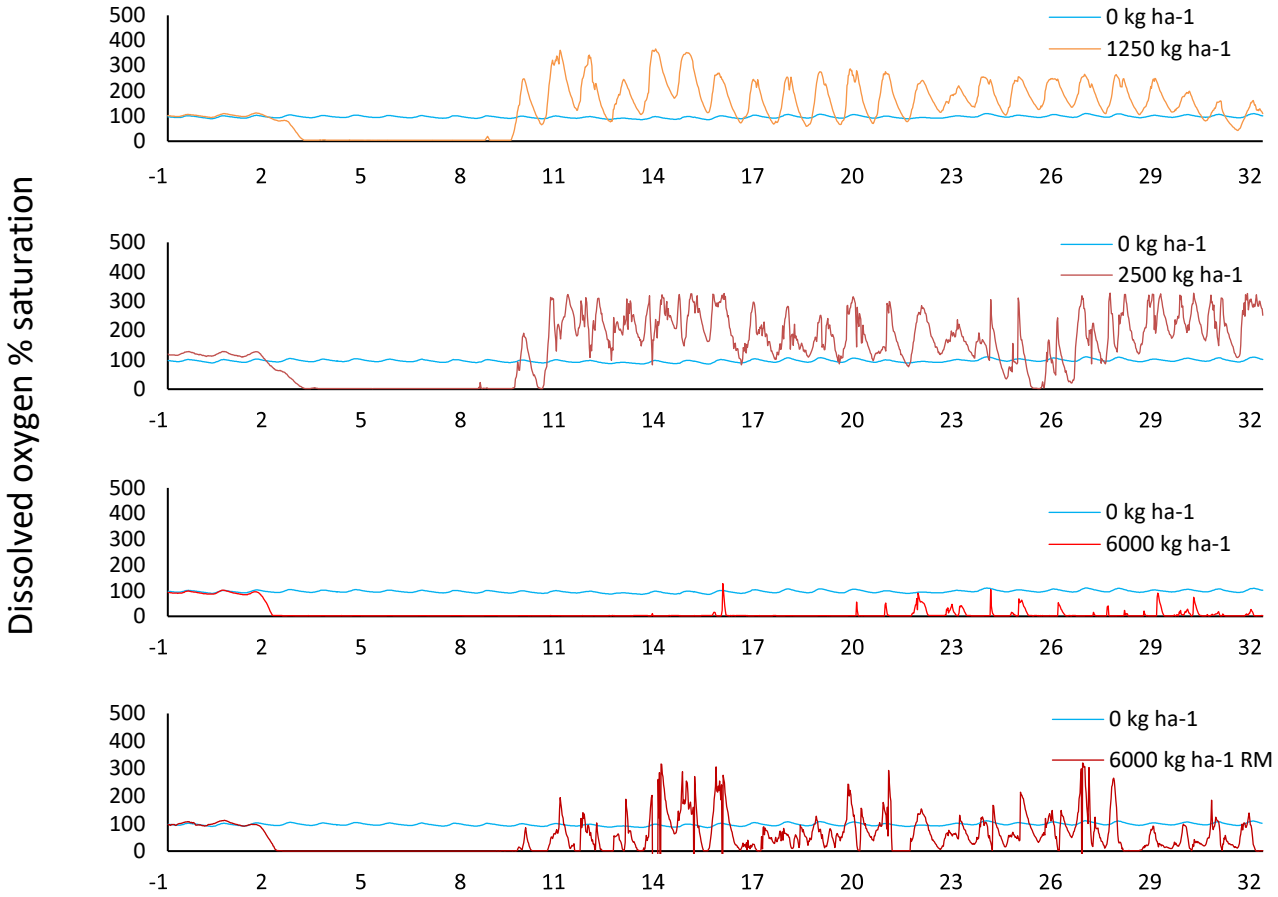

Figure 13. Dissolved oxygen levels as % saturation for each of the biomass treatments compared to the control during the mesocosm experiments. Initial readings were recorded the day prior to application of fish biomass treatments (Day -1).

E. coli and total coliforms

Anaerobic bacteria such as *Escherichia coli (E. coli)* and total coliforms all increased with increasing carp biomass. *E. Coli* and total coliforms differed most from the control on day 3 and gradually dropped to control levels by day 11 for total coliforms 28 for E. coli (Figure 14).

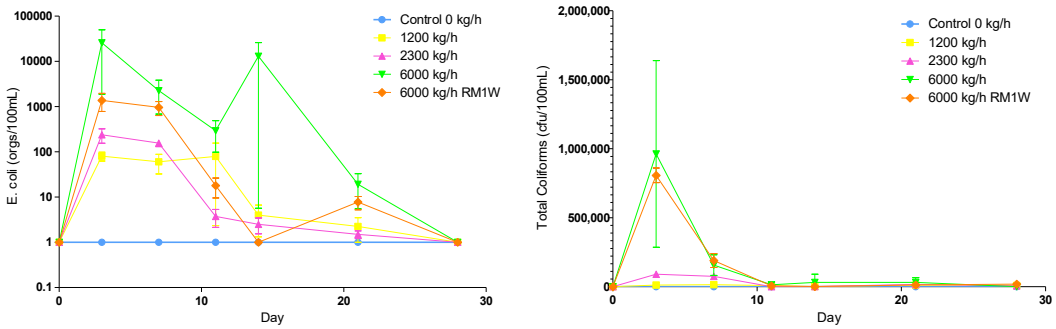

Figure 14. E. coli and total coliforms plate counts during the experiment. Error bars represent ± standard error mean (n = 4).

Bacterial Community Diversity

The diversity of bacterial communities was compared between mesocosm treatments and time points. Alpha diversity, measured by Chao1 values, remained stable in the control mesocosms throughout the experiment ($F = 1.21$, $df = 2$, $p > 0.05$), but by the first sample occasion on the 01/12/2016 had increased significantly in the medium ($F = 13.08$, $df = 1$, $p < 0.01$) and both the high biomass treatments (high; $F = 16.61$, $df = 1$, $p < 0.01$, removed; $F = 48.07$, $df = 1$, $p < 0.01$) compared to the control mesocosms. Throughout the experiment, alpha diversity was statistically indistinguishable between control and low biomass treatments ($p = > 0.05$). By the second sample occasion on 08/12/2016, alpha diversity in only the "removed" biomass mesocosms remained statistically higher ($F = 17.70$, $df = 1$, $p < 0.01$) compared to controls (Figure 15). At the final sample occasion performed on the 14/12/2016, alpha diversity across all treatments were statistically indistinguishable from control mesocosms ($p <0.05$) and both high and high/removed treatments were also statistically indistinguishable.

PERMANOVA was employed to statistically compare bacterial community composition between treatments, with significant differences observed between sample time points ($F = 5.17$, $perm = 9999$, $p < 0.001$), treatments ($F = 5.84$, $perm = 9999$, $p < 0.001$), and the time/treatment interaction ($F = 2.78$, $perm = 9999$, $p < 0.001$). The bacterial communities within the control mesocosms were statistically different from all carp biomass treatments ($p < 0.001$). The bacterial communities within low and medium carp biomass treatments were

not significantly different from each other (p >0.05) but were significantly different to the high carp biomass and the high/removed treatments (p < 0.001).

Principal Components Analysis (Figure 16) revealed that bacterial communities in control mesocosms remained stable over the duration of the experiment and were distinct from the treatment mesocosms. Conversely, the composition of bacterial communities within 1250 – 6000 kg ha^{-1} carp biomass treatments were similar at the first sampling point but by the second sample occasion 1,200 and 2,300 kg ha^{-1} carp biomass treatments diverged notably from 6,000 kg ha^{-1} carp biomass treatments. Interestingly, after removal of carp biomass from the 6,000 kg ha^{-1} carp biomass treatments at day eight of the experiment, the general composition of bacterial communities in carp-removed treatments converged with those belonging to 0 – 1,200 kg ha^{-1} carp biomass treatments. Moreover, bacterial communities in the 6,000 kg ha^{-1} carp biomass treatments remained distinct from lower biomass treatments for the duration of the study.

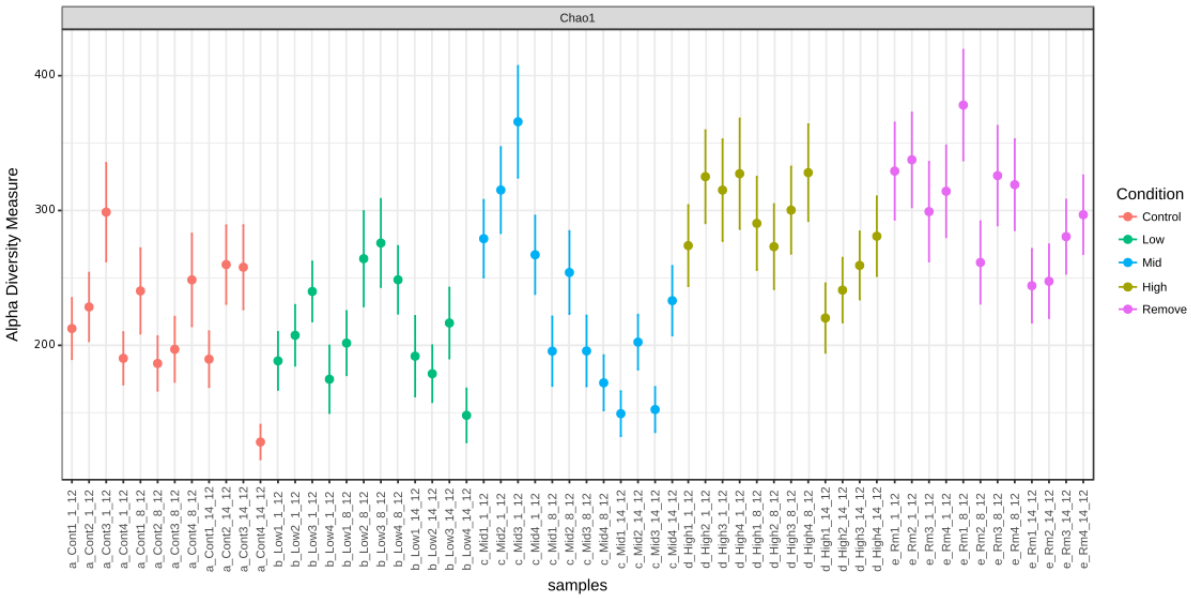

Figure 15 Box plots depicting alpha-diversity of bacterial communities using the Chao1 diversity measure.

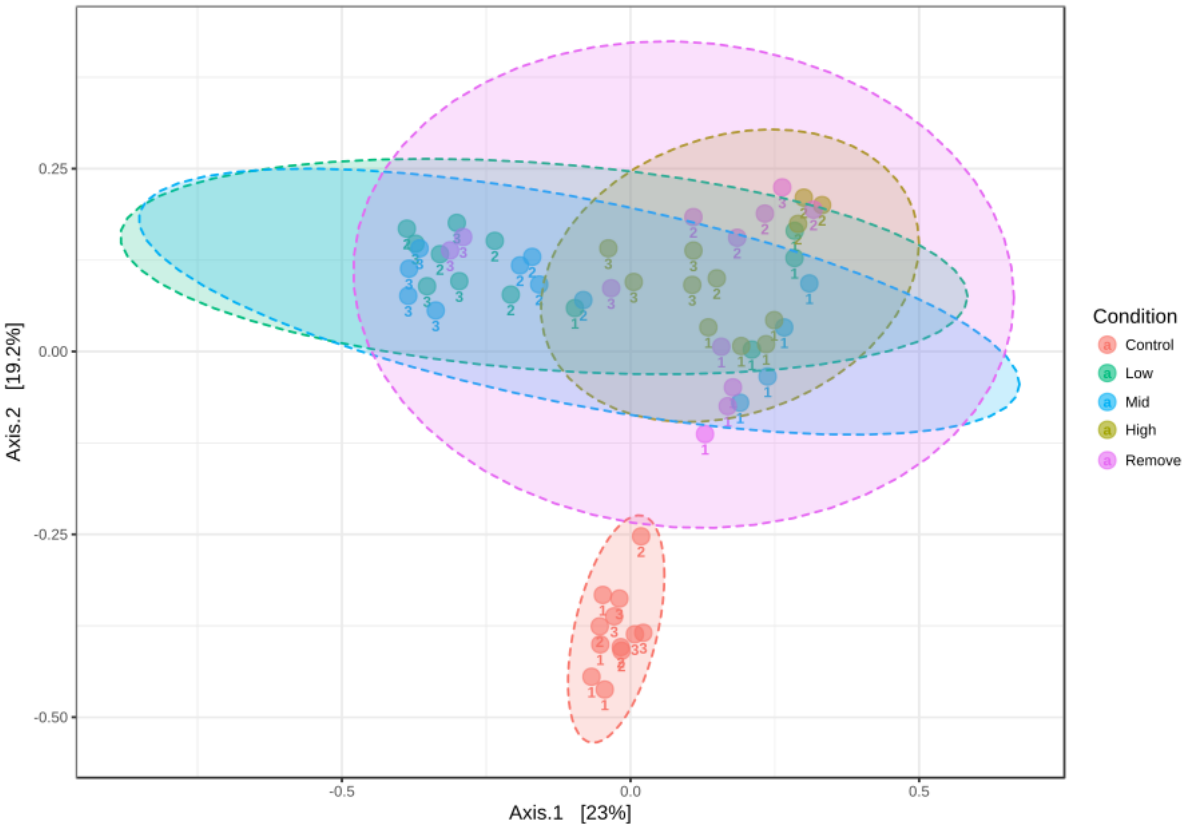

Figure 16 The phylogenetic relationship of bacterial community assemblages are compared between mesocosm treatments using a Principal Components plot depicting beta-diversity

Bacterial Community Composition

In the control mesocosms, the composition of bacterial communities remained relatively stable across sample occasions at days 7, 14 and 21 compared to carp biomass treatments. Bacteria belonging to the Proteobacteria phylum were the most relatively abundant, representing between 50.6 – 65.7% of bacterial communities, and maintained a stable population across the study period. Within Proteobacteria, OTUs assigned to the Betaproteobacteria and Alphaproteobacteria classes were the most abundant, ranging in relative abundance from 28.8 – 47.6% and 15.7 – 22.0% respectively. The most notably abundant Proteobacteria OTU was Limnohabitans, a bacterium widely distributed and common in freshwater environments, which comprised 21.1% of all bacterial OTUs at day seven and rose to 34.5% at day 21. Limnohabitans was the key driver of dissimilarity between

control and carp biomass treatments, contributing 4.84%, 5.1%, 5.1% and 5.2% of dissimilarity between 1,200, 2,300, 6,000 kg and 6,000 kg ha^{-1} removed treatments respectively.

OTUs affiliated with Actinobacteria were the next most relatively abundant bacteria, comprising 21.0% of the bacterial community at day 7, reducing to 12.6% by day 21. The most abundant OTUs affiliated with the Actinobacteria phylum belonged to the genera HGCL_clade, which is ubiquitously observed in the surface waters of lakes and rivers globally (Aguilar et al., 2018). OTUs matching to HGCL_clade comprised 4.4% of the bacterial community at day 7, diminishing to 1.5% by day 21.

The relative abundance of Bacteroidetes comprised between 8.4 – 18.8% of bacterial OTUs in the control mesocosms, notably less than what was observed in all carp biomass treatment mesocosms. The most abundant OTUs within Bacteroidetes were matched to the genus Pseudarcicella, which increased in relative abundance across the study period, ranging from 0.1% at day 7, to 9.7% at day 21 in controls mesocosms. Interestingly, cyanobacteria OTUs, typically common in surface waters of freshwater rivers and lakes, were absent from bacterial communities within controls.

The bacterial communities of all carp biomass treatments were dominated almost exclusively (>90%) by Proteobacteria and Bacteroidetes across all three sample occasions. Proteobacteria exhibited the highest abundance in high carp biomass treatments at day seven of the study (6,000 kg ha^{-1}; 83.4%, 6,000 kg ha^{-1} removed; 77.7%), and was the most dominant phylum in all carp biomass treatments by day 21 of the study (1,200 kg ha^{-1}; 52.6%, 2,300 kg ha^{-1}; 77.0%, 6,000 kg ha^{-1}; 63.4%, 6,000 kg ha^{-1} removed; 60.8%). Bacteroidetes abundance exhibited an inverse pattern to Proteobacteria, being higher in the low carp biomass treatment at day seven (1,200 kg ha^{-1}; 73.1%) compared to high biomass treatments (6,000 kg ha^{-1}; 14.4%, 6,000 kg ha^{-1} removed; 17.6%), and peaking in relative abundance at day 14 within 2,300 kg ha^{-1} and 6,000 kg ha^{-1} (carp removed) treatments at 51.9 and 77.8% respectively.

Freshwater bacterial taxa that dominated bacterial community composition within control mesocosms were far less abundant in mesocosms treated with carp biomass. Limnohabitans comprised a maximum of 6.5% relative abundance at day seven within the 2,500 kg ha^{-1} mesocosms but were otherwise low relative abundance (< 4%) or absent from other carp

biomass treatments and sample occasions. Other signature lake bacteria including Limnobacter, HGCL_clade and Pseudarcicella displayed very similar abundance patterns to Limnohabitans, as seen in Figure 15 and Figure 17.

Bacterial communities within the low carp biomass treatment mesocosms were characterised by high relative abundance across three bacterial OTUs and high dissimilarity across sample occasions. At day seven, Flavobacterium represented 44.4% of the bacterial community, and Runella (26.1%) was the next most dominant. At day 14, Flavobacterium abundance had decreased to <1%, replaced by Roseococcus (26.6%) and Runella (7.7%). At the last sample occasion (day 21) Runella had become the most relatively abundant OTU (21.8%) and Roseococcus the second most dominant (13.0%).

At day seven and 14 bacterial communities inhabiting the medium carp biomass treatment mesocosms were strikingly distinct from low and high carp biomass treatment mesocosms. Flavobacterium was the most relatively abundant OTU at day seven, contributing 25.1% of the bacterial community. Additionally, two OTUs: Arcobacter, representing 11.6% of the bacterial community and contributing 3.3% dissimilarity to low carp biomass treatment mesocosms, and Hydrogenophaga (11.6%) were dominant at day seven. Interestingly, Arcobacter was mostly absent from other treatments, and this was the only treatment and sample occasion where Arcobacter represented greater than 5% of bacterial communities. At day 14 bacterial communities were dominated by Roseococcus (31.6%) and Fluviimonas (14.1%) while Flavobacterium, which represented more than 25% of the community at day seven, was now ~1%. At day 21 Runella (16.7%) and Roseococcus (9.7%) were now the most dominant OTUs, while OTUs dominant at days seven and 14 were each less than 1% of the bacterial community.

Bacterial communities within the high carp biomass concentration mesocosms 6,000 kg ha^{-1} and 6,000 kg ha^{-1} with carp carcasses removed at day 14, exhibited the lowest overall dissimilarity between treatments (average dissimilarity 74.17%). At day seven, Rhodobacter was the most dominant OTU in both treatments (49.9% and 41.0% respectively), and a further two OTUs were also relatively abundant compared to other OTUs, including Cloacibacterium (6.5% and 12.7% respectively) and Flavobacterium (6.8% and 4.0% respectively). However, at the next sampling occasion at day 14, which was following the removal of carp on day 8, bacterial community composition began to diverge between 6,000 kg ha^{-1} and carp carcass

removed treatments. While dominant OTUs including Flavobacterium (32.8% and 28.2% respectively) and Cloacibacterium (20.7% and 29.2%) remained very similar, the relative abundance of Rhodobacter (21.4% and 0.9% respectively) and Fluviimonas (0.2% and 14.8%) were highly dissimilar. The removal of carp carcasses appears to have led to a bacterial community where dominance was shared across five OTUs, including Flavobacterium (12.5%), Runella (9.4%), Roseococcus (9.2%), Hydrophenophaga (7.6%) and Cloacibacterium (6.0%). Conversely, in the 6,000 kg ha^{-1} treatment without carcass removal, three OTUs were distinctly dominant, including Hydrogenophaga (20.8%), Cloacibacterium (17.9%) and Rhodobacter (13.3%).

Rare and transient OTUs

Throughout the study and across treatments, several OTUs that were generally rare or absent abruptly became notably higher in relative abundance, albeit temporarily. OTUs that exhibited notably transient patterns in mesocosms treated with carp carcasses include: Arcobacter, which reached 23.6% of total bacterial community at day seven within the 2,500 kg ha^{-1} treatment, and 4.8% in the 6,000 kg ha^{-1} (removed) treatment at day 7 but was otherwise less than 1%; Sediminibacterium that reached 10.3% in 1,200 kg ha^{-1} treatment at day 14 and 9.0% in the 2,300 kg ha^{-1} treatment at day 14 but was otherwise less than 2%; Lewinella which was 4.4% and 10.6% in 1,200 kg ha^{-1} treatment at days seven and 14 respectively; and Clostridium sensu stricto 13, rare but present across all treatments, having highest relative abundance in 1,200 kg ha^{-1} treatment at day 14.

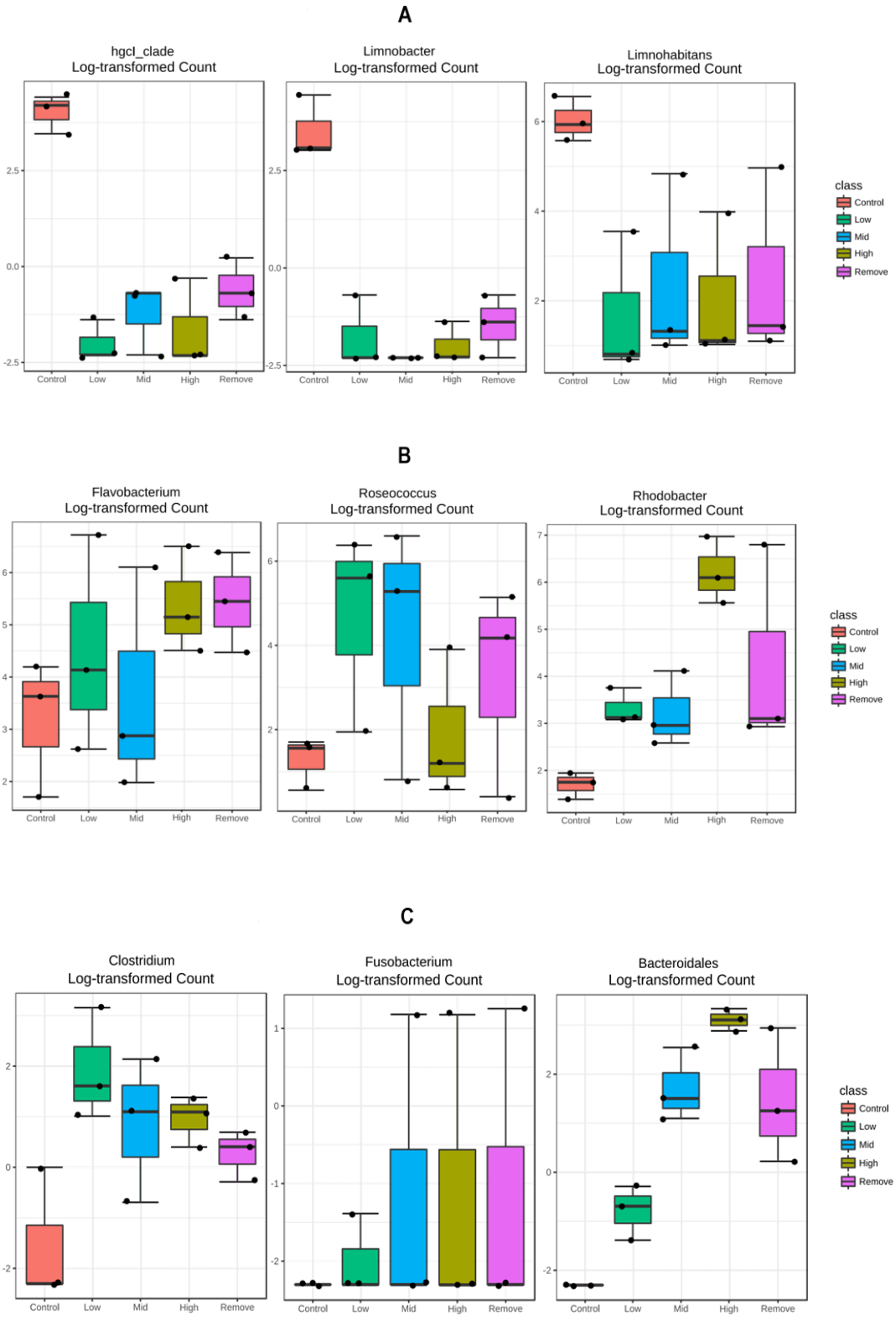

Figure 17: 16S rRNA gene amplicon results depicting changes in key bacterial populations associated with the addition of dead carp in treatments ranging from 1200 – 6000 kg ha^{-1}. (A) Signature lake bacteria groups, (B) Environmental coptiotrophs (C) Fish gut signature bacterial groups.

MIB and Geosmin

The results showed an increase in the geosmin and to a lesser extent MIB in the third and fourth week for the 1200 and 2300 kg ha^{-1} treatments (Figure 18). The cyanobacteria *Pseudanaebena* and *Merismopedia* both correlated significantly with geosmin and MIB spikes during the experiment (Figure 19).

The extended local similarity pipeline was used to explore significant correlations between microbes and taste and odour compounds MIB and geosmin. A total of 36 OTUs (33 bacterial OTUs and 3 algal species) co-varied significantly with geosmin, and 45 OTUs (40 bacterial OTUs, and 4 algal species and one Archaea OTU) with MIB. One Cyanobacteria – *Pseudanabaena*, which is known to produce geosmin and MIB under certain environmental conditions, co-varied significantly with geosmin (SCC = 0.43, p = <0.01) and MIB (SCC = 0.39, p = 0.01), albeit while at very low relative abundance. One green algae species – *Selenastrum*, also correlated significantly with geosmin (PCC = 0.44, p = <0.01).

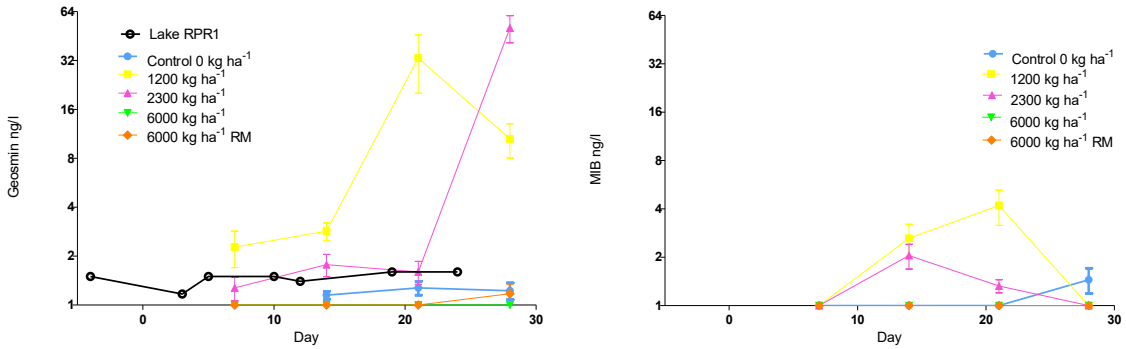

Figure 18 Geosmin and MIB levels during the experiment. Error bars ± standard error mean, n=4 (Lake RPR1 n=1)

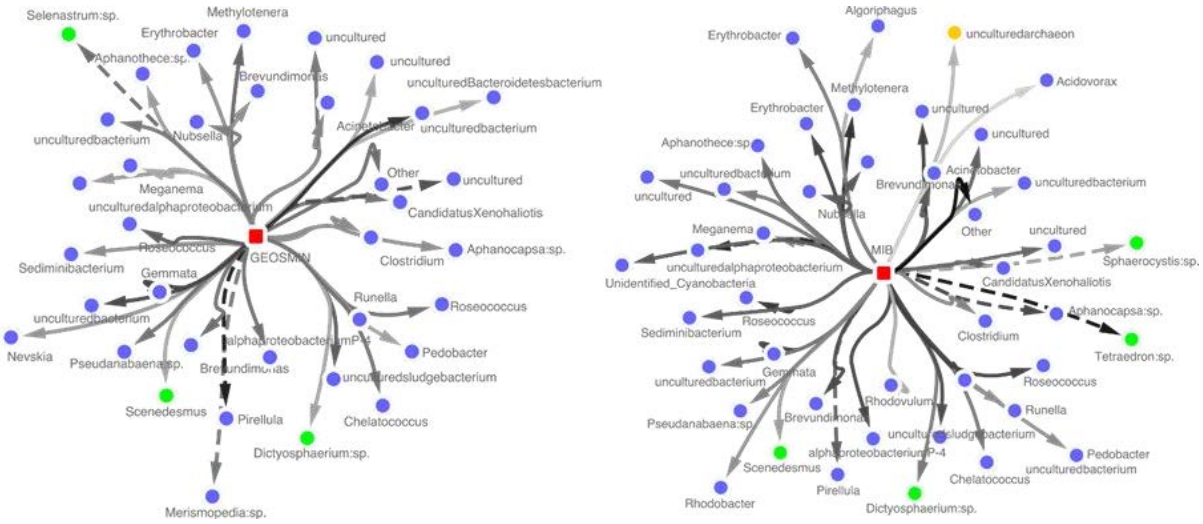

Figure 19. Network analysis, depicting covariance correlations between bacterial OTUs (blue nodes), algal cell counts (green nodes), and MIB and Geosmin (red central nodes). Edge darkness is weighted by Pearson's correlation coefficient (PCC) or Spearman's correlation coefficient (SCC) score, whereby stronger correlations are represented by dark edges.

3.5 Discussion

This mesocosm study demonstrates that high amounts of carp carcasses, as would be expected after controlled virus release fish kill resulted in significant microbial shifts. This shows that fish kills associated with the release of the Cyprinid herpesvirus 3 (CyHV-3) virus may lead to rapid and profound changes to MCC in the short term with a density dependent shift to heterotrophic bacterial groups commonly associated with eutrophic and poor water quality environments.

3.5.1 Water quality changes

Water quality changes from addition of dead fish lead to the depletion of dissolved oxygen in all treatments, with anoxic conditions in all treatments. Chapter 2 showed huge nutrient increases followed by increases in chlorophyll a and alga biomass. This water quality change is typical following a fish kill (Kushlan, 1974, Boros et al., 2015). The removal of carcasses from 6000 kg ha^{-1} RM treatment led to a noticeable increase in dissolved oxygen compared to 6000 kg ha^{-1} (Figure 13). This stopped further breakdown in the water of the carcass by aerobic bacteria (Boyd, 2008, Hlordzi et al., 2020).

3.5.2 Change in bacterial community composition

Sequencing of the 16S rRNA amplicon confirmed that shifts in the environment were closely paired with changes in microbial assemblage structure. Changes were seen from a community typical of fresh water lake environments, comprising oligotrophic bacteria such as Limnobacter, Limnohabitans and the hgcl_clade (Llirós et al., 2014, Guo et al., 2021) to a more eutrophic signature, including higher relative abundances of environmental copiotrophs such as Flavobacterium, Roseococous and Rhodobacter (Figure 17). These bacteria typically bloom following pulse inputs of nutrient substrate which can be of allochthonous (e.g. rainfall catchment runoff) or autochthonous (e.g. algal exudates) sources (Teufel et al., 2017). Flavobacterium species are chemoheterotrophic and play a role in mineralizing various types of organic matter (carbohydrates, amino acids, proteins, and polysaccharides) (Parte et al., 2011), and appear during episodes of high bacterial production (Eiler and Bertilsson, 2007). During the experiment there was large increases in algal biomass in carcass added treatments (Pera et al., 2021) and algal-derived organic matter can result in correlations between algal and bacterial community dynamics (Paver et al., 2013, Parulekar et al., 2017). Roseobacter can utilise labile substrates in the form of algal exudates or degradation products of Flavobacteria (Teufel et al., 2017). Rhodobacterales are also key biofilm formers on surfaces (Gäb et al., 2020). There were also increases in bacteria potentially associated with fish guts such as Arcobacter, Clostridium and Bacteroides (Sugita et al., 1990, Rathlavath et al., 2016) (Figure 17). Populations of Bacteroidetes are specialized for successive decomposition of algal-derived organic matter (Teeling et al., 2012) and the anoxic conditions of some of the mesocosms are the preferred environmental conditions for Clostridium (Edwards et al., 2013). This shift from oligotrophs to copiotrophs, are associated with more eutrophic conditions and are generally adapted to using a resource rapidly when available (Koch, 2001) and typical anaerobic microbial decomposition of organic matter (Kristensen et al., 1995).

Chapter 2 and Pera et al (2021) found that although there were large nutrient inputs of N, P and C, this didn't relate to an increase in cyanobacteria although other algal groups did bloom (Figure 10). This could be related to the relative increases of cyanobactericidal bacteria which can through direct or indirect attack from Bacteroidetes (Flavobacterium) and interactions between algae and cyanobactericidal bacteria (Miyashita et al., 2019, Yang et al., 2020). Common carp intestinal microflora are usually dominated by Firmicutes and Proteobacteria (Mulyani et al., 2018) including the genera *Enterobacteriaceae, Pseudomonas* and

Bacteroides (Sugita et al., 1985, Sugita et al., 1990, Sugita and Mizuki, 2012). Many of these heterotrophic bacteria are important in the breakdown of organic matter, but have also been linked to disease (Galloway and Cohen, 2021) and opportunistic pathogenic bacteria increase as carcass decomposition proceeds (Su et al., 2022).

3.5.3 Public health

Farming and pastoral properties within the MDB are left to their own devices to secure adequate domestic water supplies and ensure that the water being used is not a health risk (ILWS-CSU, 2018). Therefore, detection of changes in the quality of raw source water due to fish kills may be missed by agricultural water users, potentially posing a serious health threat to livestock, farmers and consumers of farm produce. *E. coli* as an indicator for faecal contamination in drinking and recreational freshwater is supported by both epidemiological studies and Quantitative Microbial Risk Assessment (NHMRC, 2008, EPA, 2012, Leonard and Eaton, 2021). *E. coli* exhibits strong correlations to swimming-associated gastroenteritis (Cabelli et al., 1982, Federigi et al., 2020). During this study *E. coli* levels in some mesocosm treatments were above World Health Organization considered high risk (>100MPN/100ml) for drinking (Odonkor and Mahami, 2020) for 7 days. This indicates that microbial shifts associated with the breakdown of high densities of carp biomass could pose health risks for farming and pastoral properties within the MDB without access to treated drinking water and for recreational activities such as swimming. Even after peaking on day 3 *E. coli* levels stay above controls for 20 days.

Other bacteria including Vibrionaceae, Campylobacter, Arcobacter and Shewanella were detected in carp treatments, and are known as both animal and human pathogens (Snelling et al., 2006, Sharshar and Azab, 2008, Paździor et al., 2019, Mudadu et al., 2021). Several species of Vibrionaceae are pathogenic, and infections in humans can arise from water exposure and consumption of seafood produce (Baker-Austin and Oliver, 2018, Frehse and Sylla, 2023). Pathogenic Shewanella species are considered to be the causative agent of shewanellosis (deep ulcers associated with hemorrhagic bullae on the lower extremities), in different freshwater fish species and causing serious health disorders in humans (Kon and Rai, 2017, Müller et al., 2023). Human infection of Shewanella was reported on the Murray River in South Australia in 2017 (Keane, 2017) indicating it can be an issue in this region.

Sequences matching *Clostridium botulinum* and Enterobacteriaceae *Shigella* genus were detected across treatments. *Shigella* requires ingestion of fewer than 100 bacterial cells to cause an infection, depending on the health of the host (Levinson, 2020). Botulinum spores may become active when conditions suit, including conditions exacerbated by climate changes such as prolonged drought and low to no oxygen coupled with high nutrient environments such as after a fish kill (Espelund and Klaveness, 2014, NCCP, 2019). Zhou et al., (2021) found several potential pathogenic genera from fish decomposing carcasses including Comamonas, Acinetobacter, Bacteroides, Pseudomonas, Clostridium, Brevundimonas and Delftia showing the transfer from dead animals to water environment. 16s sequencing does not discriminate between dead and viable/culturable cells, however the *E. Coli* counts as an indicator support the survival of other anaerobic pathogens in the treatments.

3.5.4 Taste and Odour

The absence of MIB and only low concentration of geosmin in day 7 samples suggests putrescene and cadaverine, odour compounds produced from decaying organic matter, were potentially the main odour producing compounds early in the experiment. These are likely to be produced by bacteria such as Enterobacteria, Clostridium and Fusobacterium which are known to produce cadaverine and putrescine (Allison and Macfarlane, 1989, Soksawatmaekhin et al., 2004, Berthoud et al., 2022, Sakanaka et al., 2022).

The second half of the experiment saw spikes in MIB and geosmin. The cyanobacteria *Pseudanaebena* and *Merismopedia* both correlated significantly with geosmin and MIB spikes during the experiment (Figure 19) and both species are known to produce the odour compounds (Youn et al., 2020, Jeong Hwan et al., 2023). Geosmin and MIB can act as a warning to predators to indicate unpalatable or toxic bacteria (Stensmyr et al., 2012, Zaroubi et al., 2022). Geosmin and MIB production by cyanobacteria can be influenced by light intensity and water temperature (Wu and Jüttner, 1988, Saadoun et al., 2001). Increased geosmin or MIB synthesis can occur when cyanobacterial growth is inhibited under less favourable conditions (Wang and Li, 2015). The environment of the mesocosms in weeks 3 and 4 saw increases in algal biomass which could have led to increased competition or a change in the light intensity of the mesocosms. The emergence of cyanobactericidal bacteria such as Flavobacterium suggest multiple factors may have created poor growth conditions for cyanobacteria which could further trigger increased MIB and geosmin production. It is unclear

if current water treatment plants would have the capacity to remove the taste and odour issues associated with a mass fish mortality events and whether disinfection would still be manageable (Fabris, 2017). The removal of the carcasses from the water body within a day of them dying or floating to the surface would result in better outcomes for both environmental and public health perspectives.

3.6 Conclusions

The addition of carp carcasses to mesocosms resulted in a reduction in oxygen levels and a change in the microbial composition. There were also increases in then taste and odour compounds MIB and geosmin. Results showed a shift from oligotrophs to copiotrophs utilizing organic material, algal-derived organic matter and algal exudates or degradation products of other bacteria. The presence of indicator and pathogenic bacteria of public health and environmental concerns occurred in carp treatments.

Chapter 4: The rise and fall of carp; carcass movement through depth in lakes and interactions with scavengers.

Statement of contribution to co-authored chapter and paper to be submitted to Environmental Biology of Fishes

This chapter includes the co-authored as follows:

My contribution to the paper included data pre-processing, analysis and interpretation, and manuscript writing.

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4.1 Abstract

In lakes the location of dead fish will determine where decomposition and nutrients released will occur. This is also where possible interactions with scavengers will occur. The proposed release of the Cyprinid herpesvirus-3 (CyHV-3) in Australia will lead to mass common carp *Cyprinus Carpio* mortality and will provide lakes with a large organic matter and nutrient pulse. However, it is not well understood where this may occur in the water column. To evaluate how fish carcasses may move through depth over time, dead carp were allowed to sink to different depths in a lake and monitored. Fish surveys were also performed at different depths to determine the possibility of scavenging. If there was no interference by scavengers, all the dead carp in water depth of 0.5-1 m floated to the surface the next day. In 10 m of water, 66% and in 20 m, 33% of dead carp floated after 3-4 days. Scavenging of dead carp occurred at 5m depth where interactions were observed with catfish and carcasses also exhibited signs of eel predation. There was no sign of fish scavenging at 10 m and 20 m, however, decapods were sighted near carcasses at all depths. Lake surveys showed a reduction of potential scavenging fish below 10 m, suggesting dead carp that sink below that depth would only be decomposed by invertebrates and microbes and may contribute to nutrient loads in the hypolimnion. Based on this data, any clean-up of a CHV-3 carp fish kills would need to occur for at least a week as carcasses could continue to rise from depths for up to 6 days post-death.

4.2 Introduction

Fish can be important nutrient sinks in aquatic ecosystems due to their large size and potential longevity (Kitchell et al., 1975, Sereda et al., 2008). In deep-sea marine environments, sinking cetacean and large elasmobranch carcasses, known as food falls, are important carbon-input for the benthic communities (Higgs et al., 2014, Sumida et al., 2016). Food falls in freshwater lakes lead to nutrient transfers across ecosystem boundaries such as post salmon spawning-derived nutrients which impact on the aquatic bacterial community structure and microbial community metabolism (Payne and Moore, 2006, Premke et al., 2010, Lujan et al., 2022). The fate of decomposing fish carcasses within a lake can play an important role in determining which area of a lake is supplied the nutrient pulse, as breakdown by scavengers and microbes will contribute to support carbon cycling within lakes, returning carbon to the biosphere (Hewson et al., 2003, Carney et al., 2016, Arora-Williams et al., 2018, Barton et al., 2019). Large carrion sources differ in the way they provide carbon and nutrients compared to macroscopic organic aggregates (MOA) or wild fires followed by extreme rainfall events (Neris et al., 2021). Large carrion sources concentrate carbon and nutrients around the carcass rather than evenly spreading it through the water column (MOA or lake snow) (Grossart et al., 1997, Bižić-Ionescu et al., 2018). Furthermore, large carrion sources are available to scavenging fish and invertebrates (Redondo-Gómez et al., 2022) compared to MOA which is accessed by zooplankton and bacteria (Weiss et al., 1996, Tang et al., 2009).

In Australia, fish kills are generally associated with anoxic or hypoxic events in rivers. A large scale fish kill event in 2019 on the Darling River near the town of Menindee was caused by a water column mixing event associated with extreme temperatures and high fish biomass (Science, 2019, Vertessy et al., 2019). More commonly, fish kills are attributed to flooding events which wash organic material into waterways, causing black water events where oxygen is consumed by microbes (Liu et al., 2020, Thiem et al., 2020, Thiem et al., 2021). Severe hypoxic blackwater events are non-discriminatory to species and can kill a large portion of the aquatic life (Small et al., 2014). This prevents interactions between the animal carrion and scavenger species such as eels, catfish and decapods as they too can be killed during the anoxic events (Thiem et al., 2020, Stuart et al., 2021). Pulse nutrient releases related to fish kills in Australian freshwater systems are not an annual cycle and are more related to specific events (Science, 2019, Sheldon et al., 2022). Therefore, it is uncertain as to how larger scavenging necrobiomes, which tend to be opportunistic, will interact with a

potentially large-scale fish kill in an ecosystem that is not adapted (Woollard et al., 1978, Smith, 2004, Benbow et al., 2020). It is also unclear if the carrion feeders will be able to process such large volumes or if the carcasses decompose in deep water out of the reach of scavengers (Schneider, 1998, Whitmore, 2003).

All lakes have some level of fish mortality that provides a supply of carcasses to the aquatic ecosystems (Benbow et al., 2020). In Australia, the Cyprinid herpesvirus-3 (CyHV-3) is being looked at as a biological control agent for invasive carp (McColl, 2016). The potential release of CyHV-3 in Australia, being species specific, may lead to mass deaths of common carp *Cyprinus carpio*, that will act as a nutrient pulse at least for the first year and potentially over several years, with each prevailing year possibly smaller than the previous (Hedrick et al., 2006, Michel et al., 2010, Thresher et al., 2018). These kills would be similar to salmon spawning events in feeder streams (Mathewson et al., 2003) and cuttle fish in shallow marine bays of South Australia (Arkhipkin, 2013). But unlike those examples, the Australian freshwater aquatic ecosystems have not evolved for mass death of specific species and the following nutrient pulses may not be utilised effectively (Wipfli et al., 1998, Nagasaka et al., 2006).

Invertebrates and vertebrates breakdown organic materials in aquatic ecosystems and are crucial in carrion decomposition (Coull, 1999, Beasley et al., 2012, Cifoni et al., 2021). Scavenger-driven fish carcass decomposition and phosphorus recycling sequestered a relatively large fraction (up to 33% in catfish and 36% in crayfish) of total carcass P in their bodies (Boros et al., 2020). The yabby *Cherax destructor* and freshwater prawn *Macrobrachium spp* are found in swamps, streams, rivers and lakes at low to medium elevations throughout the Murray-Darling Basin, Australia (Merrick, 1993, Hawking, 1997, Jones, 2002). Yabbies are omnivorous and feed on carrion. Densities of yabbies can be as high 500 –1500 kg ha^{-1} in ponds (Mills and McCloud, 1983, Sang et al., 2011, McCormack, 2014). Freshwater eels *Anguilla reinhardtii* and *Anguilla australis* are the apex predator in most coastal drainages of south-eastern Australia. Freshwater eel abundances and can be high, comprising a large proportion of the fish biomass of rivers and lakes (Sloane, 1984, Merrick, 1993, Allen, 2003). As carp are the only species effected by the CyHV-3 virus (Kenneth and St, 2013) in south eastern Australia, this would leave crustaceans and large aquatic scavengers like turtles, freshwater eels, freshwater catfish *Tandanus tandanus* and Murray Cod *Maccullochella peelii* to feed on the dead carp (Santori et al., 2020).

Native Australian freshwater fish generally evolved from marine ancestors over a relatively short time period, from the late Cretaceous (MacDonald, 1978, Unmack, 2001, Tims et al., 2021) and have not specifically evolved to live in lakes, which in Australia were generally constructed in the last century (Beasley, 1988). Lakes differ to rivers as they are generally much deeper and are more heavily influenced by limnological processes such as solar radiation, wind, stratification and develop an anoxic hypolimnion over the warmer months (Payne et al., 1990, Gray et al., 2020). If carcasses of the carp sink to the sediments in deeper waters out of the euphotic zone that may change the rate at which carcass nutrients are remineralized and available to pelagic primary producers (Premke et al., 2010). The pulsed nutrients could lead to variation in thermocline depth in stratified lakes by increasing nutrient loading along a productivity gradient (Briddon et al., 2023) and changes in anoxia due to increases in total phosphorus (Deeds et al., 2021). This in turn can change the phytoplankton community (Lofton et al., 2022). Thus, a virus induced fish kill could lead to significant deposition of bioavailable nutrients. There is a dearth of research on how carp carcasses respond to different water pressure levels from varying depths. Understanding the resting place of the dead carp will aid in determining which trophic zone will be impacted and what management approach is needed to deal with that impact.

This study examined the time a dead carp will spend on the sediment at the bottom of a lake at different depths. It also investigated what depths fish species are found, and the likelihood of those species being potential scavengers. It will also cover interactions with scavengers and dead carp carcasses potential role of shifting basal resource availability. It is hypothesised that the depth the dead carp carcasses sink to at the lake bed affects the time the carcass will spend there before a build-up of gasses from bacteria production causes bloating and the carcass to resurface (Whitmore, 2003, Gäb et al., 2020). It is also hypothesised that some of the carcasses will be consumed by scavengers and that depth will affect distribution of scavengers and their interactions with common carp carcass.

4.3 Materials and methods

Study Site for Dead Carp Resuspension

The study was performed at the north-eastern side of Prospect Reservoir (Figure 20), in western Sydney, NSW, Australia. Prospect Reservoir measures 26 m high and 2.2 km long and has a storage volume of 2,892×103 m^3 (Beasley, 1988). The reservoir is part of Sydney's drinking water supply system. The reservoir is destratified with a bubble plume aerator which results in a uniform temperature and dissolved oxygen profile (Figure 21) at water quality site RRP1 (Figure 12) is approximately 50 m west of the 20 m site. Over the last 30 years the reservoirs mean nutrient levels were 0.007mg L^{-1} for total phosphorus and 0.270 mg L^{-1} for total nitrogen (WaterNSW).

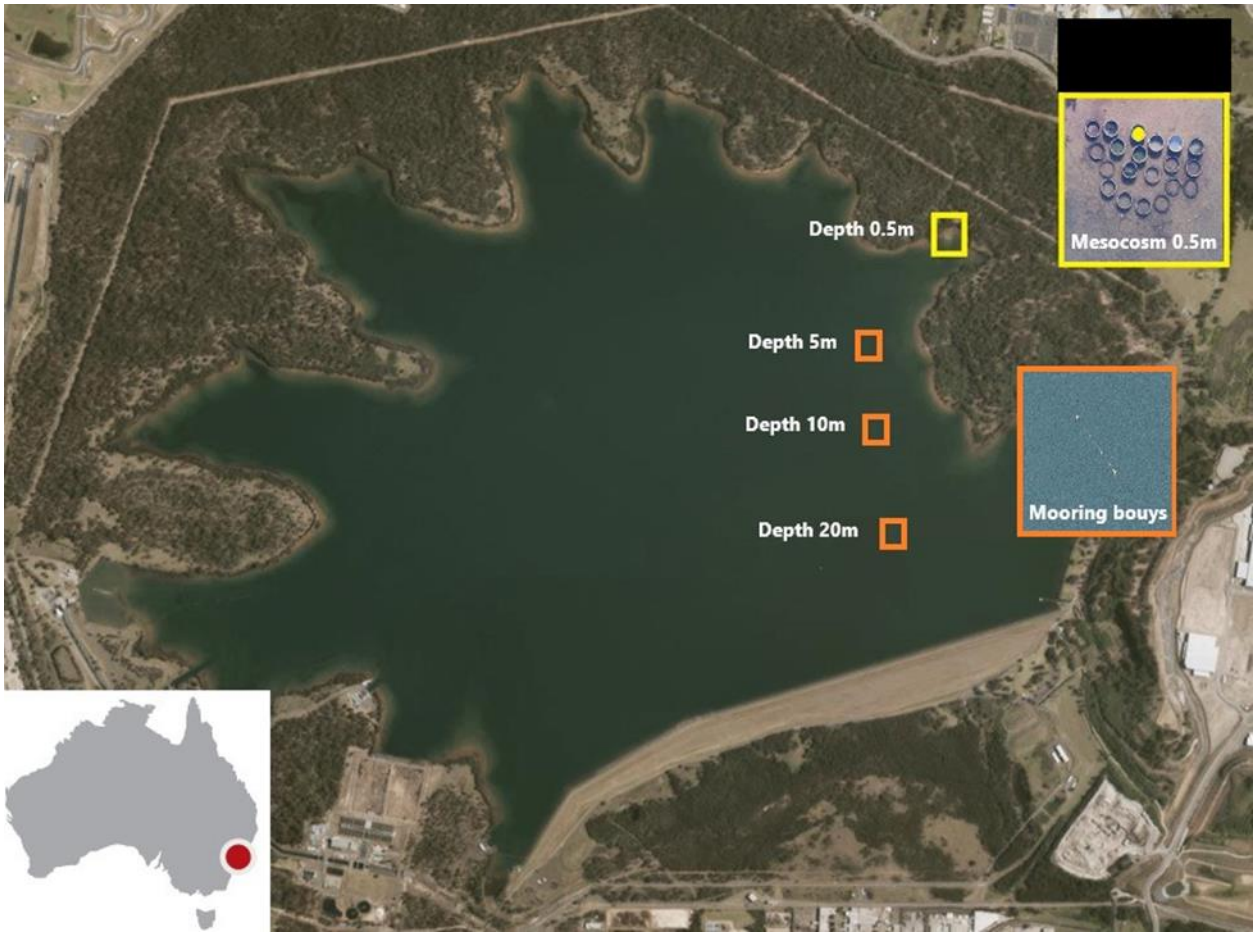

Figure 20: Location of the mooring buoys and mesocosm within Prospect Reservoir, Australia.

Unpublished DPI Fisheries and WaterNSW data shows that Prospect Reservoir contains large bodied fish from both eastern drainage rivers and the Murray-Darling Basin. Eastern drainage river fish in Prospect Reservoir include the long-finned eel and the Australian Bass *Percalates novemaculeata*. Murray-Darling Basin drainage fish include eel, tail catfish and Murray cod. The decapod freshwater shrimp *Macrobrachium* is also common throughout the reservoir.

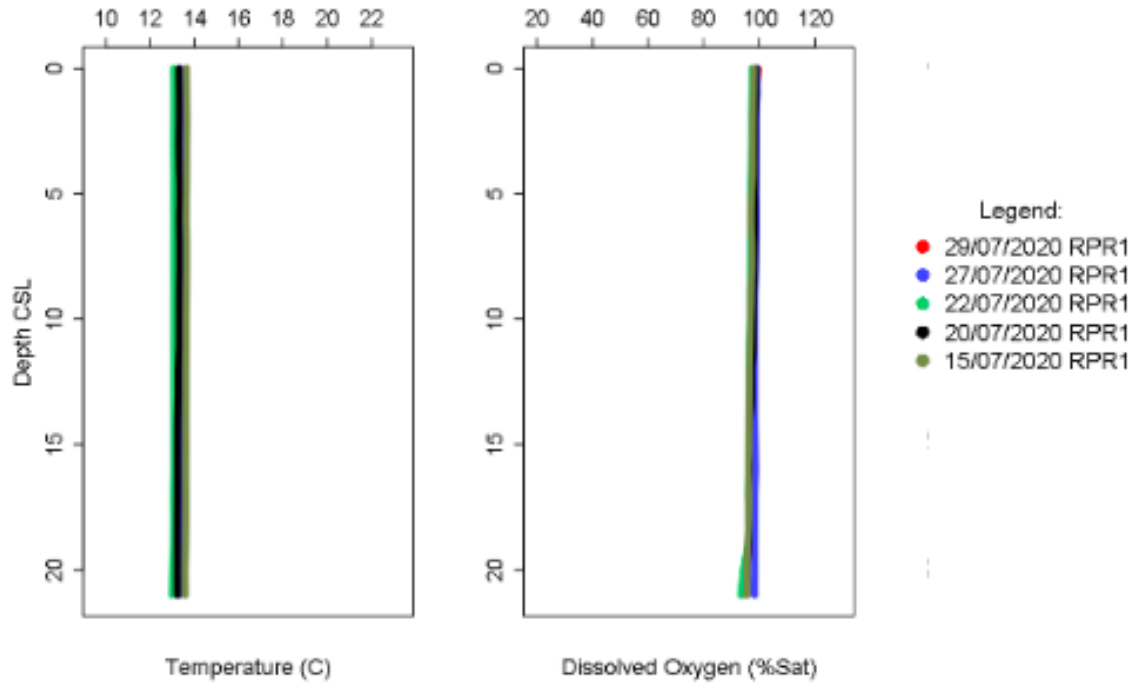

Figure 21. Temperature and dissolved oxygen profiles at site RPR1 on Prospect Reservoir from 15/07/2022 to the 29/7/2020.

Experimental Design

The experiment was performed between 27th May 2020 and 3rd July 2020. To assess the effect of depth on resuspension, dead common carp were sunk to the lake bottom at depths of 0.5 – 1 m, 5 – 6 m, 10 – 11 m, and 19 – 20 m. At each depth six replicate carp were sunk. Carcasses were negatively buoyant so gradually sunk until they rested at the bottom of the lake. At the 0.5 m depth carp were placed in a mesocosms consisting of a cylindrical outer shell made from rolled steel (COLORBOND®) 2.5 m in diameter and 1.2 m in height and were checked daily. For the 5 m, 10 m and 20 m depths a buoy system was developed where two buoys were anchored +/- 1 m depth and separated by 10 m for the 5 m and 10 m depth and 20 m

separation for the 20 m depth (Figure 22). This was to reduce the likelihood of entanglement of the tethered dead carp. It was noted that there was a bed of Chara at 5 m, a multicellular green alga, which covered the sediment. The Chara bed was between 0.5– 1 m thick. The carp were dense enough to flatten the Chara and rest on the lake bottom. An acoustic VR2W monitoring receiver (InnovaSea, Canada) was attached at the midpoint of each anchoring rope to detect depth data transmissions from acoustic transmitters attached to each carcass (Figure 22). Two empty bait traps (500mm length x 250mm width x 250mm height, funnel entrance 50mm) were placed at each depth close to the dead fish, to determine if there was the presence of invertebrate scavengers such as yabbies or prawns.

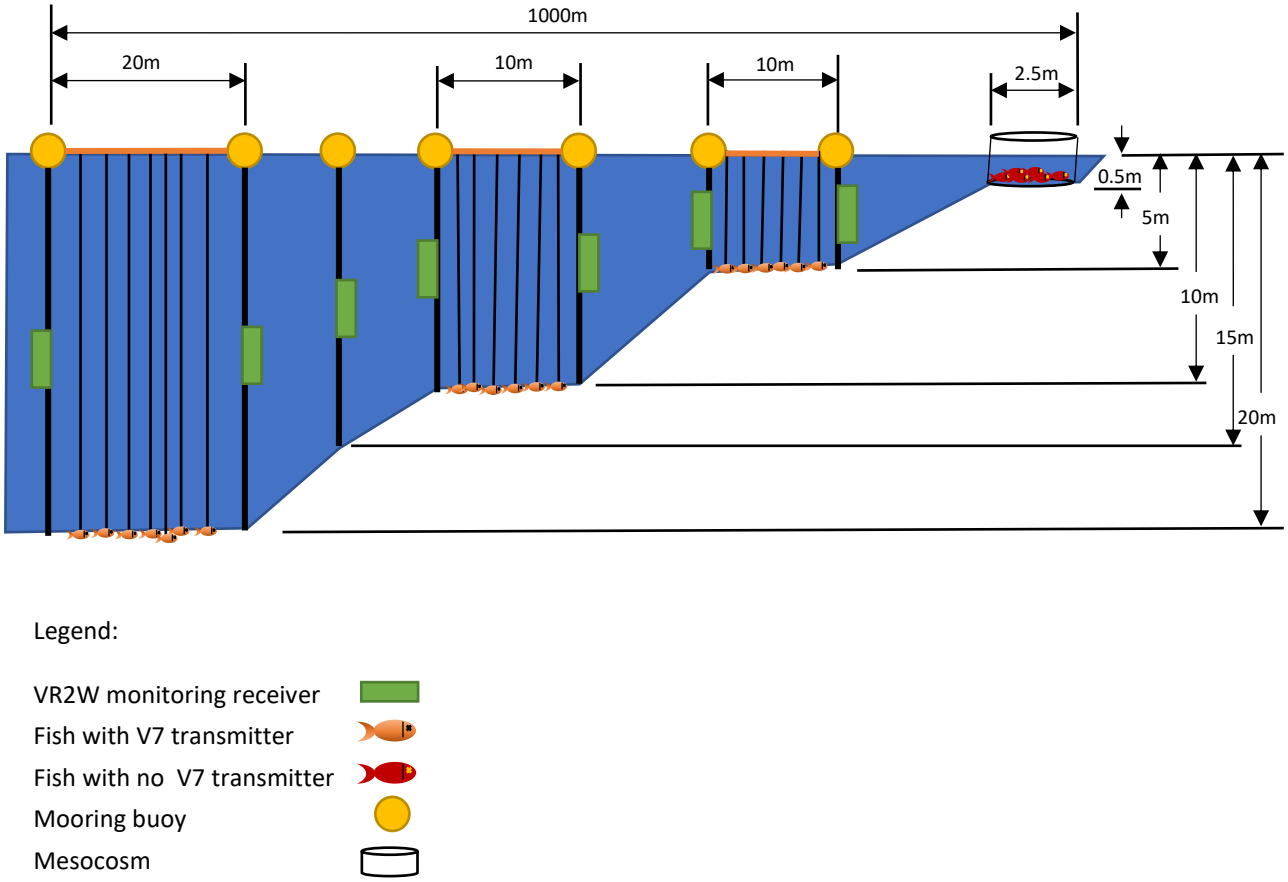

Figure 22: Schematic diagram representing the resuspension experiment showing buoys, receivers and fish with and without transmitters.

Sampling procedures and analysis

Fish were collected from the Prospect catchment, euthanised and pressure sensing acoustic transmitters (V7P transmitter, InnovaSea, Canada) were attached to each carcass placed at 5, 10 and 20 m depths. Data on the depth and the time/date was recorded from each individual carcass every five minutes on the array of monitoring receivers placed adjacent to each of the experimental depths (Figure 22). Fish lengths and weights ranged from 150 – 660 mm TL and 0.3 – 4.1 kg. Due to limitations of fish available generally smaller fish were used for the 0.5m depth (0.09 – 2.2 kg).

To supplement the data collected by the acoustic receivers, common carp were visually checked at the surface (V) and underwater (U) with a 600TVL Underwater Fishing Camera, and GOPRO Hero10X. The cameras were used to monitor for potential interactions between the carcasses and scavengers. Cameras were lowered at each site and depth, and each carcass was viewed looking for signs of scavenging, such as bite marks, missing fish or sightings of scavengers. These sites were visited on day 2 (V), 3 (VU), 5 (V), 7 (VU) 9 (V), 13(VU), 15(V), 20(VU), and 21 (V). Two empty bait traps were placed within 2 m of the carcasses at 5, 10 and 20 m depth to examine decapod depth distribution.

Table 5: Weight and length of common carp placed different depths

	Depth 0.5-1m		Depth 5-6m			Depth 10-11m				Depth 19-20m	
Fish number	length mm	weight kg	tag number	length mm	weight kg	tag number	length mm	weight kg	tag number	length mm	weight kg
1	470	1.9	1303370	560	3	1303368	480	1.4	1303365	660	4.1
2	470	1.5	1303371	630	4.1	1303369	500	2.4	1303367	560	2.6
3	240	0.5	1303372	470	1.6	1303374	580	2.2	1083424	410	1
4	220	0.5	1083417	460	1.5	1083421	550	2.6	1057404	450	1.8
5	150	0.25	1083426	530	2.4	1083413	450	1.1	1057403	590	3.5
6	280	0.7	1083409	470	1.6	1083422	510	2.2	1303411	520	2.4
Average	305	0.9		520	2.4		512	2.0		532	2.6

Scavenger depth distribution

Gill netting fish surveys were conducted at Lake Burrinjuck and Burragorang in April 2018 at different depths to determine scavenging teleost distributions (Figure 23). Prospect Reservoir was not included as it is smaller and artificially mixed and does not represent most lakes in

NSW which thermally and oxygen stratify. Both lakes were artificially dammed, with Burrinjuck being the oldest built in 1912 with a maximum depth of 60 m and a volume of 1,000 (GL) (Walter et al., 2001). Lake Burragorang was completed in 1960 with a maximum depth of 101 m and a volume of 2,027 GL (Beasley, 1988). At that time of the year the thermocline at Lake Burragorang was between 20 – 25 m. Stratification was not known for Lake Burrinjuck but based on historical data the thermocline would be expected at a similar depth to that of Lake Burragorang (Lawrence et al., 2000).

Figure 23: Locations of Lake Burrinjuck and Lake Burragorang in south eastern Australia where scavenger depth distribution studies were conducted.

Panel gill nets 30 m x 2 m (10 m panels) stretch mesh size = 100 mm x 30 mm mesh, were set to sample the littoral zone and benthic offshore habitat as per (Stuart et al., 2021) (Figure 24). Four survey sites in Lake Burrinjuck and three survey sites within Lake Burragorang were selected greater than 2 km apart and representative of the habitat variability in each impoundment. Each site had five contour zones: 0 – 3 m, 6 m, 12 m, 18 m and 25 m depth

within 100 m of the shore (Figure 24). In the 0 – 3 m contour zone, 1 demersal panel net was placed at a depth of 2 m within 10 m of the shore. At each of the 6 m, 12 m, 18 m and 25 m contour zone, 1 demersal panels net was deployed. Mesh nets were set ~4h prior to sunset and first inspected at dusk; fish captured had their length, weight and species recorded and were released >200 m from the nets. All nets were retrieved the following morning between 8 am to 10 am. Fish captured at the dusk inspection and final retrieval were pooled for analysis. A two factor ANOVA was performed to examine the effect of species and depth-of-capture on abundance within the two reservoirs. Data were $\log_{10}$ transformed to meet assumptions of normality (Figure 24).

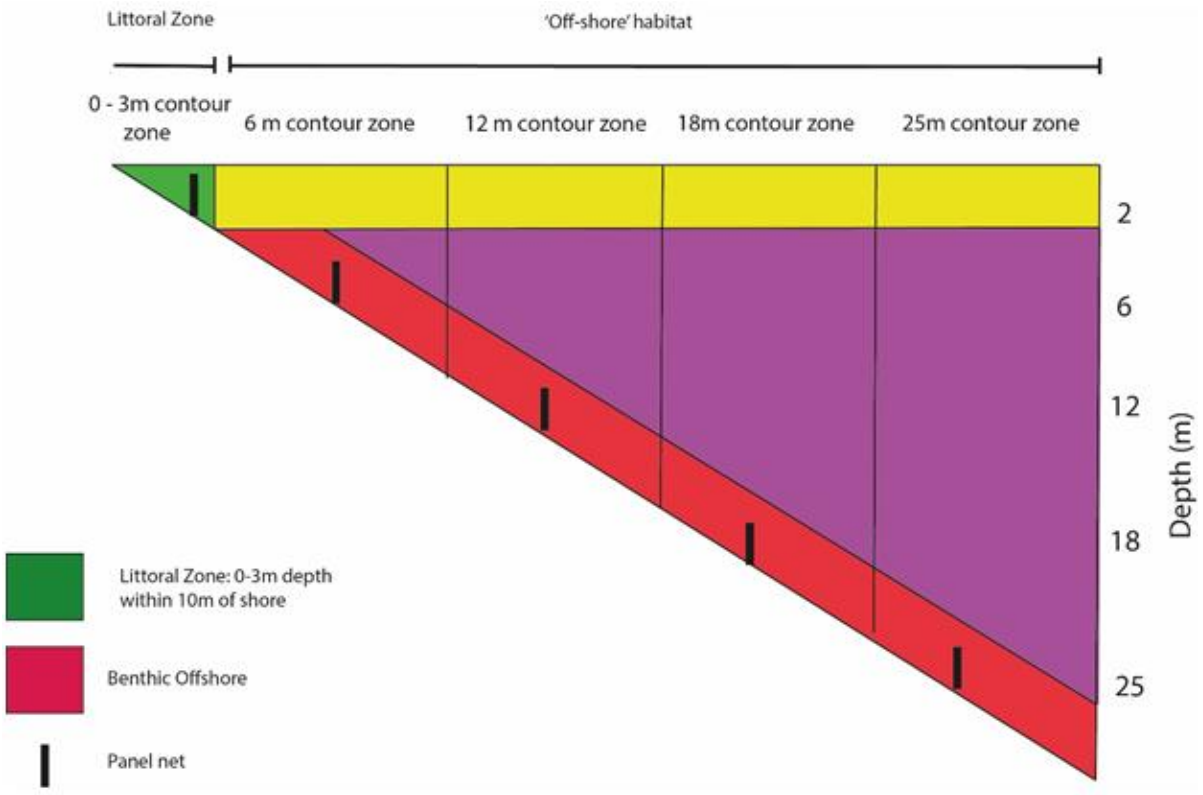

Figure 24: Schematics of net deployment depths within Lake Burrinjuck and Lake Burragorang used to examine fish species depth utilisation.

4.4 Results

Dead Common Carp resuspension and scavenger interactions

0.5 – 1m depth

All dead common carp carcasses sank once placed in the water and all rose to the surface within 24 hrs. Avian scavengers were not part of the study but on day three a swamp harrier *Circus approximans* was sighted on the edge of the mesocosm looking at the floating fish. It was unable to access the fish as it mesocosm was covered with a mesh.

5m depth

Video monitoring on day three showed three of the carcasses placed at 5 m were eaten, with only the skull remaining. The other two carcasses were almost entirely removed, with just the dorsal barb remaining. Logged depth data showed the fish tag 1083417 moving after half a day (Figure 25). Fish tags 1083409 and 1083370 were removed (eaten) a day later with the loggers resting on top of the Chara bed (Figure 25). The fourth common carp carcass, tag 1303370, had its stomach removed, while the two remaining were still intact with no visible signs of scavengers, though some bloating was observed. By day four, the three remaining common carp carcasses seemed to be entangled and when removed on day nine no carcasses were attached to the tether (Figure 25). Video surveillance observed a freshwater catfish feeding on the skull of fish 1083417. Freshwater shrimp *Macrobrachium* were sited near carcasses both on video and captured in bait traps placed nearby.

10m

Common carp carcasses were checked on day three and were still intact with no visible signs of predation by scavengers. Four common carp carcasses rose to the surface between day three and four (Figure 25). Two fish remained at the bottom with no signs of scavenging. *Macrobrachium spp.* were caught in bait traps near the carcasses.

20m

Common carp carcasses were checked on day three and were still intact with no visible signs of interactions with large scavengers nor were there any observed on video. Two carp carcasses (tags 105403 and 1303365) rose the surface after 5 days while the remaining four stayed at the bottom (Figure 25). *Macrobrachium* were caught in bait traps near the carcasses.

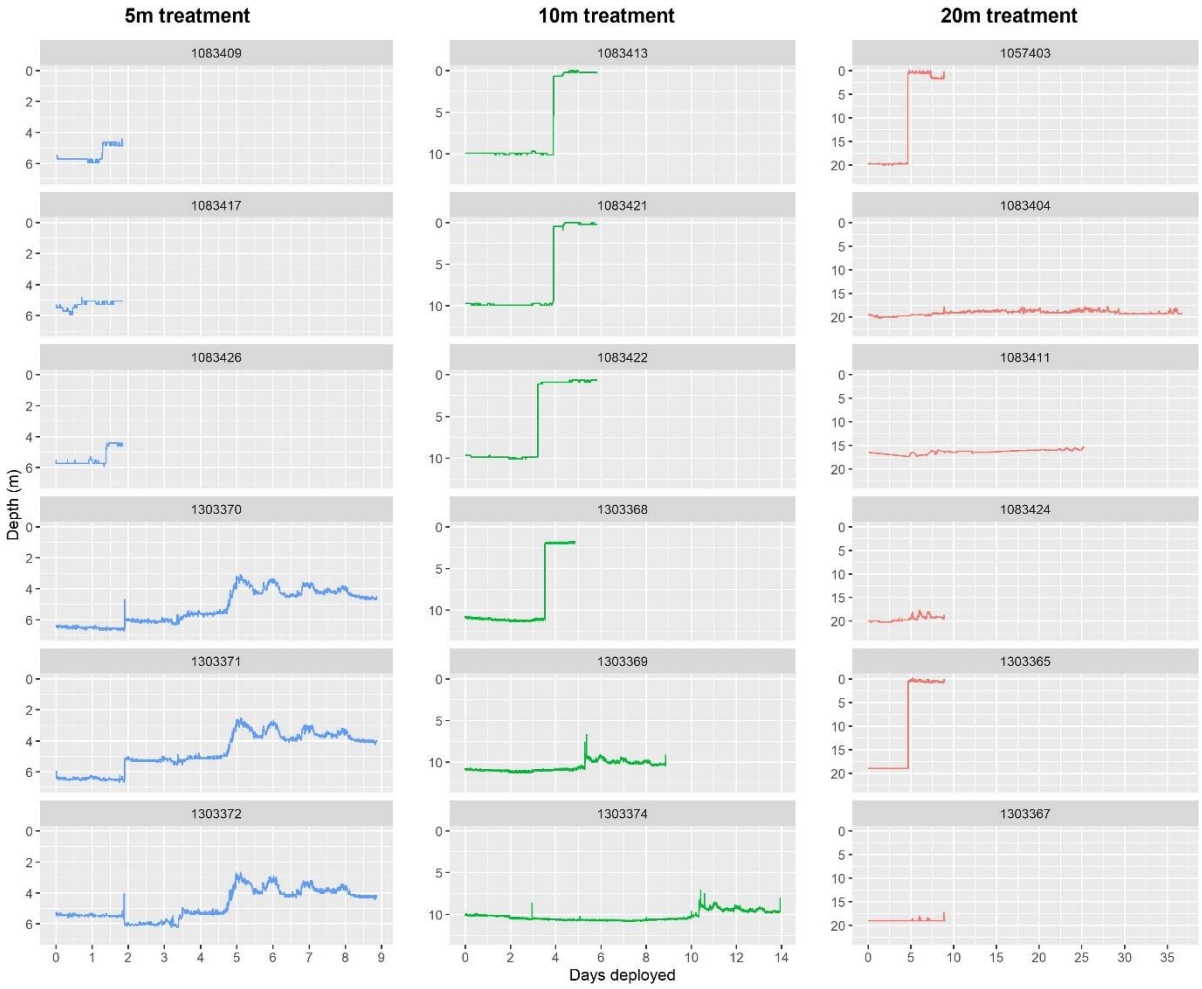

Figure 25: Vertical movement of common carp carcasses affixed with depth sensing transmitters deployed at 5m, 10m and 20m depth within Prospect Reservoir, Australia.

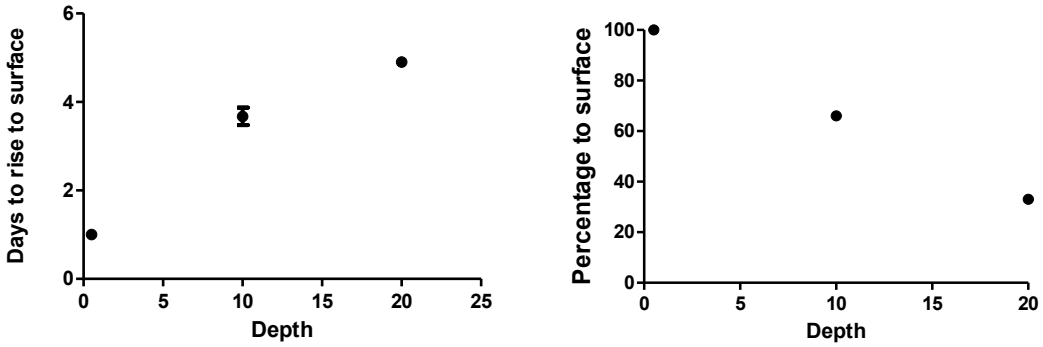

Figure 26: Plot of days of carcass to rise from depth and percentage that rose from depth. 5m depth not included on the plots as all carrasess at this depth were eaten.

Scavenger depth distribution

Within Lake Burrinjuck water depth had a significant effect on scavenger abundances ($F_{4,117}$ =2.59, P<0.05). Within Lake Burragorang a significant interaction was observed between scavenger species and water depth ($F_{32,63}$ =2.50, P<0.001).). Within each lake no fish scavengers were captured in panel gill nets set at 25 m depth. Abundances of fish at 18 m were significantly less than abundances at the shallower depths (Figure 27). For Lake Burragorang, native fish such as silver perch, Australian bass and freshwater catfish were restricted to the top 12 m, with only alien species, including common carp and trout captured below 12 m (Figure 27). At Lake Burrinjuck fish were captured below 18 m with more alien species such as carp and redfin found at 12 m and 18 m. Native fish including silver perch were found to inhabit the top 3 m with golden perch the only species found between 0-18 m.

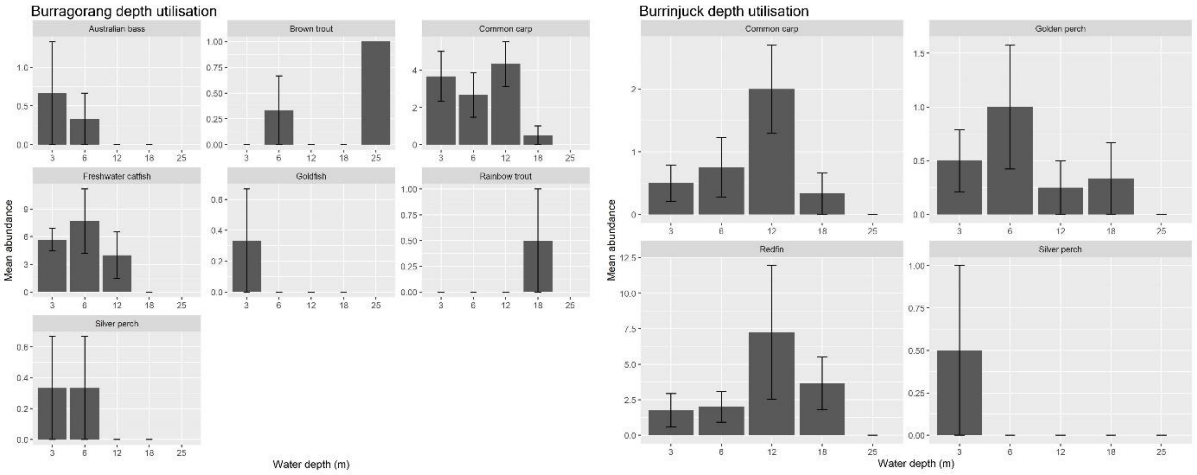

Figure 27: Depth utilisation of fish species captured within demersal pannel gill nets set in Lake Burragorang (n=3 sample sites) and Lake Burrinjuck (n=4 sample sites). Error bars ± standard error.

Table 6: Results of 2-way ANOVA analysis examining the effect of water depth and species con fish catch.

Significance is denoted as 0.001 ***, 0.01 **, 0.05 *.

Lake Burrinjuck	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Species	8	2.6983	0.3373	12.496	7.82e-13 ***
Water depth	4	0.2798	0.0699	2.591	0.0402 *
Species: Water depth	32	1.1595	0.0362	1.342	0.131
Residuals	117	3.1581	0.027		
Lake Burragorang					
Species	8	4.629	0.5786	25.384	< 2e-16 ***
Water depth	4	0.457	0.1142	5.01	0.001433 **
Species: Water depth	32	1.823	0.057	2.499	0.000958 ***
Residuals	63	1.436	0.0228		

4.5 Discussion

This chapter examines interactions between scavengers and dead carp carcasses on the bottom of the lake, and the impact pressure from different depths has on the vertical movement of carcasses. This study found that increasing depth of a carcass increased the time it took to resurface and reduced the number of carcasses that returned. This study found large scavenging fish present down to 12 m depth, but the main interactions with carcasses were at 5 m. Only crustaceans were found at 20 m depth.

Water depth and carcass resuspension

This study found that the depth a dead common carp carcass sank to (or died at) affected the time it would take for the carcass to return the surface. The greater the depth, the less likely the carcass would return to the surface, and if it did, it would take longer to return with increasing depth. Dead fish that sank in shallow waters (0.5-1 m depth) all floated to the surface after a day.

At 10 m saw over 66% of the fish return to the surface while only 33% rose from the sediment at 20 m depth. This may be due to microbial driven gas bloating overcoming hydrostatic pressure (Gäb et al., 2020). This suggests carcasses which sink to depth below thermoclines such as in Lake Burragorang and Burrinjuck are less likely to return to the surface, due to greater hydrostatic pressures and lower temperatures slowing microbial activity (Elder and Smith, 1988, Schneider, 1998, Gäb et al., 2020, Wu et al., 2021). Further, there is likely less or no interactions with large scavengers at these depths. These carcasses may act as nutrient sinks for the microbial fauna below the thermocline (Hewson et al., 2003), causing increases in phosphorus and faster declines in oxygen levels (Deeds et al., 2021).

Time spent at the bottom of the lake and the depth at which the common carp carcass settles can influence the interaction with scavengers and the probability of the carcass resurfacing (Schneider, 1998, Gäb et al., 2020). Dead fish that sank to 5 m in this study were in the littoral zone, an area with high levels of scavenger activity (Chidami and Amyot, 2008). Video evidence showed a freshwater catfish feeding on the remains of a carcass and evidence of eel predation (Chandrasena et al., 1997). Four fish were eaten within the first two days while the other fish were still intact, but they were eventually eaten by day 4. Video filming found no evidence of scavengers at 10 m and 20 m depths but shrimp were seen in the vicinity. Fish at these depths that did not float remained intact on the bottom of the lake. No bite or tear marks were visible and the skin was still intact, but soft tissue had reduced and this was likely caused by slow microbial activity (Elder and Smith, 1988, Schneider, 1998, Gäb et al., 2020, Wu et al., 2021).

Stratification of lakes will heavily influence carcass activity and movement, and this will change seasonally. As the hypolimnion of many lakes is anoxic during summer it will prevent access by scavengers such as crustaceans, eels and catfish (Elder and Smith, 1988). Artificially destratified lakes like Prospect Reservoir do allow invertebrates and fish to potentially access the carcasses at greater depths due to greater oxygen penetration, however based on the acoustic data (Figure 6) and video surveillance they had little influence on the fate of carcasses at 20m.

Scavenging of dead fish

Freshwater catfish were seen feeding on one of the 5 m dead carcasses in Prospect Reservoir. There were no sightings of eels feeding at Prospect Reservoir but long finned eels have been seen feeding in other studies such as Botany wetlands in Sydney (Chandrasena et al., 1997, Pinto et al., 2005). Eels do not have cutting teeth so most food is swallowed whole, however they are able to feed on large prey or carrion (Jellyman, 2022) by tearing food by rotational spinning (Jellyman, 1989, Greenhalgh, 1999). A carp removal project used netting to remove carp and found eels were feeding on live and dead carp in the gill nets set by commercial fishermen (Pinto et al., 2005). During that project, carp were found with only the head remaining in the net due to scavenging by eels similar to fish tag 1083417. Eels feed by accessing soft organs via the anus of the dead carp. This matched wounds on fish tag 1303370. There was no sign of eel scavenging at the 20 m site suggesting the eels don't frequent these depths at the time of the study. This may be as increased hydrostatic pressure alters aerobic metabolism inhibiting of oxidative phosphorylation, which induces modification of membrane phospholipid composition, which has an energy cost associated, as seen in European eels *Anguilla anguilla* (Sébert et al., 2009). Eels make up a large proportion of the biomass in eastern drainage rivers and were plentiful enough for indigenous Australians to make fish traps specifically for eels over 6000 years ago (Smith et al., 2019). These numbers would mobilise and feed on the carp carcasses as they did in shallow lakes of Botany wetlands (Pinto et al., 2005) but they would unlikely scavenge in deeper lakes below 10 m.

Drainages of the Murray-Darling Basin do not have eels but do other have other large vertebrates like freshwater catfish, Murray cod and turtles (Rogers and Ralph, 2010). No Murray cod were sighted feeding on carcasses in Prospect Reservoir, but it would be expected they would feed on the common carp when they are in the final stages of the CyHV-3 infection, when loss of the osmoregulatory functions of the gills cause the carp to die (Michel et al., 2010). However, it is uncertain whether Murray cod will feed on large dead carp. Freshwater catfish and turtles are known detritus feeders (Clunie and Koehn, 2001, Santori et al., 2020) and freshwater catfish were sited feeding on one of the carcasses during this study. Scavenging of carcasses by the freshwater catfish are similar to findings from experiments in North America with the Brown and Blackhead catfish *Ameiurus nebulosus* and *Ameiurus melas* (Chidami and Amyot, 2008, Boros et al., 2020). This suggests freshwater catfish and Murray cod could scavenge in western lakes though the lake survey data suggests they are

unlikely to be found below 12 m (Figure 27). Trout and redfin were found at deeper depths, but this is due to hunting behaviour which involves waiting in dim light at the bottom of the open water light zone, to ambush shoals of smaller fish, and both are not known to be scavengers (Greenhalgh, 1999). Freshwater shrimps were found at all depths. If there is high biomass of large crustaceans like *Macrobrachium* shrimp and yabbies they could aid in the rapid decomposition of the carcasses but this would only be in oxygenated waters above the thermocline (Pascali et al., 2020).

Common carp that sink within the littoral zone will be accessible to scavenging by vertebrates and invertebrates, and by trapping a portion of carcass-derived nutrients in their bodies, will exclude access to primary producers in the short term (Boros et al., 2020). Due to stratification of many lakes and the hypolimnion being anoxic, carp that sink to deeper parts of the lakes will be consumed by microbes and decompose over a longer period of time. The resting place of the carcasses will be important for determining where nutrients will be allocated, in the hypolimnion or the epilimnion (Özkundakci et al., 2011).

Terrestrial scavenger species were not targeted in this study but the interaction of the Swamp Harrier with the floating fish near the 0.5-1 m treatment shows that terrestrial scavengers like raptors and other mammals will have an important role with the carcasses when they float to the surface (Orihuela-Torres et al., 2022). It is acknowledged that scavenging of carrion will continue when the carp reach the surface and the shoreline (Redondo-Gómez et al., 2022). Orihuela-Torres (2022) found the introduction dead carp carcasses on the shoreline of a Spanish wetland were eaten by a variety of vertebrate species. These species included Red fox *Vulpes Vulpes,* Wild boar *Sus scrofa* and the Brown rat *Rattus norvegicus* which are all found in Australia (West, 2018) and other species such as Water Rat, Gulls, Magpies and Eagles which have equivalent species in Australia (Simpson and Day, 1998, Van Dyck and Strahan, 2008)

4.6 Conclusion

Field monitoring of carp fate at different depths revealed once the carcass had sunk to the lake bottom interactions with large scavengers was limited to above 5 m, and beyond that depth, microbes were the main driver of decomposition. Depth of the carcass in the lake was significant in determining if it would resurface, with carcasses less likely to float from microbial bloating with increased pressure at greater depths.

These finds have a significant impact on any clean-up of carcasses post virus release or any general fish kill. To confirm removal of the majority of carcasses, monitoring of the lake should be done 2-3 days after the first carcasses are seen floating, and monitoring should continue in lakes deeper than 5m for at least another 3 days, to collect any remaining carcasses that float up from deeper depths. This will safeguard against carcass induced nutrient enrichment, undesirable water quality and microbial responses.

Chapter 5: Estimating Fish/Carp biomass in lakes and reservoirs: Evaluation of DIDSON transects as a rapid biomass estimation method

Statement of contribution to co-authored published chapter and paper to be submitted to Knowledge and Management of Aquatic Ecosystems

This chapter includes the co-authored details as follows:

My contribution to the paper included data pre-processing, analysis and interpretation, and manuscript writing.

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5.1 Abstract

Estimating biomass of fish and species composition is important in determining the state of aquatic ecosystems. Common carp *Cyprinus carpio* biomass in Australian water bodies is often measured as indicator of the health of ecosystem with high biomass seen as a poor outcome. Also the mass mortality from a cyprinid herpesvirus 3 could pose a significant risk to the ecosystem in areas with carp biomass. The aim of this study was to evaluate the utility of Dual-Frequency Identification Sonar (DIDSON) transects as a rapid fish biomass estimation method. Specifically, this study compared the biomass of large bodied fusiform fish like common carp in lakes. Line transect methodologies and spatial mapping techniques were employed in lakes of south-eastern Australia to determine approximate biomass of fish. DIDSON proved to be an effective hydroacoustic technique to determine carp biomass within water storage reservoirs and lakes. Lake size and depth were compared to biomass to determine if they influenced biomass of fusiform fish. Results indicated shallow lakes had a higher biomass of common carp compared to larger deeper lakes. This is important in determining water bodies which will be at a higher risk of water quality issues if there is a fish kill.

5.2 Introduction

Common carp were first introduced into Australia in the mid 1800's though remained relatively contained until the introduction of the 'Boolara' strain in the 1960's. They are now widely established throughout the Murray Darling Basin and many reservoirs and lakes of south eastern Australia (DPI, 2010, Gilligan et al., 2010, Koehn et al., 2020). Common carp biomass has been estimated at 80% of the total fish biomass in many waterways of south-east Australia (Harris and Gehrke, 1997) and can achieve biomasses as high as 3144 kg ha^{-1} (Harris and Gehrke, 1997). Within Australia and elsewhere throughout the world it has been demonstrated that carp impact upon numerous aspects of aquatic ecosystem health, including water quality and biodiversity values (Matsuzaki et al., 2009, Weber and Brown, 2009, Bajer and Sorensen, 2015, Weber and Brown, 2016). They are now considered a major pest species with significant impacts on the ecosystem (Koehn et al., 2018, Stuart and Conallin, 2018). To reduce abundances of common carp within Australian waterways the release of Cyprinid Herpesvirus-3 (CyHV-3) has been proposed as a biological control (NCCP 2022). CyHV-3 is a highly contagious viral disease that causes significant mortality in all forms and subspecies of Cyprinus carpio, (Hedrick et al., 2006).

Methods for surveying fish biomass in lakes include towed video cameras (TVC) (Lauth et al., 2004) and hydroacoustic sensing equipment (Djemali et al., 2009, Lian et al., 2018). TVC can provide qualitative and quantitative assessment but the accuracy of the field of view is highly dependent upon turbidity. Many shallow lakes in Australia can be highly turbid if there is a large biomass of common carp (Roberts et al., 1995), plus the cryptic behaviour of fish would mean that in clearer water fish may flee before the camera records their presence (Li et al., 2020). Hydroacoustic sensing equipment includes single beam, multi beam and sidescan sonar systems (Moursund et al., 2003, Boswell et al., 2007, Guillard and Vergès, 2007, Liu et al., 2023). DPI Fisheries trialed a biomass estimation method using a Biosonic hydroacoustic sounder (BHS), to determine size class and biomass in recreational lakes (DPI, 2021). These systems may have issues in very shallow sections of lakes (Girard et al., 2020, Baran et al., 2021) making it unsuitable for determining carp biomass given their preference for shallow edges of lakes (Bajer et al., 2011, Crichigno et al., 2016).

Dual-frequency Identification Sonar (DIDSON, Sound Metrics, Bellevue, WA, USA) is an underwater acoustic camera providing real time video imagery that can be recorded for later

analysis. It is used in fisheries research for observations of fish behaviour, estimates of fish abundances and fish size due to its ability to identify fish in turbid or dark water as well as high clarity systems (Moursund et al., 2003, Baumgartner et al., 2006, SOUNDMETRICS, 2008, Boys et al., 2013, Martignac et al., 2015).

Fish community structure is an indicator of the ecological status of water bodies (Karr, 1981) and comparisons of fish community structure among water bodies, or among years are indicative of the ecological status or ecological changes (De Leeuw et al., 2003). Fish biomass is a characteristic often associated with the productivity of a system (Hanson and Leggett, 1982) and an increase in biomass can be sometimes linked to fish diversity (Samarasin et al., 2015). However, biomass can also be influenced by distribution of species abundance and the environment, not just species richness and associated with fish assemblages dominated by a few generalist species of a high trophic level, who are able to exploit both the benthic and pelagic energy pathway (Maureaud et al., 2019).

Understanding the biomass of carp in lakes and reservoirs is crucial in determining the impact a virus induced fish kill would have on water quality and ecosystem function. Pera et al. (2021) found that a large biomass of dead carp over 250 kg ha^{-1} could have severe negative effects on water quality. The NCCP developed a model-based approach to provide an estimate of carp abundance, biomass and density at basin and continental scales (Stuart et al., 2021). Stuart et al. (2021) used long-term electrofishing data to estimate carp biomass. Due to the coarse spatial modelling, extrapolating estimates from the modelled data to a specific lake without any historic survey data can be imprecise. Given fish biomass can vary spatially between lakes (Koehn et al., 2016), and temporally within lakes due to fluctuations in water levels and population dynamics, finer scale resolution of carp densities is required. Traditional fish survey techniques using netting and electrofishing are labour intensive and require specifically trained personnel. A rapid assessment method that can be conducted without highly qualified staff would be of high value, particularly in drinking water reservoirs that can have fluctuating water levels. The use of DIDSON in this study for the rapid assessment of fish biomass is a novel hydroacoustic technique, as most prior applications were involved in fish behaviour and migration studies (Baumgartner et al., 2006, Boys et al., 2011).

This study designed and assessed a rapid survey method and to measure low (<200kg/ha), moderate (200-400kg/ha) and high (>400kg/ha) levels of fish biomass and density using

common carp as a case study species. The primary objective of this study is to compare and validate the DIDSON transect method (DTM) as a rapid biomass survey method that is accurate enough to determine biomass levels. Within the context of the NCCP and proposed release of CyHV-3 in Australia, this survey method would help identify reservoirs that are of high risk of water quality issues from mass fish kills. Furthermore, trying to determine if biomass and lake size changes the risk profile of a carp herpes virus induced fish kill

5.3 Materials and Methods

Study location

The study area incorporated drinking water lakes and smaller lakes within NSW and the Australian Capital Territory (ACT) from varying catchments and altitudes from 8 m to 660 m above sea level (Figure 28). The drinking water storages were; Prospect Reservoir (Georges River catchment), Wingecarribee Reservoir, Fitzroy Falls Reservoir and Lake Burragorang (Hawkesbury-Nepean catchment). Several smaller lakes such Upper Stranger Pond, Lake Isabella (Murrumbidgee catchment), Plumpton Pond (Hawkesbury-Nepean catchment), Wattle Grove Lake and Lake Gillawarna (Georges River catchment) were included. Lakes were chosen based on historical records of carp being present at difference densities.

Prospect, Wingecarribee, Fitzroy Falls and Lake Burragorang are part of Sydney's drinking water catchment. These reservoirs can vary in storage volume depending on water demand and rainfall. Lake Burragorang is the largest at 7500 ha with a maximum depth of 90 m and forms up to 80% of Sydney's drinking water supply (Beasley, 1988). Although much smaller reservoirs such as Wingecarribee 625 ha with maximum depth 9 m deep and Fitzroy Falls 522 ha with a maximum depth of 6 m, form a crucial chain for water transfers from the Shoalhaven Scheme via a network of rivers, pipes and canals to the Nepean catchment (Beasley, 1988). Prospect Reservoir 525 ha with a maximum depth of 21 m is located close to the geographical centre of Sydney and for many years was the final stop of the distribution network (Beasley, 1988). It now acts as a backup reservoir for adjustment of water quality and quantity before entering the main water treatment plant at Prospect. These lakes all form part of Sydney

water drinking water storage and as such are monitored to comply with Australian drinking water guidelines (ADWG, 2011).

Two lakes were sampled within the Murrumbidgee catchment of the Murray-Darling Basin. These included Lake Isabella and Upper Stranger Pond which are small with a surface area of 4.4 ha with a maximum depth of 4 m, and 5 ha with a maximum depth of 2 m respectively.

Plumpton Pond, Wattle Grove Lake and Lake Gillawarna are all within Greater Sydney. These artificial lakes where primarily designed for storm water retention and to enhance green space in highly urban environments. They are the smallest of lakes in this study ranging in area and depth from Plumpton Lake at 0.6 ha and 2 m maximum depth, Lake Gillawarna 1.6 ha and maximum 2 m depth and Wattle Grove Lake 2.6 ha and a maximum depth 2 m.

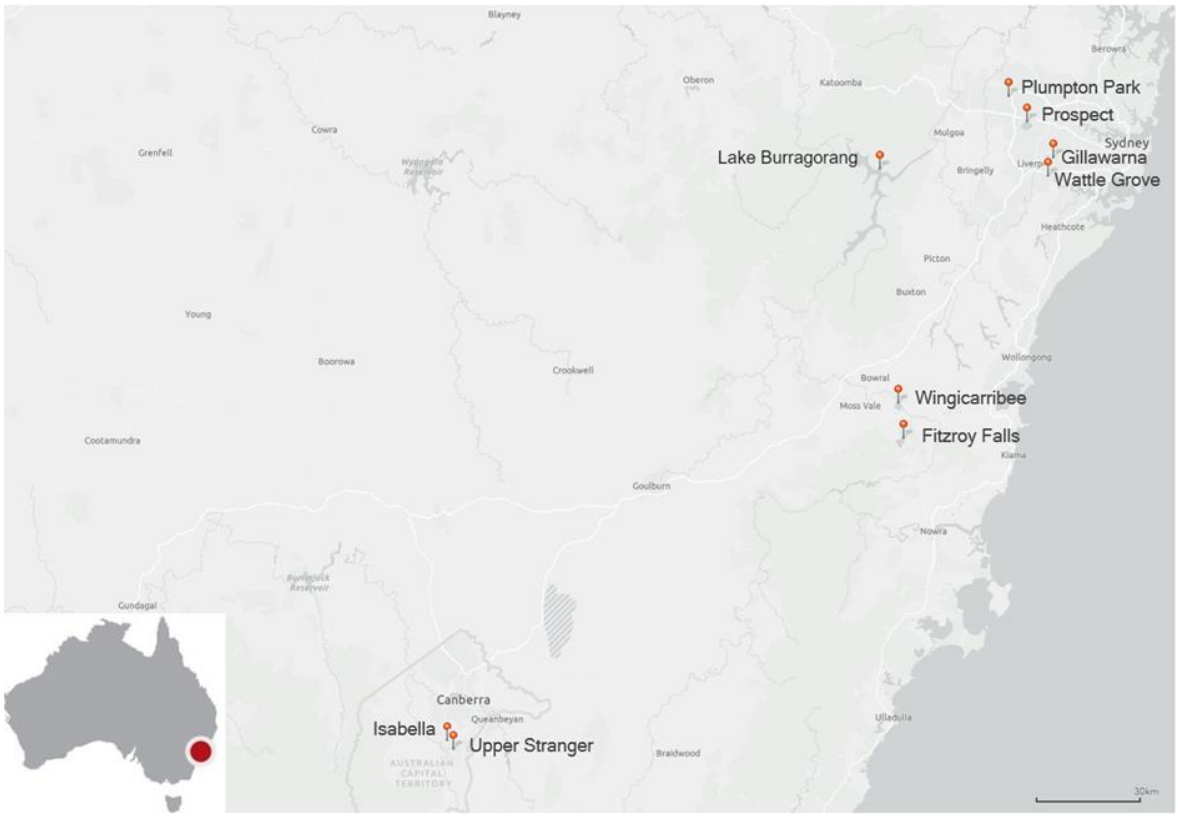

Figure 28 Locations of lakes examined for fish biomass estimates.

200 kHz sonar & 800 kHz downscan sonar

Hydroacoustic georeferenced line transect data was collected with a Lowrance HDS7 Gen3 GPS integrated echo-sounder with a total-scan transducer. At each site, sonar recordings were collected. A track was recorded during each transect and displayed on the Lowrance head-unit in 'split screen mode' to assist with transect spacing. Mapping was conducted with different boats depending on lake size. A flat bottom v punt (4.8 m long) was used for smaller lakes (Plumpton, Gillawarna and Wattle Grove) and a Stacer (5.5 m long) v hull for the larger lakes (Burragorang, Prospect, Wingecarribee and Fitzroy Falls), both with a transom mounted transducer. A flat bottom punt (3.3 m long) was used for Isabella Pond. Sonar logs were recorded at a consistent speed of 1-2 knot using an outboard petrol engine. The sonar recordings were logged at a rate of 15 to 20 data signals s^{-1} at 200 kHz with a 208-beam angle; 800 kHz downscan imagery was simultaneously recorded. The sounder display was monitored to ensure the unit was detecting bottom and producing a clear reading. Start and end times were recorded along the transect. Manual processing of the georeferenced 200 kHz sonar and 800 kHz downscan sonar was performed in 'ReefMaster Sonar Viewer' version 1.0.36.0 software package (https://reefmaster.com.au/).

800 kHz sidescan sonar

During each transect, sidescan imagery was simultaneously recorded using the same Lowrance HDS7 Gen3 GPS integrated echosounder with a total-scan transducer. Sidescan sonar imagery was collected at 800 kHz with a range of 2-3 times the water depth. The outermost transect was conducted in a direction to ensure the transom mounted sidescan transducer had a clear view towards the bank, without the outboard motor leg blocking the sonar signal. Although unable to detect fish, the system was used as reference and to determine if there were any anomalies detected by the DIDSON.

DIDSON Transect Method (DTM)

During each transect a gunnel mounted forward facing DIDSON collected aquatic bathymetry imagery. The DIDSON was submerged 0.2-0.3 m below the water surface and aimed

downwards at an angle of between 10-40 degrees from horizontal depending upon water depth (a larger angle was used during deeper transects). DIDSON recordings were synchronised with the sonar recordings allowing for standardisation when comparing each of the methods. The DIDSON data was collected at 1.8 MHz (high-frequency mode) with a window start of 2.5 m, maximum range of 12 m and frame rate of 7 FPS. The DIDSON imagery was analysed using a line transect methodology. Each fish that was observed from the DIDSON footage was recorded and length measured using the measuring tool within the DIDSON softwareV5 26.06 (Figure 29). Using the measure tool, a box is drawn around the fish to determine the approximate length. Length would be estimated using the length, height or diagonal of the box depending on the orientation of the fish. To determine variability within the measurement of a fish, a sample fish was measured multiple times (4) as it moved across the viewing screen. A standard deviation was calculated from the mean (CL95%).

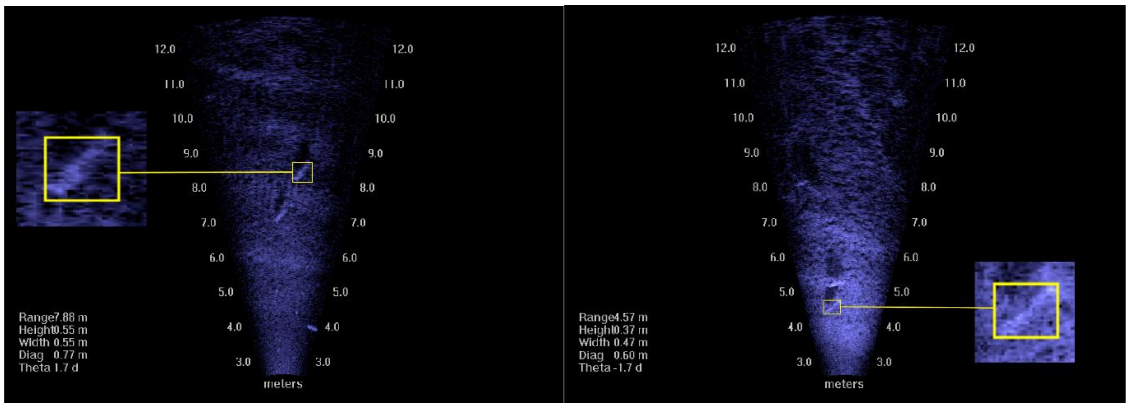

Figure 29. Boxed fish with distance from sonar and length, height and diagonal of box.

Weight was determined from existing length-weight relationships (NSW Fisheries unpublished data) (Figure 30).

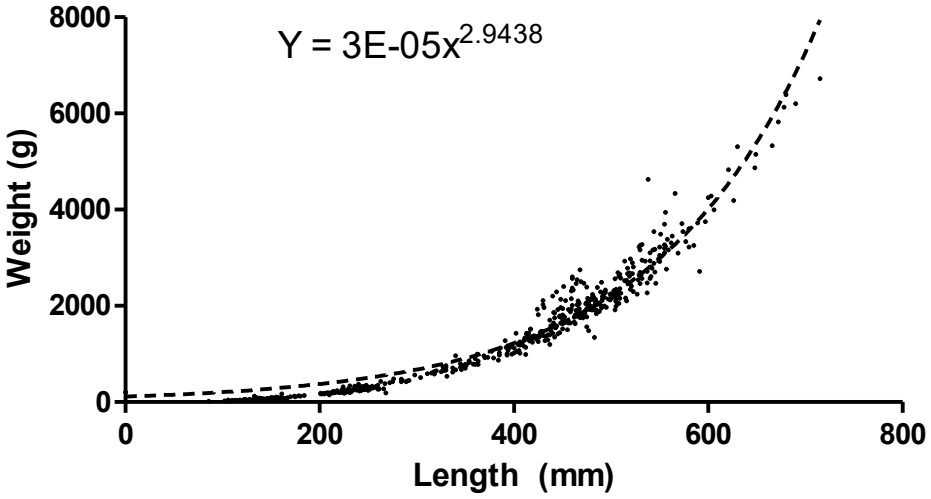

Figure 30. Historical common carp weight and length data from DPI fisheries data.

It was not possible to identify all species of fish from DIDSON footage however certain fish shapes such as eel *Anguilla reinhardtii* or *A. australis* and freshwater catfish *Tandanus tandanus* were easier to determine. Eels and catfish fish were distinguishable due to their swimming motion and their rounded tails. Fish were categorized as either eel *Anguilla reinhardtii* or *A. australis*, freshwater catfish *Tandanus tandanus* or unknown fusiform (bullet or torpedo shape (Blake, 2004). Unknown fusiform fish species were all grouped together, 200 mm in length or greater would include cyprinids (common carp and goldfish *Carassius auratus*), percichthyids (Australian bass *Percalates novemaculeata* and Murray cod *Maccullochella peelii*), salmonids (brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss*) and terapontids (silver perch *Bidyanus bidyanus*). Depending on the water body and based on previous electrofishing surveys, species other than carp would be combined in an overall biomass of fusiform fish. The electrofishing surveys biomass of common carp percentage of total fish biomass would be used to calculate common carp biomass from the DTM.

Transects covered a range of habitats relative to their abundance within the lake to provide a representative estimate. These habitats included soft and rocky substrates, macrophyte beds, in lake structures as well as shallow and deeper parts of the lake. In deeper lakes approximately 50% of the transects would be performed in water deeper than 4m.

Validating methodology

To validate estimates of biomass, the DTM was compared with other techniques in Lake Gillawarna and Lake Burragorang. Two water bodies were sampled in conjunction with the NCCP biomass estimates in lakes and reservoirs (Stuart et al., 2021). The NCCP study chose two methods in shallow lakes (Gillawarna <4 m), with carp abundance estimated through a depletion method and mark-recapture data. The density and biomass estimates per hectare are compared to the catch per unit sampling effort for the standardised sampling technique used by the Sustainable Rivers Audit (SRA) for electrofishing (MDBC, 2004) to provide a correction factor. In deep lakes like Lake Burragorang (>4 m) the study used a depth utilisation model (Stuart et al., 2021).

The DTM was performed within 24 hr prior to the depletion and mark-recapture assessment. To assess the effectiveness of the DTM it was performed in Upper Stranger Lake before it was drained and the absolute number of carp present in the pond were counted. Likewise, in Plumpton Lake the DTM was carried out before carp removal was done by the local council. To compare the actual biomass removed from lakes to transects recorded in the field, carp biomass would be considered low if below 200 kg ha^{-1}, moderate between 200-400 kg ha^{-1}, high between 400 kg ha^{-1} and above. This was based on observed impacts on dissolved oxygen from a fish kill at those biomasses (Pera et al., 2021).

Relationship between lake size and common carp biomass

Lakes would be assessed on risk of a virus caused common carp fish kill once biomass is known. This would be based on the relationship of increasing biomass and the dissolved oxygen consumption data from Chapter 2 (Figure 3 and Figure 4). The data showed common carp carcass biomass over 250 kg ha^{-1} resulted in all the oxygen being consumed.

Statistical analysis

The relationship between DTM carp biomass and other biomass methodologies was analysed by linear correlation. Biomass of carp and depth and surface area of lake were analysed by non-linear correlation. Where a significant correlation was found, the relationship was defined using linear regression after first checking residuals plots to ensure the data met the

relevant assumptions. Error bars plotted on graphs represent standard error of the mean (SEM). GraphPad Prism 5 was used to perform the linear regressions and create graphs.

5.4 Results

Biomass Validation methodology

The comparison of DTM to other methods for determining biomass, including depth utilisation, depletion mark recapture and removal and weighing common carp had a linear regression relationship Y=0.004738X - 0.3986 and r^2=0.98 (Figure 31).

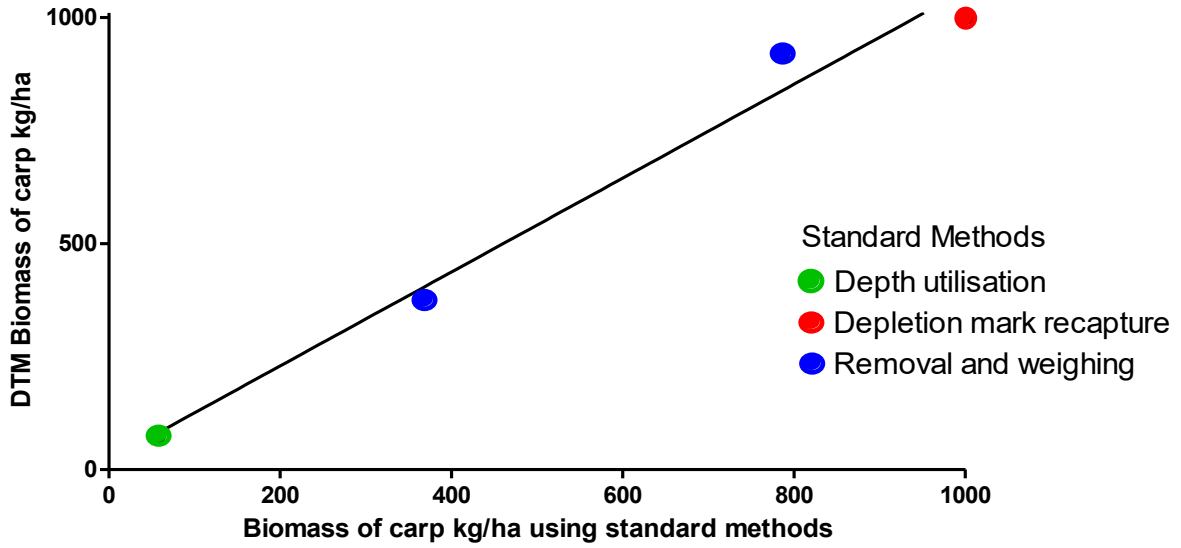

Figure 31. Linear relationship of DTM biomass to other biomass calculation methods including 1) Depth utilisation, 2) Depletion mark recapture, 3) Removal and weighing.

Low biomass <200kg/ha

In Lake Burragorang Stuart et al. (2021) used the depth utilisation model to estimate biomass/density. Stuart et al. (2021) found that mean carp biomass was 57 kg/ha while the DTM survey calculated fusiform fish biomass was 97kg ha^{-1}. Fish surveys by DPI found five fusiform fish species in the lake, namely Common Carp, Silver Perch, Australian Bass, Brown and Rainbow Trout. Based on the presence of other fusiform fish and carp making up 91% of

total electrofished fish biomass, it would equate to 88 kg ha^{-1} of carp in Lake Burragorang. Both methods assessed the biomass of carp as 'low' (<200kg ha^{-1}).

Moderate biomass 200-400 kg ha^{-1}

Isabella Pond was drained and all carp were weighed by ACT Environment, Planning and Sustainable Development Directorate as part of their rehabilitation of the pond (ACT Environment, 2018). Based on the surface area of 4.4 ha of the pond at 100% capacity, biomass was calculated at 250 kg ha^{-1}. At the time of the DTM survey the lake was in the process of being drawn down. The approximate surface area was 3 ha, based on the reduction in surface area would equate to a biomass of 367 kg ha^{-1}. The DTM survey calculated fusiform fish biomass was 397kg ha^{-1} (Figure 32). Fish surveys by ACT Environment, Planning and Sustainable Development Directorate found the only fusiform fish in the lake was common carp (ACT Environment, 2018). Both methods had moderate estimates for carp in Upper Stranger Pond.

High biomass >400 kg ha^{-1}

At Lake Gillawarna the depletion experiment conducted by Stuart et al. (2021) estimated the carp biomass at 1000 kg ha^{-1} while the DTM survey calculated it at 956 kg ha^{-1}. Electrofishing surveys conducted on the lake by Stuart et al. (2021) found the only fusiform fish in the lake was common carp. Both methods both had high biomass estimates for Lake Gillawarna (Figure 32).

The Plumpton Lake DTM survey calculated the biomass of common carp to be 923 kg ha^{-1}. Carp removal from the lake via electrofishing for three days estimated a total of 786 kg ha^{-1} present. Both methods had high biomass estimates for Plumpton Lake (Figure 32). Electrofishing surveys showed the only fusiform fish in the lake was common carp in both lakes.

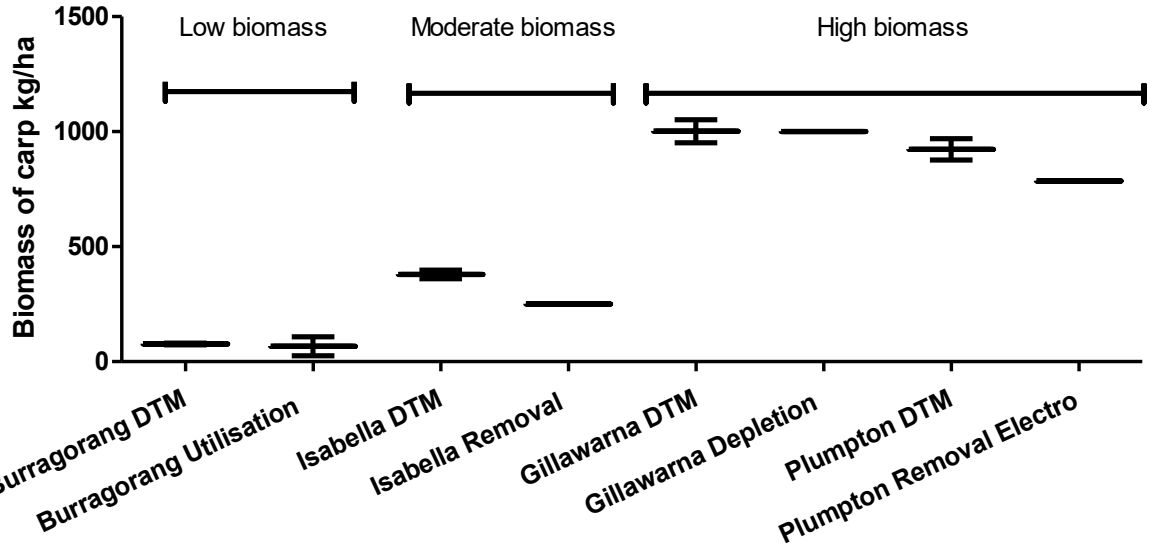

Figure 32. Estimates of biomass by various methods. DTM (CL95%) compared to NCCP depth utilisation at Lake Burragorang (CL95%), Lake Gillawarna depletion mark recapture (CL95%), and removal of carp from Plumpton Lake via electrofishing (Approx. 80-90% carp weighed) and draining of Isabella Pond(100% of carp weighed).

Biomass survey of lakes

A total of eight lakes were surveyed using the DTM. Data from Upper Stranger Pond is from weighed fish from the drained lake. Biomass of carp within lakes varied from the lowest at Fitzroy Falls Reservoir, which had a biomass of 48 kg ha^{-1}, with the highest Lake Gillawarna at 1002 kg ha^{-1} (Figure 5). Smaller lakes from 0.5 to 5 ha in surface area had a high biomass of carp, greater than 500 kg ha^{-1}, with the exception being Upper Stranger Pond (4.4 ha), which had carp biomass in the moderate range of 250 kg ha^{-1} (Figure 32). Isabella Pond differed from the other small ponds as it had a maximum depth of 4 m while the others ranged from 1.5-2 m.

The larger lakes with depth greater than 6 m all had low carp biomass ranging from 48 kg ha^{-1} to 147 kgha^{-1} (Figure 33).

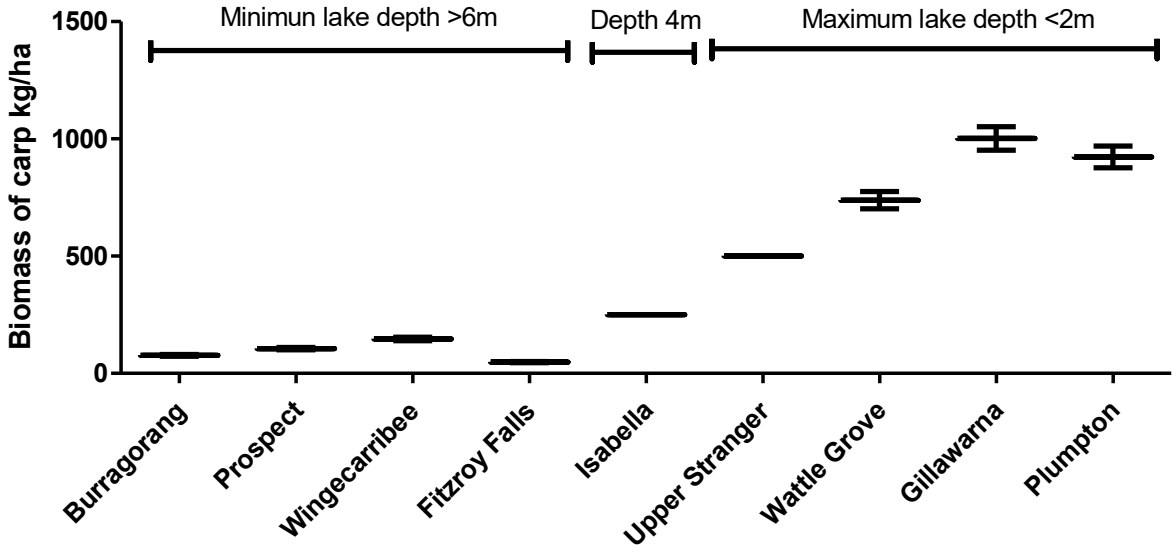

Figure 33. Biomass of lakes using DTM (CL95%). Upper Stranger and Isabella are actual biomass from a drained lake.

Common carp biomass and maximum lake depth

Common carp biomass (kg/ha) was plotted as a function of maximum lake depth. A nonlinear exponential growth equation was fitted to the data and provided the best fit.

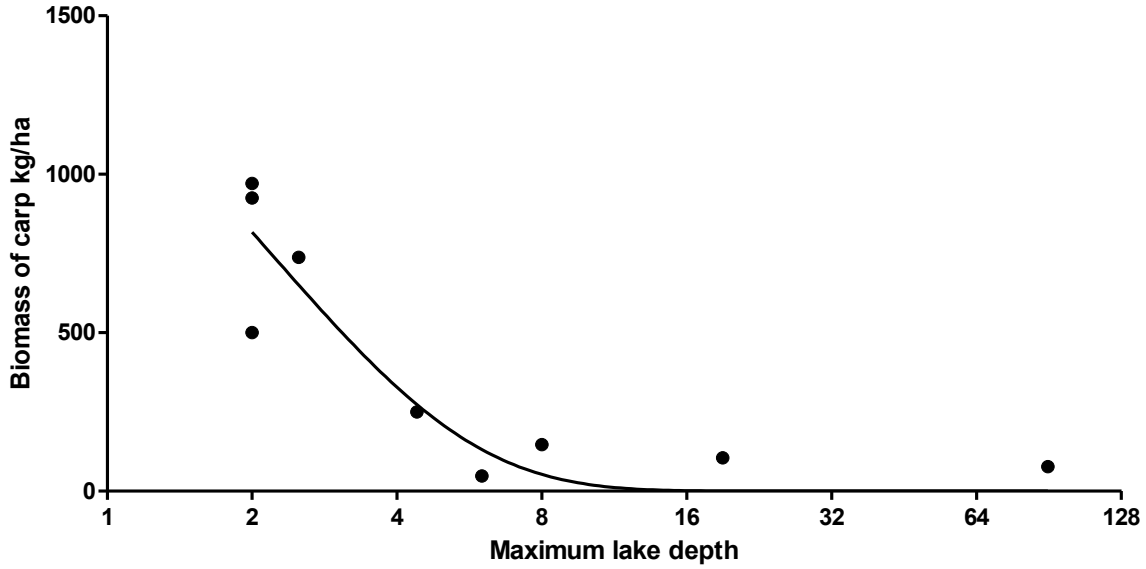

Figure 34. Common carp biomass in kg/ha as a function of maximum lake depth

5.5 Discussion

The DTM survey was developed as a rapid method for determining carp biomass in lakes. Its main purpose was to determine if lakes had low (0-200 kg ha^{-1}), moderate (200-400 kg ha^{-1}) or high (>400 kg ha^{-1}) biomass of common carp. Comparisons from four lakes with different biomass levels showed the method to be precise enough for that assessment (Figure 32) and with further development could be more accurate at determining fish species with improvements in the DIDSON models.

Comparing DTM to other biomass methods

To estimate fish biomass, there are three methods available in the deeper lakes, the DTM, depth utilisation and the BHS. Depth utilisation involves netting and electrofishing and typically involves setting and retrieving nets over multiple days and 2-3 staff. However, a benefit of this method, is that it provides information on species composition and relative abundances. This is not possible with the BHS or the DTM as absolute species differentiation is not possible. The DTM is limited due to resolution of images, but can be used to differentiate fish with different body types and/or modes of swimming i.e. eels vs perch species. The BHS will work at depths from 0.5 m down to 2000 m, whereas the DTM was best suited to <6 m deep, but could theoretically reach down to 12 m, thus, leaving large areas of deep lakes not surveyed. However, it is effective in the littoral zone where the largest proportion of common carp are found (Bajer et al., 2011) and differential habitat utilisation by carp in deep storages showed a strong preference for relatively shallow water with a catch per unit effort (CPUE) decline between 2 and 6 m of 61.9% and a 86% decline at 12 m (Stuart et al., 2021). In shallow lakes an electrofishing catch per unit sampling effort can be used as well as the DTM and the BHS.

The main advantage of the DTM is in reducing staff time spent in the field and staff knowledge requirements of fish handling and identification when compared to electrofishing catch per unit sampling effort and depth utilisation. The DTM is able to give sufficient information on the biomass of common carp, allowing water managers to be able to assess the risks of a virus induced fish kill.

Biomass of common carp vs lake size

Data from the surveys showed that smaller shallower lakes had a greater biomass than deeper larger lakes (Figure 33). Furthermore, maximum lake depth showed a relationship with carp biomass (Figure 34). This may be due to; 1) Shallow lakes as a percentage have a larger littoral zone (Jeppesen et al., 1997, Nõges, 2009) so would be relatively more productive than deeper lakes (Howard-Williams and Lenton, 1975); 2) turbidity from carp in high numbers (Zambrano et al., 2001, Weber and Brown, 2009) would offer protection to juvenile carp from predators (Richardson et al., 1995, Mutethya and Yongo, 2021) and; 3) the shallow lakes surveyed did not have predatory fish such as Australian bass or Murray cod that could moderate common carp abundances (Koehn, 2004).

My study shows that a virus related common carp fish kill in shallow small lakes is at more risk of water quality issues than larger deeper lakes (Pera et al., 2021). In small shallow lakes the fish would die, sink to the bottom and resurface the next day (Chapter 4). At the estimates of common carp densities gained from this study it is likely that if fish are not removed from smaller lakes the decomposition of the dead common carp could result in hypoxic conditions throughout the lake potentially killing other fish species and invertebrates (Kushlan, 1974, Science, 2019, Pera et al., 2021, Sheldon et al., 2022).

Biomass estimates of common carp in larger deeper lakes ranged from 48–148 kg ha^{-1} (Figure 33). At these levels hypoxia as a result of virus-related common carp fish kill would likely not occur (Pera et al., 2021). However, these biomass levels were based on the lakes at full storage and will differ when lakes are considerably lower in volume. Many regional lakes vary in level, based on demand from water intensive primary industries and changes in inflow volumes (Austin et al., 2020, Colloff and Pittock, 2022). Lake levels during the last drought fell to between 2 and 14% (Doyle, 2019, Drevikovsky, 2020, Environment, 2022). Based on such a large drop in lake surface area this would concentrate the number of common carp to a level that would have implications to water quality if there were a common carp fish kill. This concentration of common carp would change the biomass per hectare from levels with minimal water quality changes to levels which could cause lake wide hypoxia (Pera et al., 2021). Determination of risk using littoral zone ratio versus lake surface area, could be useful in estimating biomass of a lake at full storage and then determining what happens when lakes

are drawn down to determine effective new biomass and implication to the release of the virus of a fish kill in that storage.

A national estimate of carp biomass for MDB was done by the NCCP, using historic CPUE electrofishing data but the precision of the biomass estimates was broad (Stuart et al., 2021). Impact from structures such as weirs means on only 40–50% of the MDB's main stem rivers remain free flowing (Koehn et al., 2020). Given the diminished effectiveness of boat-electrofishing expected at water depths > 3 m (Reid et al., 2021), the DTM survey offers a method that could validate or more accurately assess areas above weirs where historic electrofishing data was used.

The DTM survey prior to introduction of the virus, or in areas where poor water quality from a combination of higher temperatures and high algal biomass, would give water managers a better knowledge of the oxygen demand in that system. This would also be beneficial in areas where there have been numerous fish kills such as the Darling River (Sheldon et al., 2022, Durrant-Whyte, 2023) by being able to assess the remaining fish community without the added stress of more tradition techniques such as electrofishing or netting.

5.6 Conclusions

The study found the DTM survey offers a quick, easily repeatable, consistent and cost-effective method for determining fish biomass in lakes, particularly in shallow lakes <4 m deep. It also determined that small lakes had a high biomass of between 500-1000 kg ha^{-1} of common carp. If a CyHV-3 fish kill occurred in these small lakes it could have catastrophic implications to water quality and the ecosystems. Larger lakes had common carp biomasses of between 45-140 kg ha^{-1} and are unlikely to have major water quality and ecological issues from a CyHV-3 fish kill. An understanding of common carp biomass within individual lakes will provide insight to the risk of water quality issues associated with a CyHV-3 related mass kill.

Chapter 6: General discussion

This thesis examined the influence of fish kills of common carp on lakes such as from a species-specific virus induced fish kill. Manipulative field mesocosm experiments were carried out to examine the possible implications of a CyHV-3 fish kill on water quality, nutrients and algal growth as well as microbial community structure and pathogens. Monitoring studies were also conducted to characterise carcass movement throughout the lake depth profile and better understand the fate of dead fish and potential interactions with scavengers. Finally, surveys of carp biomass in lakes were conducted to better understand biomass as a function of lake area and then related this to risk from a CyHV-3 fish kill. Collectively, these studies provide insights into the effects of a CyHV-3 fish kill may have in lakes, the fate of the carcasses and the risks in different sized lakes.

6.1 Dissolved oxygen response to dead carp input

Chapter 2 and 3 showed the changes in dissolved oxygen from the input of different amounts of common carp carcasses. The mesocosm experiment showed that at levels above 500 kg ha^{-1} the dissolved oxygen concentration went to zero and at 250 kg ha^{-1} levels dropped to 3-4 mg L^{-1}. Most aquatic plants and animals require oxygen above 5 mg L^{-1} to survive (Bozorg-Haddad et al., 2021). Juvenile Australian native fish have some ability to survive long-term hypoxic conditions in the range of 3-4 mg L^{-1} (Gilmore et al., 2019) and short term levels up to 48 hrs at ranges from 2.4 – 3.3 mg L^{-1} depending on the species (Small et al., 2014). The ability of native fish to cope with hypoxic conditions may also be reduced by anthropogenic activities in the Murray-Darling Basin (MBD) such as eutrophication and consequent algal growth from excess runoff of nutrients (nitrogen and phosphorus) into local waterways (Thorburn et al., 2003, Jenny et al., 2016, Isaza et al., 2021). Extremely low to zero DO levels continued for between 4- 14 days for higher carp biomass treatments and in some treatments diel fluctuations from 35 - 200% saturation present a challenge to aquatic life (Andersen et al., 2017) .

A CyHV-3 species specific fish kill in small shallow lakes or large lakes at low lake levels has the potential of creating hypoxic conditions for multiple days. Biomass of common carp and lake depth are important as they influence the extent and severity of the oxygen demand from the carcasses. Much of the MBD has been modified into a series of long deep weir pools

(Whitworth et al., 2012, Beavis et al., 2023) and these weir pools pose significant impact to fish migration and the ability of fish to escape adverse conditions. These pools are ideal conditions for thermal stratification and act like long thin lakes (Tekile et al., 2015, Science, 2019, Beavis et al., 2023) and in low flow conditions stratification can leave a very shallow epilimnion (Sheldon et al., 2022). These sections of the river will be at high risk of fish kills due to prevailing conditions and a CyHV-3 release could trigger a larger fish kill due to increase dissolved oxygen demand (Davie and Pera, 2021, Ellis et al., 2022, Sheldon et al., 2022).

6.2 Phytoplankton changes in response to dead carp

Chapter two examined phytoplankton response to different biomass of carp carcasses. The field based mesocosm showed the algal biomass response as chlorophyll a had a linear correlation with carcass biomass as was the case for nitrogen and phosphorus concentrations. There was a trend towards picoplankton dominating in high biomass treatments and this may be due to high levels of nutrients that supported small algal cell growth due to competitive advantage due to greater surface area to volume ratio (Hillebrand et al., 2022). It is also possible that allelopathic effects of macrophytes in Prospect Reservoir may be inhibiting certain algal species from growing (Mohamed, 2017, Colville and Rohlfs, 2019, Maredová et al., 2021). The experiment was done at a relatively low salinity of 200 $\mu S\ cm^{-1}$ and low turbidity <5 NTU with a small relatively natural catchment (Pera, 2020). The large response by phytoplankton to the common carp fish kill should pose a significant warning to other areas in Australia with large carp numbers. Recent estimates of common carp biomass across the MDB suggest large parts of the river system and water bodies have biomass levels (Stuart et al., 2021) which would promote significant phytoplankton growth (Pera et al., 2021).

The MDB is a significantly altered catchment (Barrett and Ansell, 2003, Leblanc et al., 2012). The MDB has salinity levels varying from 45- 6000 $\mu S\ cm^{-1}$ (Jolly et al., 2001, Hart et al., 2020). Increased salinity can favour toxic algal species and exacerbate fish kills (Free et al., 2023, Sobieraj and Metelski, 2023). In 2022 the Oder river in Poland experienced a fish kill along hundreds of kilometres which was driven by toxic golden algal species *Prymnesium parvum*, the bloom forming due to hydrological conditions (low water levels and low flows) and atmospheric conditions (high temperatures, lack of precipitation) (Absalon et al., 2023). It caused the mass extinction of fish along the middle and lower section of the river (Free et al., 2023). This an example of the collapse of an ecosystem when high nutrient loads enter into

waterways that are already stressed with climatic extremes. As large sections of the MBD have been modelled to have biomass levels of carp exceeding 250 kg ha^{-1} (Stuart et al., 2021), given the high oxygen consumption of carcasses from chapter 2 and Pera et al., (2021), large sections of the MDB would be at risk of aquatic ecosystem collapse (Marshall et al., 2018, Steward et al., 2019).

The variation in water quality in the MDB may see phytoplankton respond differently to fish kills depending the location (i.e. Darling River vs the Murray River). The Darling River upstream of weir 32 near the town of Menindee is known as a eutrophic section of the river and has a history of cyanobacterial blooms (Mitrovic et al., 2011, Science, 2019, Sheldon et al., 2022). In March of 2023 low oxygen levels were caused by more than a million fish dying (Loughran et al., 2023). The majority of the fish were not removed and the carcasses were allowed to sink to the bottom of the river. Three months later in July 2023 at the water quality sampling site Talyawalka at Menindee-Pooncarie Road, there was potentially toxic cyanobacterial count of 24,818,333 cells mL^{-1} , and sites stretching 200 km downstream with toxic count cyanobacterial above 400,000 cells mL^{-1} (WaterNSW, 2023). Typically, those downstream sites in June have toxic count cyanobacterial counts between 0-2,000 cells mL^{-1}. The July 2023 result represents a 200 fold increase on a typical winter counts. These results on the Darling River show that the microbial response to a large fish kill may go beyond the month of the mesocosm experiment. The flow condition in rivers will contribute to the toxic phytoplankton response post CyHV-3 release and in particular flows when the fish kills happen.

Increase in average daily summer temperatures and reductions in flows during drought will also favour more stratified environments in rivers and lakes (Mitrovic et al., 2003, Carey et al., 2012). The likely increase in primary productivity from the CyHV-3 common carp fish kill will reduce light penetration and create a deeper anoxic hypolimnion (Ficke et al., 2007, Zastepa et al., 2023). This will reduce habitable zones for aerobic aquatic life such as fish, and lead to an environment similar to Weir 32 weir pool on the Darling River near Menindee that led to 3 fish kill events (Science, 2019, Vertessy et al., 2019, Sheldon et al., 2022).

Based on the timing of the release of CyHV-3 the outcome to non-target aquatic biota could vary significantly. A low flow, high temperature scenario is likely to pose the most significant risk to non-target aquatic biota in both lakes and rivers. Monitoring of temperature, dissolved oxygen and flow will be critical in preventing non-target species from being impacted.

6.3 Bacterial responses to dead carp input

Chapter 3 showed high carcass biomass overshadowed the complex interactions in lakes between autotrophs (autotrophic phytoplankton) and heterotrophic bacteria and mixotrophic phytoplankton. It reversed the normal lake resource model for bacterial growth in low nutrient environments (Wang et al., 2007, Edwards, 2019). Instead, without any stoichiometric constraints bacteria shifted from signature lake bacterial groups to copiotrophs and anaerobic bacteria such as Clostridium and *E. Coli*. This was shown to be a response to the higher available organic carbon and nutrients from the carp carcasses. The increases in available nutrients allowed aerobic bacteria to bloom causing oxygen levels to reduce and then favour anaerobic bacteria. The study site at Prospect reservoir is considered an oligotrophic environment, however the resulting dominant bacteria post fish decomposition would be difficult to predict in other mesotrophic and eutrophic reservoirs. These microbial shifts will potentially alter nutrient cycling, which would impact system productivity and trophic state (Sagova-Mareckova et al., 2021, Baumann et al., 2022). Depending on the environmental conditions prior to a fish kill, it may lead to different microbial shifts and potentially multiple microbial pathogenic or toxic cyanobacterial responses (Killberg-Thoreson et al., 2014, Carney et al., 2015, Sheldon et al., 2022, Free et al., 2023). However, it would be reasonable to presume that the increase in nutrients and organic carbon would again shift to bacterial groups such as copiotrophs and anaerobic bacteria. The anoxic environment caused by bacterial decomposition will also impact on other aquatic life if dissolved oxygen levels to fall below 4 mg L^{-1} (Gilmore et al., 2019, Bozorg-Haddad et al., 2021).

6.4 End fate of carcasses and interactions with scavengers

Chapter 4 examined the fate of carp carcasses in lakes at different depths. It highlighted the importance of where the carcasses sink to, or deaths occur. In the carcasses at lower depths, it was less likely they would float back to the surface and also the less likely there would be interactions with scavengers. Carcasses which sank in shallow water (< 5 m) were more likely to be scavenged while ones that weren't scavenged were more likely to float to the surface.

Potential aquatic scavengers of the eastern and western draining rivers and lakes are eels and freshwater catfish. However, the biomass of these are only a tenth of the biomass of common

carp carcasses, and it is likely they would be overwhelmed with the amount of carcass available. The carcasses that float to the surface would be accessible to land and avian scavengers such as seagulls and raptors, but the Menindee fish kills 2018-19 (Sheldon et al., 2022) and 2023 fish kill did not see large scale migration of theses scavengers although there were some seagulls (Williams and Schulz, 2023). With the drop in dissolved oxygen these fish kills impacted the whole of the aquatic biota including large aquatic scavengers and included crustaceans (Williams and Schulz, 2023). The western drainage has potentially higher biomass of yabbies (Mills and McCloud, 1983, Sang et al., 2011, McCormack, 2014) which will aid in the breakdown of carcasses but only if the dissolved oxygen levels remain high enough for them to survive. If there is anoxia in the system there would be no scavenging of the carcasses by large teleost scavengers such as eels and freshwater catfish, or small invertebrate scavengers as they would not survive the anoxic conditions.

A clean up the carcasses would be the best response post fish kill. It would remove potential nutrients and prevent fueling microbial blooms (Pera et al., 2021). Based on the data most of the carcasses that would resurface would do so by 4 days (Figure 25). In lakes less than 3 m in depth a cleanup could remove 100% of carcasses and at least 50% in lakes 10 m or deeper. This data should be taken into consideration for other types of fish kills. Large fishkills such as the 2018-19 and 2023 Darling River near Menindee, Australia (Sheldon et al., 2022) and Oder River in Poland (Absalon et al., 2023, Sobieraj and Metelski, 2023).

6.5 Lake size and carp biomass

Chapter 5 looked at the biomass of common carp in relation to lake size. Both surface area and depth play a role in determining the number of common carp in lakes (Stuart et al., 2021). In my surveys, It found shallow lakes with a maximum depth of 2 m had significantly higher biomass of common carp. The levels of the common carp in these lakes would be considered a high risk of catastrophic water quality impacts if 80% or more of the carp were to die from a CyHV-3 release. Decomposition of the common carp would cause near zero oxygen levels for days (Pera et al., 2021). Generally, common carp biomass is calculated based on surface area not volume (Gilligan et al., 2010, Stuart, 2019). Larger lakes deeper than 6 m, based on increased volume to surface are ratio (Tilzer, 1990), had biomass levels of carp that would not cause oxygen depletion to levels that would impact other aquatic fauna. However, carcasses that sink below the thermocline are less likely to rise (Chapter 4) and may become an energy

source for hypolimnial bacteria, with potential effects on aquatic carbon and nutrient cycling. Many of the western lakes in NSW have a primary role of irrigation water supply (Kingsford, 2000) and levels can vary quickly and dramatically. Lake Burrendong capacity varied from 5% to 130% in two years (Thackray, 2022). Based on a biomass of 100 kg ha^{-1} at full storage at 5% storage volume the estimated biomass of carp would be 1500 kg ha^{-1}. This illustrates that even large lakes, dependent on storage volume, can have biomass levels considered a high risk of a catastrophic water quality impact. Rivers can also experience reduced or zero flows that can lead to only refuge pools remaining (Rohlfs et al., 2021). This would lead to a concentration of fish in the refuge pools with high biomass levels and stressed conditions.

6.6 Comparison to other types of fish kills

The aim of the thesis was to better understand changes to ecosystems after a CyHV-3 virus release. Many of the learnings from this thesis are directly relatable to fish kills from other factors such as blackwater, toxic algae and extreme environmental conditions. Global climate change is increasing the likelihood of extreme weather events including longer and more severe droughts, floods and thermal extremes (Stott, 2016, Till et al., 2019, Clarke et al., 2022, Yuan et al., 2023, Zachariah et al., 2023). These events will increase the occurrence of large scale fish kills such as in Darling River near Menindee where prolonged drought and low flows lead to a scenario where there were large scale fish kills (Science, 2019, Sheldon et al., 2022). This drought related fish kill in 2018-19 was followed by another fish kill in 2023 following a long period of flooding (Loughran et al., 2023, Ormonde, 2023). In 2022 thousands of dead fish were found floating in the 840 km Oder River, which runs from the Czech Republic to the Baltic Sea along the border between Germany and Poland (Absalon et al., 2023, Free et al., 2023). Fish kills such as these will likely follow the same order of succession as seen in the mesocosm experiments depending on biomass. As the dead fish decompose oxygen levels will drop with increasing microbial activity. This would then be followed by increased algal growth, especially in summer. The results from the mesocosm experiment can be used to assess other fish kills if the size of the kill and the size of the water body are known.

6.7 Management recommendations

Preparation is the key when dealing with fish kills and knowledge of the biomass of carp within lakes will enable water operators to estimate the size of the clean-up. That knowledge combined with a rapid deployment of resources for removal and disposal of carcasses will ensure water quality impacts are reduced. This study has shown that post fish kill oxygen levels begin to drop with within two days and the dead fish will resurface within one to four days depending on the depth they sank to. If a virus was used as a biocontrol of carp, preparations would need to be made in resourcing of equipment and trained personnel to remove dead carp from the waterways and monitoring of water quality. If it is not possible to remove the dead carp then monitoring should be done to determine the water quality impacts and preparations for closing access to water ways or informing the public of potential health threats. The level of risk at lakes could be determined based on the size of lake and the biomass of carp from chapter 5.

6.8 Recommendations for further studies

Sediment based mesocosm to see if there is a greater influence from benthic microbe Bacteria 16s QPCR

While there was clearly a change in the microbial community from the addition of common carp carcasses to the mesocosms (Chapter 3), it is difficult to determine what influence microbes in the sediment would contribute to the microbial community response from carcass input as the mesocosms did not include contact with the benthos. Studying changes with sediment contact would allow more anaerobic microbes to source the nutrients and by combining 16s with the addition of quantitative polymerase chain reaction QPCR allow a better understanding microbial biomass.

When common carp are in the final stages of the CHV-3 virus will Murray cod and long finned eel predate on the compromised fish?

This study has provided crucial insights into the dynamics of large aquatic predators such as long finned eel and catfish and their scavenging on the common carp carcass. Healthy common carp generally do not have predators once they reach a certain size, but a fish in the late stages of the CHV-3 virus will be struggling to swim as the virus impacts its ability to

breath from the mucus coating on the fishes' gills. Distressed common carp may attract the attention of large predators who seek out prey which are vulnerable. Murray cod are one of the only fish within Australian freshwater systems which are large enough to eat adult common carp.

Land based scavenger interactions with carcasses

A major finding of this thesis was the importance of scavenging of carcasses by aquatic scavengers (Chapter 4). There are potentially land based scavengers which could interact with the carcasses. It was not clear how or if the scavengers will contribute to the removal of carcass nutrients away from the waterbody. Transfer of these nutrients to the land may be a significant pathway. Research directly measuring the different types of land-based scavengers and their interactions with common carp carcasses is still a key research gap in understanding how carp carcasses will affect land food web dynamics.

6.9 Conclusions

The Cyprinid herpesvirus-3 (CyHV-3) release in Australia with an associated mass common carp mortality may have serious effects on water quality in lakes. To evaluate the impact of a virus induced fish kill, different biomasses of dead common were placed into 2000 L mesocosms within Prospect Reservoir, Australia for up to 40 days. Decaying carp created anoxic conditions within all treatments except the 250 kg ha^{-1} which dropped to 30% oxygen saturation. Higher biomass of carp led to longer periods of anoxia. Total nitrogen increased from 0.25 to 1.5-30 mg L^{-1} while total phosphorus rose from 0.01 to 0.05-5.0 mg L^{-1} in treatments. Chlorophyll a level increased from <5 µg L^{-1} to between 100-1000 µg L^{-1} in treatments. Mean nutrient levels (TN and TP), chlorophyll-a and phytoplankton biovolume all showed a significant ($P<0.05$) linear relationship with carp biomass. There were increases in signature bacterial copiotrophs, fish gut bacteria and pathogenic genera of bacteria were detected during carcass decomposition. Taste and odour compounds geosmin and MIB correlated with the cyanobacteria *Pseudanaebena* and *Merismopedia*.

The fate of carcasses in the water column was heavily influenced by the depth to which they sank. Any carcasses that sank in the littoral zone would either float to the surface or be fed on by scavengers. Beyond the littoral zone and into deeper water the likelihood of scavenger interactions decreased and carcasses were less likely to float to the surface. Lake size and

depth were compared to biomass to determine if they influenced biomass of fusiform fish. and shallow lakes had a higher biomass of common carp compared to larger deeper lakes. This is important in determining which lakes will be at a higher risk of water quality issues if there is a fish kill.

The concepts and knowledge developed in this thesis contribute to increased understanding of the possible impacts from a fish kill in lakes and reservoirs. This includes the ecological responses to subsequent nutrient pulses and low oxygen concentrations, influencing water quality and biotic processes. These results establish the need to quantify risk in lakes prior to CyHV-3 release to better prepare for support and cleanup programs. It also contributes to a better understanding of the impacts of fish kills from other causes such as black water events and extreme environmental conditions.

References

ABELL, J. M., ÖZKUNDAKCI, D. & HAMILTON, D. P. 2010. Nitrogen and Phosphorus Limitation of Phytoplankton Growth in New Zealand Lakes: Implications for Eutrophication Control. *Ecosystems,* 13**,** 966-977.

ABSALON, D., MATYSIK, M., WOŹNICA, A. & JANCZEWSKA, N. 2023. Detection of changes in the hydrobiological parameters of the Oder River during the ecological disaster in July 2022 based on multi-parameter probe tests and remote sensing methods. *Ecological Indicators,* 148**,** 110103.

ACT ENVIRONMENT, P. A. S. D. D. 2018. *Carp-free local pond to be re-stocked with native fish* [Online]. Available: https://www.cmtedd.act.gov.au/open_government/inform/act_government_media_releases/esdd/2018/carp-free-local-pond-to-be-re-stocked-with-native-fish [Accessed].

ADAMEK, M., SYAKURI, H., HARRIS, S., RAKUS, K., BROGDEN, G., MATRAS, M., IRNAZAROW, I. & STEINHAGEN, D. 2013. Cyprinid herpesvirus 3 infection disrupts the skin barrier of common carp (Cyprinus carpio L.). *Vet Microbiol,* 162**,** 456-470.

ADÁMEK, Z. & MARŠÁLEK, B. 2013. Bioturbation of sediments by benthic macroinvertebrates and fish and its implication for pond ecosystems: a review. *Aquaculture International,* 21**,** 1-17.

ADWG 2011. *Australian Drinking Water Guidelines 6 : National Water Quality Management Strategy / National Health and Medical Research Council, National Resource Management Ministerial Council,* Canberra, ACT, National Health and Medical Research Council.

AGUILAR, P., DORADOR, C., VILA, I. & SOMMARUGA, R. 2018. Bacterioplankton composition in tropical high-elevation lakes of the Andean plateau. *FEMS Microbiol Ecol,* 94.

ALLEN, G. R. 2003. *Field guide to the freshwater fishes of Australia / G.R. Allen, S.H. Midgley, M. Allen,* Perth, W.A, Western Australian Museum.

ALLISON, C. & MACFARLANE, G. T. 1989. Influence of pH, nutrient availability, and growth rate on amine production by Bacteroides fragilis and Clostridium perfringens. 55**,** 2894-2898.

ANDERSEN, M. R., KRAGH, T. & SAND-JENSEN, K. 2017. Extreme diel dissolved oxygen and carbon cycles in shallow vegetated lakes. 284**,** 20171427.

ANDERSON, M. J. 2001. A new method for non-parametric multivariate analysis of variance. 26**,** 32-46.

ANGELER, D. G., ALLEN, C. R., BIRGÉ, H. E., DRAKARE, S., MCKIE, B. G. & JOHNSON, R. K. 2014. Assessing and managing freshwater ecosystems vulnerable to environmental change. *AMBIO,* 43**,** 113-125.

ANSARI, A. & GILL, S. 2014. *Eutrophication: Causes, Consequences and Control Volume-2*.

ARALAR, M. L., E. SANTIAGO, A., C. GONZAL, A., B. SANTIAGO, C., EGUIA, M. R., F. BALDIA, S. & PALISOC JR, F. Incidence and causes of mass fish kill in a shallow tropical eutrophic lake (Laguna de Bay, Philippines). 9th International Conference on the Conservation and Management of Lakes, 2001 Japan. Shiga Prefectural Government, pp. 233–236.

ARKHIPKIN, A. I. 2013. Squid as nutrient vectors linking Southwest Atlantic marine ecosystems. *Deep Sea Research Part II: Topical Studies in Oceanography,* 95**,** 7-20.

ARORA-WILLIAMS, K., OLESEN, S. W., SCANDELLA, B. P., DELWICHE, K., SPENCER, S. J., MYERS, E. M., ABRAHAM, S., SOOKLAL, A. & PREHEIM, S. P. 2018. Dynamics of microbial populations mediating biogeochemical cycling in a freshwater lake. *Microbiome,* 6**,** 165.

ASSEMANY, P. P., CALIJURI, M. L., COUTO, E. D. A. D., DE SOUZA, M. H. B., SILVA, N. C., SANTIAGO, A. D. F. & CASTRO, J. D. S. 2015. Algae/bacteria consortium in high rate ponds: Influence of solar radiation on the phytoplankton community. *Ecological Engineering,* 77**,** 154-162.

AUSTIN, E. K., RICH, J. L., KIEM, A. S., HANDLEY, T., PERKINS, D. & KELLY, B. J. 2020. Concerns about climate change among rural residents in Australia. *Journal of Rural Studies,* 75**,** 98-109.

AYERS, E. L. 2010. *Differential Decomposition in Terrestrial, Freshwater, and Saltwater Environments: A Pilot Study.* M.A, Texas State University--San Marcos.
BAJER, P. & SORENSEN, P. 2013. Using Boat Electrofishing to Estimate the Abundance of Invasive Common Carp in Small Midwestern Lakes. *North American Journal of Fisheries Management,* 32**,** 817-822.
BAJER, P. G., CHIZINSKI, C. J. & SORENSEN, P. W. 2011. Using the Judas technique to locate and remove wintertime aggregations of invasive common carp. 18**,** 497-505.
BAJER, P. G. & SORENSEN, P. W. 2012. Using Boat Electrofishing to Estimate the Abundance of Invasive Common Carp in Small Midwestern Lakes. *North American Journal of Fisheries Management,* 32**,** 817-822.
BAJER, P. G. & SORENSEN, P. W. 2015. Effects of common carp on phosphorus concentrations, water clarity, and vegetation density: a whole system experiment in a thermally stratified lake. *Hydrobiologia,* 746**,** 303-311.
BAKER-AUSTIN, C. & OLIVER, J. D. 2018. Vibrio vulnificus: new insights into a deadly opportunistic pathogen. 20**,** 423-430.
BALDRY, I. 2000. Effect of common carp (Cyprinus carpio) on aquatic restorations.
BARAN, R., BLABOLIL, P., ČECH, M., DRAŠTÍK, V., FROUZOVÁ, J., HOLUBOVÁ, M., JŮZA, T., KOLIADA, I., MUŠKA, M., PETERKA, J., PRCHALOVÁ, M., ŘÍHA, M., SAJDLOVÁ, Z., ŠMEJKAL, M., TUŠER, M., VEJŘÍK, L. & KUBEČKA, J. 2021. New way to investigate fish density and distribution in the shallowest layers of the open water. *Fisheries Research,* 238**,** 105907.
BARRETT, J. & ANSELL, D. The practicality and feasibility of establishing a system of freshwater protected areas in the Murray-Darling Basin. Proceedings of the World Congress on Aquatic Habitat Congress, 2003. 601-613.
BARTON, P. S., EVANS, M. J., FOSTER, C. N., PECHAL, J. L., BUMP, J. K., QUAGGIOTTO, M. M. & BENBOW, M. E. 2019. Towards Quantifying Carrion Biomass in Ecosystems. *Trends Ecol Evol,* 34**,** 950-961.
BARWICK, M. 2016 2016. *RE: Personel communcations with National Carp Control Plan (NCCP) Coordinator 2016.*
BAUMANN, K. B. L., THOMA, R., CALLBECK, C. M., NIEDERDORFER, R., SCHUBERT, C. J., MÜLLER, B., LEVER, M. A. & BÜRGMANN, H. 2022. Microbial Nitrogen Transformation Potential in Sediments of Two Contrasting Lakes Is Spatially Structured but Seasonally Stable. *mSphere,* 7**,** e0101321.
BAUMGARTNER, L. J., REYNOLDSON, N., CAMERON, L. & STANGER, J. 2006. Assessment of a Dual-frequency Identification Sonar (DIDSON) for application in fish migration studies. 84**,** 1-33.
BEAMISH, F. W. H. 1964. RESPIRATION OF FISHES WITH SPECIAL EMPHASIS ON STANDARD OXYGEN CONSUMPTION: III. INFLUENCE OF OXYGEN. 42**,** 355-366.
BEASLEY, J. C., OLSON, Z. H. & DEVAULT, T. L. 2012. Carrion cycling in food webs: comparisons among terrestrial and marine ecosystems. 121**,** 1021-1026.
BEASLEY, M. 1988. *The sweat of their brows : 100 years of the Sydney Water Board, 1888-1988 / Margo Beasley,* Sydney, The Board.
BEASLEY, M. 1998. *The sweat of their brows. 100 years of the Sydney Water Board*, Water Board, Sydney, Illawarra, Blue Mountains.
BEAVIS, S., WONG, V., MOSLEY, L., BALDWIN, D., LATIMER, J., LANE, P. & LAL, A. 2023. Water quality risks in the Murray-Darling basin. *Australian Journal of Water Resources,* 27**,** 85-102.
BELLINGER, E. G. & SIGEE, D. C. 2015. *Freshwater algae: identification, enumeration and use as bioindicators*, John Wiley & Sons.
BENBOW, M. E., RECEVEUR, J. P. & LAMBERTI, G. A. 2020. Death and Decomposition in Aquatic Ecosystems. 8.
BENNER, R. A., JR., STARUSZKIEWICZ, W. F. & OTWELL, W. S. 2004. Putrescine, cadaverine, and indole production by bacteria isolated from wild and aquacultured penaeid shrimp stored at 0, 12, 24, and 36 degrees C. *J Food Prot,* 67**,** 124-33.

BERTHOUD, H., WECHSLER, D. & IRMLER, S. 2022. Production of Putrescine and Cadaverine by Paucilactobacillus wasatchensis. 13.
BHATERIA, R. & JAIN, D. 2016. Water quality assessment of lake water: a review. *Sustainable Water Resources Management,* 2**,** 161-173.
BICON 2023. Australian Government's Biosecurity import conditions. *Australian Government Department of Agriculture, Fisheries and Forestry*.
BILBY, R. E., BEACH, E. W., FRANSEN, B. R., WALTER, J. K. & BISSON, P. A. 2003. Transfer of Nutrients from Spawning Salmon to Riparian Vegetation in Western Washington. *Transactions of the American Fisheries Society,* 132**,** 733-745.
BILLARD, R. 1999. *The carp: biology and culture*, Springer Science & Business Media.
BIŽIĆ-IONESCU, M., IONESCU, D. & GROSSART, H.-P. 2018. Organic Particles: Heterogeneous Hubs for Microbial Interactions in Aquatic Ecosystems. 9.
BLAKE, R. W. 2004. Fish functional design and swimming performance. 65**,** 1193-1222.
BOROS, G., CZEGLÉDI, I., ERŐS, T. & PREISZNER, B. 2020. Scavenger-driven fish carcass decomposition and phosphorus recycling: Laboratory experiments with freshwater fish and crayfish. 65**,** 1740-1751.
BOROS, G., TAKÁCS, P. & VANNI, M. J. 2015. The fate of phosphorus in decomposing fish carcasses: a mesocosm experiment. 60**,** 479-489.
BOROS, G., TÁTRAI, I., GYÖRGY, Á. I., VÁRI, Á. & NAGY, A. S. 2009. Changes in internal phosphorus loading and fish population as possible causes of water quality decline in a shallow, biomanipulated lake. *International review of hydrobiology,* 94**,** 326-337.
BOSWELL, K. M., WILSON, M. P. & WILSON, C. A. 2007. Hydroacoustics as a tool for assessing fish biomass and size distribution associated with discrete shallow water estuarine habitats in Louisiana. *Estuaries and Coasts,* 30**,** 607-617.
BOYD, C. E. 2008. Better Management Practices in International Aquaculture. *Environmental Best Management Practices for Aquaculture.*
BOYS, C., ROURKE, M., ROBINSON, W., GILLIGAN, D. & THIEBAUD, I. 2011. Status of freshwater catfish populations and their habitat within the Cockburn River.
BOYS, C. A., ROBINSON, W., BAUMGARTNER, L. J., RAMPANO, B. & LOWRY, M. 2013. Influence of Approach Velocity and Mesh Size on the Entrainment and Contact of a Lowland River Fish Assemblage at a Screened Irrigation Pump. *PLOS ONE,* 8**,** e67026.
BOZORG-HADDAD, O., DELPASAND, M. & LOÁICIGA, H. A. 2021. 10 - Water quality, hygiene, and health. *In:* BOZORG-HADDAD, O. (ed.) *Economical, Political, and Social Issues in Water Resources.* Elsevier.
BRIDDON, C. L., METCALFE, S., TAYLOR, D., BANNISTER, W., CUNANAN, M., SANTOS-BORJA, A. C., PAPA, R. D. & MCGOWAN, S. 2023. Changing water quality and thermocline depth along an aquaculture gradient in six tropical crater lakes. *Hydrobiologia,* 850**,** 283-299.
BROOKER, M. P., MORRIS, D. L. & HEMSWORTH, R. J. 1977. Mass Mortalities of Adult Salmon, Salmo salar, in the R. Wye, 1976. *Journal of Applied Ecology,* 14**,** 409-417.
BROWN, J. H. 1981. Two decades of homage to Santa Rosalia: toward a general theory of diversity. *American Zoologist,* 21**,** 877-888.
BUSH, T., BUTLER, I. B., FREE, A. & ALLEN, R. J. 2015. Redox regime shifts in microbially mediated biogeochemical cycles. *Biogeosciences,* 12**,** 3713-3724.
CABELLI, V. J., DUFOUR, A. P., MCCABE, L. J. & LEVNI, M. A. 1982. SWIMMING-ASSOCIATED GASTROENTERITIS AND WATER QUALITY. *American Journal of Epidemiology,* 115**,** 606-616.
CAPO, E., DEBROAS, D., ARNAUD, F., PERGA, M.-E., CHARDON, C. & DOMAIZON, I. 2017. Tracking a century of changes in microbial eukaryotic diversity in lakes driven by nutrient enrichment and climate warming. 19**,** 2873-2892.
CAPORASO, J. G., KUCZYNSKI, J., STOMBAUGH, J., BITTINGER, K., BUSHMAN, F. D., COSTELLO, E. K., FIERER, N., PEÑA, A. G., GOODRICH, J. K., GORDON, J. I., HUTTLEY, G. A., KELLEY, S. T., KNIGHTS, D., KOENIG, J. E., LEY, R. E., LOZUPONE, C. A., MCDONALD, D., MUEGGE, B. D.,

PIRRUNG, M., REEDER, J., SEVINSKY, J. R., TURNBAUGH, P. J., WALTERS, W. A., WIDMANN, J., YATSUNENKO, T., ZANEVELD, J. & KNIGHT, R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods,* 7**,** 335-6.
CARBIS, C., RAWLIN, G., GRANT, P., MITCHELL, G., ANDERSON, J. & MCCAULEY, I. 1997. A study of feral carp, Cyprinus carpio L., exposed to Microcystis aeruginosa at Lake Mokoan, Australia, and possible implications for fish health. *Journal of Fish Diseases,* 20**,** 81-91.
CAREY, C. C., IBELINGS, B. W., HOFFMANN, E. P., HAMILTON, D. P. & BROOKES, J. D. 2012. Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. *Water Research,* 46**,** 1394-1407.
CARNEY, R. L., MITROVIC, S. M., JEFFRIES, T., WESTHORPE, D., CURLEVSKI, N. & SEYMOUR, J. R. 2015. River bacterioplankton community responses to a high inflow event. *Aquatic Microbial Ecology,* 75**,** 187-205.
CARNEY, R. L., SEYMOUR, J. R., WESTHORPE, D. & MITROVIC, S. M. 2016. Lotic bacterioplankton and phytoplankton community changes under dissolved organic-carbon amendment: evidence for competition for nutrients %J Marine and Freshwater Research. 67**,** 1362-1373.
CARPENTER, S. R., KRAFT, C. E., WRIGHT, R., HE, X., SORANNO, P. A. & HODGSON, J. R. 1992. Resilience and resistance of a lake phosphorus cycle before and after food web manipulation. *The American Naturalist,* 140**,** 781-798.
ÇELIK, K. & ONGUN, T. 2007. The Relationships Between Certain Physical and Chemical Variables and the Seasonal Dynamics of Phytoplankton Assemblages of Two Inlets of a Shallow Hypertrophic Lake with Different Nutrient Inputs. *Environmental Monitoring Assessment* 124**,** 321-330.
CHANDRASENA, N., ECCLES, D., PERA, J. & HAWKINS, P. 1997. Botany Wetlands-Removal of European carp from ponds 5/6 (1996/97). Sydney water.
CHAO, A. 1987. Estimating the population size for capture-recapture data with unequal catchability. *Biometrics,* 43**,** 783-91.
CHIDAMI, S. & AMYOT, M. 2008. Fish decomposition in boreal lakes and biogeochemical implications. 53**,** 1988-1996.
CHISLOCK, M. F., DOSTER, E., ZITOMER, R. A. & WILSON, A. E. 2013. *Eutrophication: Causes, consequences, and controls in aquatic ecosystems* [Online]. Nature Education Knowledge [Accessed 4].
CHUMCHAL, M. M., NOWLIN, W. H. & DRENNER, R. W. 2005. Biomass-dependent effects of common carp on water quality in shallow ponds. *Hydrobiologia,* 545**,** 271-277.
CIFONI, M., BOGGERO, A., GALASSI, D. M. P. & DI LORENZO, T. 2021. An Overview of Studies on Meiofaunal Traits of the Littoral Zone of Lakes. 13**,** 473.
CLARKE, B., OTTO, F., STUART-SMITH, R. & HARRINGTON, L. 2022. Extreme weather impacts of climate change: an attribution perspective. *Environmental Research: Climate,* 1**,** 012001.
CLARKE, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. 18**,** 117-143.
CLUNIE, P. & KOEHN, J. 2001. *Freshwater catfish: A Resource Document*.
COLLOFF, M. J. & PITTOCK, J. 2022. Mind the Gap! Reconciling Environmental Water Requirements with Scarcity in the Murray–Darling Basin, Australia. 14**,** 208.
COLVILLE, A. & ROHLFS, A.-M. 2019. Investigation inpossible causes for failure of Microcyctis to grow in Prospect reservoir. WaterNSW.
CONLEY, D. J., CARSTENSEN, J., VAQUER-SUNYER, R. & DUARTE, C. M. 2009. Ecosystem thresholds with hypoxia. *Hydrobiologia,* 629**,** 21-29.
COULL, B. C. 1999. Role of meiofauna in estuarine soft-bottom habitats*. 24**,** 327-343.
CRANE, M. S. & EATON, B. T. 1996. Spring viraemia of carp virus (Rhabdovirus carpio): a biological control agent. *Controlling Carp,* 22**,** 87.
CRC, I. 2012. Carp herpesvirus as a biological control for carp in Australia *PestSmart Connect*.

CRICHIGNO, S., CORDERO, P., BLASETTI, G. & CUSSAC, V. 2016. Dispersion of the invasive common carp Cyprinus carpio in southern South America: changes and expectations, westward and southward. 89**,** 403-416.
CROOKS, J. A. 2002. Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. 97**,** 153-166.
CUCHEROUSSET, J. & OLDEN, J. D. 2011. Ecological Impacts of Nonnative Freshwater Fishes. 36**,** 215-230.
DAF 2022. Restricted invasive fish carp. *The State of Queensland, Department of Agriculture and Fisheries*.
DAVIE, A. & PERA, J. 2021. The Fish Health Risk Indicator: linking water quality and river flow data with fish health to improve our predictive capacity around fish death events. *Marine and Freshwater Research,* 73.
DAVIS, J. C. & SAMPSON, R. J. 1986. *Statistics and data analysis in geology*, Wiley New York.
DE LEEUW, J. J., NAGELKERKE, L. A. J., VAN DENSEN, W. L. T., HOLMGREN, K., JANSEN, P. A. & VIJVERBERG, J. 2003. Biomass size distributions as a tool for characterizing lake fish communities. 63**,** 1454-1475.
DEAN, T. L. & RICHARDSON, J. 1999. Responses of seven species of native freshwater fish and a shrimp to low levels of dissolved oxygen. *New Zealand Journal of Marine and Freshwater Research,* 33**,** 99-106.
DEEDS, J., AMIRBAHMAN, A., NORTON, S. A., SUITOR, D. G. & BACON, L. C. 2021. Predicting anoxia in low-nutrient temperate lakes. 31**,** e02361.
DEPARTMENT OF ENVIRONMENT, L. W. & PLANNING 2017. *Impacts of carp in wetlands : fact sheet 4,* [Melbourne, Victoria], Department of Environment, Land, Water and Planning.
DEVICTOR, V., CLAVEL, J., JULLIARD, R., LAVERGNE, S., MOUILLOT, D., THUILLER, W., VENAIL, P., VILLÉGER, S. & MOUQUET, N. 2010. Defining and measuring ecological specialization. *Journal of Applied Ecology,* 47**,** 15-25.
DJEMALI, I., TOUJANI, R. & GUILLARD, J. 2009. Hydroacoustic fish biomass assessment in man-made lakes in Tunisia: horizontal beaming importance and diel effect. *Aquatic Ecology,* 43**,** 1121-1131.
DOKULIL, M., CHEN, W. & CAI, Q. 2000. Anthropogenic impacts to large lakes in China: the Tai Hu example. *Aquatic Ecosystem Health & Management,* 3**,** 81-94.
DOYLE, K. 2019. *State of the drought shows dams empty and NSW drowning in dust* [Online]. Australian Broadcasting Corporation. Available: https://www.abc.net.au/news/2019-03-12/state-of-the-drought-is-not-good/10876716 [Accessed 25/06/2023].
DPI, N. 2010. *NSW control plan for the noxious fish carp (Cyprinus carpio) / [Aquatic Biosecurity & Risk Management Unit], NSW Government, Industry and Investment,* Orange, N.S.W, Industry & Investment NSW.
DPI, N. 2014. Freshwater pest fish in New South Wales.
DPI, N. 2021. *Hydroacoustic surveys* [Online]. Department of Primary Industries Fisheries. Available: https://recfishingresearch.visualstories.com/hydroacoustic-survey-of-nsw-impoundments [Accessed 20/06/2023].
DREVIKOVSKY, J. 2020. 'It should be scary': Regional NSW dam close to empty. *The Sydney Morning Hearld*
DRIVER, P., HARRIS, J., CLOSS, G. & KOEN, T. 2005. Effects of flow regulation on carp (Cyprinus carpio L.) recruitment in the Murray–Darling Basin, Australia. *River Research*

Applications

21**,** 327-335.
DURRANT-WHYTE, H. 2023. Independent review into the 2023 fish deaths in the Darling-Baaka River at Menindee. *Office of the NSW Chief Scientist & Engineer*.

DUTTON, C. L., SUBALUSKY, A. L., HAMILTON, S. K., ROSI, E. J. & POST, D. M. 2018. Organic matter loading by hippopotami causes subsidy overload resulting in downstream hypoxia and fish kills. *Nature Communications,* 9**,** 1951.

EDBERG, S. C., ALLEN, M. J., SMITH, D. B. & KRIZ, N. J. 1990. Enumeration of total coliforms and Escherichia coli from source water by the defined substrate technology. *Appl Environ Microbiol,* 56**,** 366-9.

EDGAR, R. C., HAAS, B. J., CLEMENTE, J. C., QUINCE, C. & KNIGHT, R. 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics,* 27**,** 2194-2200.

EDWARDS, A. N., SUÁREZ, J. M. & MCBRIDE, S. M. 2013. Culturing and maintaining Clostridium difficile in an anaerobic environment. *J Vis Exp***,** e50787.

EDWARDS, K. F. 2019. Mixotrophy in nanoflagellates across environmental gradients in the ocean. 116**,** 6211-6220.

EILER, A. & BERTILSSON, S. 2007. Flavobacteria blooms in four eutrophic lakes: linking population dynamics of freshwater bacterioplankton to resource availability. *Appl Environ Microbiol,* 73**,** 3511-8.

ELDER, R. L. & SMITH, G. R. 1988. Fish taphonomy and environmental inference in paleolimnology. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 62**,** 577-592.

ELKINS, A., BARROW, R. & ROCHFORT, S. J. E. C. 2009. Carp chemical sensing and the potential of natural environmental attractants for control of carp: a review. 6**,** 357-368.

ELLIS, I., BATES, W., MARTIN, S., MCCRABB, G., KOEHN, J., HEATH, P. & HARDMAN, D. 2022. How fish kills affected traditional (Baakandji) and non-traditional communities on the Lower Darling–Baaka River %J Marine and Freshwater Research. 73**,** 259-268.

ELSER, J. J., ANDERSEN, T., BARON, J. S., BERGSTROM, A. K., JANSSON, M., KYLE, M., NYDICK, K. R., STEGER, L. & HESSEN, D. O. 2009. Shifts in lake N:P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. *Science,* 326**,** 835-7.

ENVIRONMENT, N. D. O. P. A. 2022. *Drought stages and measures implemented during the 2017-20 drought* [Online]. NSW Department of Planning and Environment. Available: https://www.industry.nsw.gov.au/water/allocations-availability/droughts-floods/drought-update/critical-valleys-in-drought [Accessed].

EPA 2012. Recreational Water Quality Criteria *Health and Ecological Criteria Division, Office of Science and Technology, United States (U.S.) Environmental Protection Agency (EPA)*.

ESPELUND, M. & KLAVENESS, D. 2014. Botulism outbreaks in natural environments – an update. 5.

FABRIS, R. 2017. NCCP Carp Treatabilty. Water RA.

FALCONER, I. R. 1999. An Overview of problems caused by toxic blue–green algae (cyanobacteria) in drinking and recreational water. 14**,** 5-12.

FAO, F. 2012. The state of world fisheries and aquaculture. *Opportunities challenges. Food Agriculture Organization of the United Nations*.

FEDERIGI, I., BONADONNA, L., BONANNO FERRARO, G., BRIANCESCO, R., CIONI, L., COCCIA, A. M., DELLA LIBERA, S., FERRETTI, E., GRAMACCIONI, L., IACONELLI, M., LA ROSA, G., LUCENTINI, L., MANCINI, P., SUFFREDINI, E., VICENZA, T., VENERI, C., VERANI, M. & CARDUCCI, A. 2020. Quantitative Microbial Risk Assessment as support for bathing waters profiling. *Marine Pollution Bulletin,* 157**,** 111318.

FELISBERTO, S. A., LEANDRINI, J. A. & RODRIGUES, L. 2011. Effects of nutrients enrichment on algal communities: an experimental in mesocosms approach *Acta Limnologica Brasiliensia,* 23**,** 128-137.

FICKE, A. D., MYRICK, C. A. & HANSEN, L. J. 2007. Potential impacts of global climate change on freshwater fisheries. *Reviews in Fish Biology and Fisheries,* 17**,** 581-613.

FREE, G., VAN DE BUND, W., GAWLIK, B., VAN WIJK, L., WOOD, M., GUAGNINI, E., KOUTELOS, K., ANNUNZIATO, A., GRIZZETTI, B. & VIGIAK, O. 2023. An EU analysis of the ecological disaster in the Oder River of 2022.

FREHSE, R. & SYLLA, Z. 2023. 3 people have died after infection with rare flesh-eating bacteria in Connecticut and New York. Available: https://edition.cnn.com/2023/08/16/us/flesh-eating-bacteria-deaths-connecticut-new-york/index.html.
FRIEDLAND, K. D., AHRENHOLZ, D. W. & HAAS, L. W. 2005. Viable gut passage of cyanobacteria through the filter-feeding fish Atlantic menhaden, Brevoortia tyrannus. *Journal of Plankton Research,* 27**,** 715-718.
GÄB, F., BALLHAUS, C., STINNESBECK, E., KRAL, A. G., JANSSEN, K. & BIERBAUM, G. 2020. Experimental taphonomy of fish - role of elevated pressure, salinity and pH. *Scientific Reports,* 10**,** 7839.
GALIC, N., HAWKINS, T. & FORBES, V. E. 2019. Adverse impacts of hypoxia on aquatic invertebrates: A meta-analysis. *Science of The Total Environment,* 652**,** 736-743.
GALLOWAY, D. & COHEN, M. B. 2021. 38 - Infectious Diarrhea. *In:* WYLLIE, R., HYAMS, J. S. & KAY, M. (eds.) *Pediatric Gastrointestinal and Liver Disease (Sixth Edition).* Philadelphia: Elsevier.
GAVEL, A., MARŠÁLEK, B. & ADÁMEK, Z. 2004. Viability of Microcystis colonies is not damaged by silver carp (Hypophthalmichthys molitrix) digestion. *Algological studies,* 113**,** 189-194.
GILLIGAN, D., JESS, L., MCLEAN, G., ASMUS, M., WOODEN, I., HARTWELL, D., MCGREGOR, C., STUART, I., VEY, A., JEFFERIES, M., LEWIS, B. & BELL, K. 2010. *Identifying and implementing targeted carp control options for the Lower Lachlan Catchment*.
GILMORE, K. L., DOUBLEDAY, Z. A. & GILLANDERS, B. M. 2019. Prolonged exposure to low oxygen improves hypoxia tolerance in a freshwater fish. *Conserv Physiol,* 7**,** coz058.
GIRARD, M., GOULON, C., TESSIER, A., VONLANTHEN, P. & GUILLARD, J. J. A. L. R. 2020. Comparisons of day-time and night-time hydroacoustic surveys in temperate lakes. 33**,** 9.
GRAY, E., MACKAY, E. B., ELLIOTT, J. A., FOLKARD, A. M. & JONES, I. D. 2020. Wide-spread inconsistency in estimation of lake mixed depth impacts interpretation of limnological processes. *Water Research,* 168**,** 115136.
GREENHALGH, M. 1999. *Freshwater Fish*, Stackpole Books.
GRIFFITH, M., HARPER, B., MARSHAL, N., HOOPER, C. & BESLEY, C. 2014. Aquatic Environmental Assessment Program St Marys water Recycling Project. *In:* HARPER, B. (ed.). Sydney water.
GROSSART, H.-P., SIMON, M. & LOGAN, B. E. 1997. Formation of macroscopic organic aggregates (lake snow) in a large lake: The significance of transparent exopolymer particles, plankton, and zooplankton. 42**,** 1651-1659.
GUILLARD, J. & VERGÈS, C. 2007. The Repeatability of Fish Biomass and Size Distribution Estimates Obtained by Hydroacoustic Surveys Using Various Sampling Strategies and Statistical Analyses. 92**,** 605-617.
GUO, D., LIANG, J., CHEN, W., WANG, J., JI, B. & LUO, S. 2021. Bacterial Community Analysis of Two Neighboring Freshwater Lakes Originating from One Lake. 30**,** 111-117.
GUO, Y., WU, C. & SUN, J. 2022. Pathogenic bacteria significantly increased under oxygen depletion in coastal waters: A continuous observation in the central Bohai Sea. *Front Microbiol,* 13**,** 1035904.
HAAS, B. J., GEVERS, D., EARL, A. M., FELDGARDEN, M., WARD, D. V., GIANNOUKOS, G., CIULLA, D., TABBAA, D., HIGHLANDER, S. K., SODERGREN, E., METHÉ, B., DESANTIS, T. Z., PETROSINO, J. F., KNIGHT, R. & BIRREN, B. W. 2011. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Res,* 21**,** 494-504.
HAMMER, Ø., HARPER, D. A. & RYAN, P. D. 2001. PAST: paleontological statistics software package for education and data analysis. *Palaeontologia electronica 4(1), 9.*
HANSON, J. M. & LEGGETT, W. C. 1982. Empirical Prediction of Fish Biomass and Yield. 39**,** 257-263.
HARRIS, J. H. & GEHRKE, P. C. 1997. Fish and rivers in stress. *FISHERIES, N. S. W., COOPERATIVE RESEARCH CENTRE FOR FRESHWATER, E. & NEW SOUTH WALES. RESOURCE AND CONSERVATION ASSESSMENT, C. ,* : the NSW rivers survey Cronulla, NSW, NSW Fisheries Office of Conservation and the Cooperative Research Centre for Freshwater Ecology, in association with NSW Resource and Conservation Assessment Council.

HART, B., WALKER, G., KATUPITIYA, A. & DOOLAN, J. 2020. Salinity Management in the Murray–Darling Basin, Australia. 12**,** 1829.
HART, B. T., BAILEY, P., EDWARDS, R., HORTLE, K., JAMES, K., MCMAHON, A., MEREDITH, C. & SWADLING, K. 1991. A review of the salt sensitivity of the Australian freshwater biota. *Hydrobiologia,* 210**,** 105-144.
HAVENS, K. E., JAMES, R. T., EAST, T. L. & SMITH, V. H. 2003. N:P ratios, light limitation, and cyanobacterial dominance in a subtropical lake impacted by non-point source nutrient pollution. *Environmental Pollution,* 122**,** 379-390.
HAWKING, J. H. 1997. *Colour guide to invertebrates of Australian inland waters / John H. Hawking and Felicity J. Smith,* Albury, N.S.W, Co-operative Research Centre for Freshwater Ecology.
HEDRICK, R. P., WALTZEK, T. B. & MCDOWELL, T. S. 2006. Susceptibility of Koi Carp, Common Carp, Goldfish, and Goldfish × Common Carp Hybrids to Cyprinid Herpesvirus-2 and Herpesvirus-3. *Journal of Aquatic Animal Health,* 18**,** 26-34.
HEISLER, J., GLIBERT, P., BURKHOLDER, J., ANDERSON, D., COCHLAN, W., DENNISON, W., GOBLER, C., DORTCH, Q., HEIL, C., HUMPHRIES, E., LEWITUS, A., MAGNIEN, R., MARSHALL, H., SELLNER, K., STOCKWELL, D., STOECKER, D. & SUDDLESON, M. 2008. Eutrophication and Harmful Algal Blooms: A Scientific Consensus. *Harmful Algae,* 8**,** 3-13.
HENDRIXSON, H., STERNER, R. W. & KAY, A. 2007. Elemental stoichiometry of freshwater fishes in relation to phylogeny, allometry and ecology. *Journal of Fish Biology,* 70**,** 121-140.
HERTAM, S. 2010. *The effects of common carp (Cyprinus carpio L.) on water quality, algae and submerged vegetation in Delta Marsh, Manitoba.*
HEWSON, I., VARGO, G. A. & FUHRMAN, J. A. 2003. Bacterial Diversity in Shallow Oligotrophic Marine Benthos and Overlying Waters: Effects of Virus Infection, Containment, and Nutrient Enrichment. *Microbial Ecology,* 46**,** 322-336.
HICKEY, C. 2018. *Bushells Lagoon drying out is a natural occurrence say locals* [Online]. hawkesbury gazette. Available: https://www.hawkesburygazette.com.au/story/5227911/bushells-lagoon-drying-out-is-a-natural-occurrence-say-locals/ [Accessed].
HICKS, B., LING, N. & DANIEL, A. J. 2012. Cyprinus carpio L.(common carp). *A Handbook of Global Freshwater Invasive Species.* Routledge.
HICKS, B., W. OSBORNE, M. & LING, N. Quantitative estimates of fish abundance from boat electrofishing. Australian Society for Fish Biology annual conference, 2006 Darwin. pp. 11-15.
HIGGS, N. D., GATES, A. R. & JONES, D. O. B. 2014. Fish food in the deep sea: revisiting the role of large food-falls. *PloS one,* 9**,** e96016-e96016.
HILLEBRAND, H., ACEVEDO-TREJOS, E., MOORTHI, S. D., RYABOV, A., STRIEBEL, M., THOMAS, P. K. & SCHNEIDER, M.-L. 2022. Cell size as driver and sentinel of phytoplankton community structure and functioning. 36**,** 276-293.
HLORDZI, V., KUEBUTORNYE, F. K. A., AFRIYIE, G., ABARIKE, E. D., LU, Y., CHI, S. & ANOKYEWAA, M. A. 2020. The use of Bacillus species in maintenance of water quality in aquaculture: A review. *Aquaculture Reports,* 18**,** 100503.
HOWARD-WILLIAMS, C. & LENTON, G. M. 1975. The role of the littoral zone in the functioning of a shallow tropical lake ecosystem. 5**,** 445-459.
HUME, D. J., FLETCHER, A. R. & MORISON, A. K. 1983 Final Report. Carp Program Report No. 10. *Fisheries and Wildlife Division, Victoria***,** 213pp.
HUSER, B. J., BAJER, P. G., CHIZINSKI, C. J. & SORENSEN, P. W. 2016. Effects of common carp (Cyprinus carpio) on sediment mixing depth and mobile phosphorus mass in the active sediment layer of a shallow lake. *Hydrobiologia,* 763**,** 23-33.
IASPUB.EPA.GOV. 2018. *Water Treatability Database* [Online]. US EPA: US EPA. Available: https://iaspub.epa.gov/tdb/pages/treatment/treatmentOverview.do?treatmentProcessId=1934681921 [Accessed 23 Oct. 2018 2018].

ILWS-CSU 2018. Secure Safe Domestic Water for the Central and Lower Darling Region. *Report on the 2017-18 Institute for Land Water and Society, Charles Stuart University (ILWS-CSU) Community Engagement Program* Charles Stuart University.

ISAZA, D. F. G., CRAMP, R. L. & FRANKLIN, C. E. 2021. Exposure to Nitrate Increases Susceptibility to Hypoxia in Fish. 94**,** 124-142.

JAHAN, P., WATANABE, T., KIRON, V. & SATOH, S. 2003. Balancing protein ingredients in carp feeds to limit discharge of phosphorus and nitrogen into water bodies. *Fisheries science,* 69**,** 226-233.

JELLYMAN, D. J. 1989. Diet of two species of freshwater eel (Anguilla spp.) in Lake Pounui, New Zealand. *New Zealand Journal of Marine and Freshwater Research,* 23**,** 1-10.

JELLYMAN, D. J. 2022. An enigma: how can freshwater eels (Anguilla spp.) be such a successful genus yet be universally threatened? *Reviews in Fish Biology and Fisheries,* 32**,** 701-718.

JENNY, J.-P., FRANCUS, P., NORMANDEAU, A., LAPOINTE, F., PERGA, M.-E., OJALA, A., SCHIMMELMANN, A. & ZOLITSCHKA, B. 2016. Global spread of hypoxia in freshwater ecosystems during the last three centuries is caused by rising local human pressure. 22**,** 1481-1489.

JEONG HWAN, B., KIM, H. N., KANG, T. G., KIM, B.-H. & BYEON, M.-S. 2023. Study of the cause of the generation of odor compounds (geosmin and 2-methylisoborneol) in the Han River system, the drinking water source, Republic of Korea. *Water Supply,* 23**,** 1081-1093.

JEPPESEN, E., PEDER JENSEN, J., SØNDERGAARD, M., LAURIDSEN, T., JUNGE PEDERSEN, L. & JENSEN, L. 1997. Top-down control in freshwater lakes: the role of nutrient state, submerged macrophytes and water depth. *In:* KUFEL, L., PREJS, A. & RYBAK, J. I. (eds.) *Shallow Lakes '95: Trophic Cascades in Shallow Freshwater and Brackish Lakes.* Dordrecht: Springer Netherlands.

JOHNSTON, N. T., MACISAAC, E. A., TSCHAPLINSKI, P. J. & HALL, K. J. 2004. Effects of the abundance of spawning sockeye salmon (Oncorhynchus nerka) on nutrients and algal biomass in forested streams. *Canadian Journal of Fisheries and Aquatic Sciences,* 61**,** 384-403.

JOLLY, I. D., WILLIAMSON, D. R., GILFEDDER, M., WALKER, G. R., MORTON, R., ROBINSON, G., JONES, H., ZHANG, L., DOWLING, T. I., DYCE, P., NATHAN, R. J., NANDAKUMAR, N., CLARKE, R. & MCNEILL, V. 2001. Historical stream salinity trends and catchment salt balances in the Murray–Darling Basin, Australia %J Marine and Freshwater Research. 52**,** 53-63.

JONES, D. S. 2002. *A field guide to crustaceans of Australian waters / Diana S. Jones and Gary J. Morgan, Western Australian Museum,* Frenchs Forest, N.S.W ; [Perth], Reed New Holland :Western Australian Museum.

KARR, J. R. 1981. Assessment of Biotic Integrity Using Fish Communities. *Fisheries,* 6**,** 21-27.

KEANE, D. 2017. Flesh-eating bacteria strips skin off leg of River Murray boatie. Available: https://www.abc.net.au/news/2017-03-10/flesh-eating-bacteria-strips-leg-of-river-murray-boatie/8343008.

KENNETH, M. A. & ST, C. J. S. M. 2013. *Cyprinid herpesvirus 3, CyHV-3: its potential as a biological control agent for carp in Australia* [Online]. Canberra: Invasive Animals CRC. [Accessed].

KHAN, T. A. 2003. Dietary studies on exotic carp (Cyprinus carpio L.) from two lakes of western Victoria, Australia. *Aquatic Sciences,* 65**,** 272-286.

KILLBERG-THORESON, L., SIPLER, R. E., HEIL, C. A., GARRETT, M. J., ROBERTS, Q. N. & BRONK, D. A. 2014. Nutrients released from decaying fish support microbial growth in the eastern Gulf of Mexico. *Harmful Algae,* 38**,** 40-49.

KING, A., ROBERTSON, A. & HEALEY, M. 1997. Experimental manipulations of the biomass of introduced carp (Cyprinus carpio) in billabongs. I. Impacts on water-column properties. *Marine Freshwater Research,* 48**,** 435-443.

KINGSFORD, R. T. 2000. Ecological impacts of dams, water diversions and river management on floodplain wetlands in Australia. 25**,** 109-127.

KITCHELL, J. F., KOONCE, J. F. & TENNIS, P. S. 1975. Phosphorus flux through fishes. *SIL Proceedings, 1922-2010,* 19**,** 2478-2484.
KITCHELL, J. F., SCHINDLER, D. E., OGUTU-OHWAYO, R. & REINTHAL, P. N. 1997. THE NILE PERCH IN LAKE VICTORIA: INTERACTIONS BETWEEN PREDATION AND FISHERIES. 7**,** 653-664.
KOCH, A. L. 2001. Oligotrophs versus copiotrophs. 23**,** 657-661.
KOEHLER, B., VON WACHENFELDT, E., KOTHAWALA, D. & TRANVIK, L. J. 2012. Reactivity continuum of dissolved organic carbon decomposition in lake water. 117.
KOEHN, J., BRUMLEY, A. R. & GEHRKE, P. C. 2000. *Managing the impacts of carp*, Bureau of Rural Sciences Canberra.
KOEHN, J., RAYMOND, S., STUART, I., TODD, C., BALCOMBE, S., BAMFORD, H., INGRAM, B., BICE, C., BUNDRED, K., BUTLER, G., BAUMGARTNER, L., CLUNIE, P., ELLIS, I., FORBES, J., HUTCHISON, M., KOSTER, W., LINTERMANS, M., LYON, J., MALLEN-COOPER, M. & ZAMPATTI, B. 2020. A compendium of ecological knowledge for restoration of freshwater fishes in Australia's Murray–Darling Basin. *Marine and Freshwater Research,* 71.
KOEHN, J., TODD, C., THWAITES, L., STUART, I., ZAMPATTI, B., YE, Q., CONALLIN, A., DODD, L. & STAMATION, K. 2016. Managing flows and carp. *Arthur Rylah Institute for Environmental Research*

Technical Report, Series No. 255.
KOEHN, J., TODD, C., ZAMPATTI, B., STUART, I., CONALLIN, A., THWAITES, L. & YE, Q. 2018. Using a Population Model to Inform the Management of River Flows and Invasive Carp (Cyprinus carpio). *Environmental management,* 61.
KOEHN, J. D. 2004. Carp (Cyprinus carpio) as a powerful invader in Australian waterways. 49**,** 882-894.
KOLMAKOV, V. I. & GLADYSHEV, M. I. 2003. Growth and potential photosynthesis of cyanobacteria are stimulated by viable gut passage in crucian carp. *Aquatic Ecology,* 37**,** 237-242.
KOLZAU, S., WIEDNER, C., RÜCKER, J., KÖHLER, J., KÖHLER, A. & DOLMAN, A. M. 2014. Seasonal Patterns of Nitrogen and Phosphorus Limitation in Four German Lakes and the Predictability of Limitation Status from Ambient Nutrient Concentrations. *PLOS ONE,* 9**,** e96065.
KON, K. & RAI, M. 2017. Index. *In:* KON, K. & RAI, M. (eds.) *The Microbiology of Skin, Soft Tissue, Bone and Joint Infections.* Academic Press.
KRISTENSEN, E., AHMED, S. I. & DEVOL, A. H. 1995. Aerobic and anaerobic decomposition of organic matter in marine sediment: Which is fastest? 40**,** 1430-1437.
KUCZYNSKI, J., STOMBAUGH, J., WALTERS, W. A., GONZÁLEZ, A., CAPORASO, J. G. & KNIGHT, R. 2011. Using QIIME to analyze 16S rRNA gene sequences from microbial communities. *Curr Protoc Bioinformatics,* Chapter 10**,** 10.7.1-10.7.20.
KUSHLAN, J. A. 1974. Effects of a Natural Fish Kill on the Water Quality, Plankton, and Fish Population of a Pond in the Big Cypress Swamp, Florida. *Transactions of the American Fisheries Society,* 103**,** 235-243.
LAUTH, R. R., WAKEFIELD, W. W. & SMITH, K. 2004. Estimating the density of thornyheads, Sebastolobus spp., using a towed video camera sled. *Fisheries Research,* 70**,** 39-48.
LAWRENCE, I., BORMANS, M., OLIVER, R., RANSOM, G., SHERMAN, B. & WASSON, P. F. 2000. *Physical and nutrient factors controlling algal succession and biomass in Burrinjuck Reservoir : technical report* Canberra, Cooperative Research Centre for Freshwater Ecology.
LEBLANC, M., TWEED, S., VAN DIJK, A. & TIMBAL, B. 2012. A review of historic and future hydrological changes in the Murray-Darling Basin. *Global and Planetary Change,* 80-81**,** 226-246.
LEGENDRE, P. & LEGENDRE, L. 1998. Developments in environmental modelling 20. *Numerical ecology: second English edition.*
LEONARD, M. & EATON, C. 2021. Recreational Water Quality Guidelines Update. New Zealand Ministry of Health.

LEONG, J. C. 2008. Fish Viruses. *In:* MAHY, B. W. J. & VAN REGENMORTEL, M. H. V. (eds.) *Encyclopedia of Virology (Third Edition).* Oxford: Academic Press.
LEVINSON, W. E. 2020. *Review of Medical Microbiology and Immunology, Sixteenth Edition*, McGraw Hill LLC.
LI, D., HAO, Y. & DUAN, Y. 2020. Nonintrusive methods for biomass estimation in aquaculture with emphasis on fish: a review. 12**,** 1390-1411.
LI, R. A., MCDONALD, J. A., SATHASIVAN, A. & KHAN, S. J. 2019. Disinfectant residual stability leading to disinfectant decay and by-product formation in drinking water distribution systems: A systematic review. *Water Research,* 153**,** 335-348.
LIAN, Y., HUANG, G., GODLEWSKA, M., CAI, X., LI, C., YE, S., LIU, J. & LI, Z. 2018. Hydroacoustic estimates of fish biomass and spatial distributions in shallow lakes. *Journal of Oceanology and Limnology,* 36**,** 587-597.
LIGHTEN, J. & VAN OOSTERHOUT, C. 2017. Biocontrol of common carp in Australia poses risks to biosecurity. *Nature Ecology &Amp; Evolution,* 1**,** 0087.
LINTERMANS, M. 2023. Fishes of the Murray-Darling Basin. *Australin River Restoration Centre, Canberra*.
LIU, J.-M., SETIAZI, H. & SO, P.-Y. 2023. Fisheries hydroacoustic assessment: A bibliometric analysis and direction for future research towards a blue economy. *Regional Studies in Marine Science,* 60**,** 102838.
LIU, X., WATTS, R. J., HOWITT, J. A. & MCCASKER, N. 2020. Carbon and nutrient release from experimental inundation of agricultural and forested floodplain soil and vegetation: influence of floodplain land use on the development of hypoxic blackwater during floods %J Marine and Freshwater Research. 71**,** 213-228.
LLIRÓS, M., INCEOĞLU, Ö., GARCÍA-ARMISEN, T., ANZIL, A., LEPORCQ, B., PIGNEUR, L. M., VIROUX, L., DARCHAMBEAU, F., DESCY, J. P. & SERVAIS, P. 2014. Bacterial community composition in three freshwater reservoirs of different alkalinity and trophic status. *PLoS One,* 9**,** e116145.
LOFTON, M. E., HOWARD, D. W., MCCLURE, R. P., WANDER, H. L., WOELMER, W. M., HOUNSHELL, A. G., LEWIS, A. S. L. & CAREY, C. C. 2022. Experimental thermocline deepening alters vertical distribution and community structure of phytoplankton in a 4-year whole-reservoir manipulation. 67**,** 1903-1924.
LORENZO, V. & ALI, T. 2015. Experimental Evidence for the Effects of Common Carp (Cyprinus carpioL., 1758) on Freshwater Ecosystems: A Narrative Review with Management Directions for Turkish Inland Waters. *Journal of Limnology and Freshwater Fisheries Research,* 1**,** 123-149.
LOUGHRAN, B., VOLKOFSKY, A., MCCONNELL, S., BROWN, O. & ORMONDE, B. 2023. *Inquiry into Menindee fish kills expected in coming weeks, minister confirms* [Online]. ABC Broken Hill: Australian Broadcasting Corporation. Available: https://www.abc.net.au/news/2023-04-12/menindee-fish-kill-inquiry-timeline-announced/102212598 [Accessed 29/7/2023 2023].
LUJAN, D. R., TRONSTAD, L. M., BRIGGS, M. A., ALBERTSON, L. K., GLASSIC, H. C., GUY, C. S. & KOEL, T. M. 2022. Response of nutrient limitation to invasive fish suppression: How carcasses and analog pellets alter periphyton. 41**,** 88-99.
LYNCH, M. D. J. & NEUFELD, J. D. 2015. Ecology and exploration of the rare biosphere. *Nature Reviews Microbiology,* 13**,** 217-229.
MAC NALLY, R., CUNNINGHAM, S. C., BAKER, P. J., HORNER, G. J. & THOMSON, J. R. 2011. Dynamics of Murray-Darling floodplain forests under multiple stressors: The past, present, and future of an Australian icon. 47.
MACDONALD, C. 1978. Morphological and Biochemical Systematics of Australian Freashwater and Estuarine Percichthyid Fishes %J Marine and Freshwater Research. 29**,** 667-698.
MAREDOVÁ, N., ALTMAN, J. & KAŠTOVSKÝ, J. 2021. The effects of macrophytes on the growth of bloom-forming cyanobacteria: Systematic review and experiment. *Science of The Total Environment,* 792**,** 148413.

MARSHALL, B. E. & FALCONER, A. C. 1973. Eutrophication of a Tropical African Impoundment (Lake McIlwaine, Rhodesia). *Hydrobiologia,* 43**,** 109-123.
MARSHALL, J., DAVISON, A. J., KOPF, R. K., BOUTIER, M., STEVENSON, P. & VANDERPLASSCHEN, A. 2018. Biocontrol of invasive carp: Risks abound. *Science,* 359**,** 877-877.
MARTIGNAC, F., DAROUX, A., BAGLINIERE, J.-L., OMBREDANE, D. & GUILLARD, J. 2015. The use of acoustic cameras in shallow waters: new hydroacoustic tools for monitoring migratory fish population. A review of DIDSON technology. 16**,** 486-510.
MARTIKAINEN, P. J. 2022. Heterotrophic nitrification – An eternal mystery in the nitrogen cycle. *Soil Biology and Biochemistry,* 168**,** 108611.
MATHEWSON, D. D., HOCKING, M. D. & REIMCHEN, T. E. 2003. Nitrogen uptake in riparian plant communities across a sharp ecological boundary of salmon density. *BMC ecology,* 3**,** 4-4.
MATSUI, K., HONJO, M., KOHMATSU, Y., UCHII, K., YONEKURA, R. & KAWABATA, Z. I. 2008. Detection and significance of koi herpesvirus (KHV) in freshwater environments. *Freshwater Biology,* 53**,** 1262-1272.
MATSUZAKI, S. S., USIO, N., TAKAMURA, N. & WASHITANI, I. 2009. Contrasting impacts of invasive engineers on freshwater ecosystems: an experiment and meta-analysis. *Oecologia,* 158**,** 673-86.
MAUREAUD, A., HODAPP, D., VAN DENDEREN, P. D., HILLEBRAND, H., GISLASON, H., SPAANHEDEN DENCKER, T., BEUKHOF, E. & LINDEGREN, M. 2019. Biodiversity–ecosystem functioning relationships in fish communities: biomass is related to evenness and the environment, not to species richness. 286**,** 20191189.
MCCOLL, K. A. 2016. *Using herpes virus to eradicate feral fish? Carp diem!* [Online]. Available: https://blog.csiro.au/reclaiming-our-rivers-from-feral-carp/ [Accessed 2018].
MCCOLL, K. A. & CRANE, M. 2013. Cyprinid herpesvirus 3, CyHV-3: Its potential as a biological control agent for carp in Australia. *PestSmart Toolkit publication, Invasive Animals Cooperative Research Centre, Canberra, ACT*.
MCCORMACK, R. B. 2014. New records and review of the translocation of the yabby Cherax destructor into eastern drainages of New South Wales, Australia. *Australian Zoologist,* 37**,** 85-94.
MCDOWALL, R. M. 1980. *Freshwater fishes of south-eastern Australia (New South Wales, Victoria and Tasmania / edited by R.M. McDowall,* Terrey Hills, N.S.W, Reed.
MDBC 2004. *Pilot audit technical reports and supporting information [electronic resource] : Sustainable Rivers Audit / Murray-Darling Basin Commission,* Canberra, Murray-Darling Basin Commission.
MERRICK, J. R. 1993. *Freshwater crayfishes of New South Wales / John R. Merrick,* Milson's Point, N.S.W, Linnean Society of New South Wales.
MICHEL, B., FOURNIER, G., LIEFFRIG, F., COSTES, B. & VANDERPLASSCHEN, A. 2010. Cyprinid herpesvirus 3. *Emerging infectious diseases,* 16**,** 1835-1843.
MILLER, S. A. & CROWL, T. A. 2006. Effects of common carp (Cyprinus carpio) on macrophytes and invertebrate communities in a shallow lake. 51**,** 85-94.
MILLS, B. J. & MCCLOUD, P. I. 1983. Effects of stocking and feeding rates on experimental pond production of the crayfish Cherax destructor clark (Decapoda: Parastacidae). *Aquaculture,* 34**,** 51-72.
MINAMOTO, T., HONJO, M. N. & KAWABATA, Z. I. 2009. Seasonal Distribution of Cyprinid Herpesvirus 3 in Lake Biwa, Japan. 75**,** 6900-6904.
MITROVIC, S. M., HARDWICK, L. & DORANI, F. 2011. Use of flow management to mitigate cyanobacterial blooms in the Lower Darling River, Australia. *Journal of Plankton Research,* 33**,** 229-241.
MITROVIC, S. M., OLIVER, R. L., REES, C., BOWLING, L. C. & BUCKNEY, R. T. 2003. Critical flow velocities for the growth and dominance of Anabaena circinalis in some turbid freshwater rivers. 48**,** 164-174.

MIYASHITA, Y., HAGIWARA, T. & IMAI, I. 2019. The existence of cyanobactericidal bacteria and growth-inhibiting bacteria on water plants in Lake Ohnuma, Japan. *Limnology,* 20**,** 39-53.
MOHAMED, Z. A. 2017. Macrophytes-cyanobacteria allelopathic interactions and their implications for water resources management—A review. *Limnologica,* 63**,** 122-132.
MORGAN, D. K. & HICKS, B. J. 2013. A metabolic theory of ecology applied to temperature and mass dependence of N and P excretion by common carp. *Hydrobiologia,* 705**,** 135-145.
MOSLEY, L. M., PRIESTLEY, S., BROOKES, J., DITTMANN, S., FARKAŠ, J., FARRELL, M., FERGUSON, A. J., GIBBS, M., HIPSEY, M., HUANG, J., LAM-GORDILLO, O., SIMPSON, S. L., TYLER, J. J., WAYCOTT, M. & WELSH, D. T. 2023. Extreme eutrophication and salinisation in the Coorong estuarine-lagoon ecosystem of Australia's largest river basin (Murray-Darling). *Marine Pollution Bulletin,* 188**,** 114648.
MOURSUND, R. A., CARLSON, T. J. & PETERS, R. D. 2003. A fisheries application of a dual-frequency identification sonar acoustic camera. *ICES Journal of Marine Science,* 60**,** 678-683.
MUDADU, A. G., SALZA, S., MELILLO, R., MARA, L., PIRAS, G., SPANU, C., TERROSU, G., FADDA, A., VIRGILIO, S. & TEDDE, T. 2021. Prevalence and pathogenic potential of Arcobacter spp. isolated from edible bivalve molluscs in Sardinia. *Food Control,* 127**,** 108139.
MUELLER, S. & MITROVIC, S. 2015. Phytoplankton co-limitation by nitrogen and phosphorus in a shallow reservoir: progressing from the phosphorus limitation paradigm. *Hydrobiologia,* 744.
MÜHLENBRUCH, M., GROSSART, H.-P., EIGEMANN, F. & VOSS, M. 2018. Mini-review: Phytoplankton-derived polysaccharides in the marine environment and their interactions with heterotrophic bacteria. 20**,** 2671-2685.
MÜLLER, S., VON BONIN, S., SCHNEIDER, R., KRÜGER, M., QUICK, S. & SCHRÖTTNER, P. 2023. Shewanella putrefaciens, a rare human pathogen: A review from a clinical perspective. 12.
MULYANI, Y., ARYANTHA, I. N., SUHANDONO, S. & PANCORO, A. 2018. Intestinal Bacteria of Common Carp (Cyprinus carpio L.) as a Biological Control Agent for Aeromonas. *Journal of Pure and Applied Microbiology,* 12**,** 601-610.
MUTETHYA, E. & YONGO, E. 2021. A comprehensive review of invasion and ecological impacts of introduced common carp (Cyprinus carpio) in Lake Naivasha, Kenya. 26**,** e12386.
NAGASAKA, A., NAGASAKA, Y., ITO, K., MANO, T., YAMANAKA, M., KATAYAMA, A., SATO, Y., GRANKIN, A. L., ZDORIKOV, A. I. & BORONOV, G. A. 2006. Contributions of salmon-derived nitrogen to riparian vegetation in the northwest Pacific region. *Journal of Forest Research,* 11**,** 377-382.
NATIONAL CARP CONTROL PLAN. 2021. *The carp problem* [Online]. Available: https://carp.gov.au/en/the-carp-problem [Accessed 9/9/2021 2021].
NCCP 2019. Carp Biocontrol and Water Quality. *Technical Paper 3.*
NERIS, J., SANTIN, C., LEW, R., ROBICHAUD, P. R., ELLIOT, W. J., LEWIS, S. A., SHERIDAN, G., ROHLFS, A.-M., OLLIVIER, Q., OLIVEIRA, L. & DOERR, S. H. 2021. Designing tools to predict and mitigate impacts on water quality following the Australian 2019/2020 wildfires: Insights from Sydney's largest water supply catchment. n/a.
NHMRC 2008. Guidelines for managing risks in recreational water. Canberra, A.C.T. :: National Health and Medical Research Council.
NÕGES, T. 2009. Relationships between morphometry, geographic location and water quality parameters of European lakes. *Hydrobiologia,* 633**,** 33-43.
NRE 2023. Invasive Freshwater Species. *Department of Natural Resources and Environment Tasmania*.
O'DONNELL, D., WILBURN, P., SILOW, E., YAMPOLSKY, L. & LITCHMAN, E. 2017. Nitrogen and phosphorus colimitation of phytoplankton in Lake Baikal: Insights from a spatial survey and nutrient enrichment experiments. *Limnology and Oceanography,* 62.
ODONKOR, S. T. & MAHAMI, T. 2020. Escherichia coli as a Tool for Disease Risk Assessment of Drinking Water Sources. *Int J Microbiol,* 2020**,** 2534130.

ORIHEL, D. M., BAULCH, H. M., CASSON, N. J., NORTH, R. L., PARSONS, C. T., SECKAR, D. C. M. & VENKITESWARAN, J. J. 2017. Internal phosphorus loading in Canadian fresh waters: a critical review and data analysis. 74**,** 2005-2029.
ORIHUELA-TORRES, A., PÉREZ-GARCÍA, J. M., SÁNCHEZ-ZAPATA, J. A., BOTELLA, F. & SEBASTIÁN-GONZÁLEZ, E. 2022. Scavenger guild and consumption patterns of an invasive alien fish species in a Mediterranean wetland. 12**,** e9133.
ORMONDE, B. 2023. *Darling River's back-to-back floods a hindrance and opportunity for outback farmers* [Online]. Australian Broadcasting Corporation Available: https://www.abc.net.au/news/2023-03-05/darling-river-back-to-back-floods-impact-on-outback-farmers/102041308 [Accessed].
ÖZKUNDAKCI, D., HAMILTON, D. & GIBBS, M. 2011. Hypolimnetic phosphorus and nitrogen dynamics in a small, eutrophic lake with a seasonally anoxic hypolimnion. *Hydrobiologia,* 661**,** 5-20.
PARKOS III, J. J., SANTUCCI, J. V. J. & WAHL, D. H. 2003. Effects of adult common carp (Cyprinus carpio) on multiple trophic levels in shallow mesocosms. *Canadian Journal of Fisheries and Aquatic Sciences,* 60**,** 182-192.
PARTE, A., KRIEG, N. R., LUDWIG, W., WHITMAN, W. B., HEDLUND, B. P., PASTER, B. J., STALEY, J. T., WARD, N. & BROWN, D. 2011. *Bergey's Manual of Systematic Bacteriology: Volume 4: The Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes*, Springer New York.
PARULEKAR, N. N., KOLEKAR, P., JENKINS, A., KLEIVEN, S., UTKILEN, H., JOHANSEN, A., SAWANT, S., KULKARNI-KALE, U., KALE, M. & SÆBØ, M. 2017. Characterization of bacterial community associated with phytoplankton bloom in a eutrophic lake in South Norway using 16S rRNA gene amplicon sequence analysis. *PLOS ONE,* 12**,** e0173408.
PASCALI, J. P., VIEL, G., CECCHETTO, G., PIGAIANI, N., VANIN, S., MONTISCI, M. & FAIS, P. 2020. The Red Swamp Crayfish Procambarus Clarkii (the Louisiana Crayfish) as a Particular Scavenger on a Human Corpse. *J Forensic Sci,* 65**,** 323-326.
PAVER, S. F., HAYEK, K. R., GANO, K. A., FAGEN, J. R., BROWN, C. T., DAVIS-RICHARDSON, A. G., CRABB, D. B., ROSARIO-PASSAPERA, R., GIONGO, A., TRIPLETT, E. W. & KENT, A. D. 2013. Interactions between specific phytoplankton and bacteria affect lake bacterial community succession. 15**,** 2489-2504.
PAYNE, F. E., THORNTON, K. & KIMMEL, B. Reservoir Limnology: Ecological Perspectives. 1990.
PAYNE, L. & MOORE, J. 2006. Mobile scavengers create hotspots of freshwater productivity. 115**,** 69-80.
PAŹDZIOR, E., PĘKALA-SAFIŃSKA, A. & WASYL, D. 2019. Phenotypic Diversity and Potential Virulence Factors of the Shewanella Putrefaciens Group Isolated from Freshwater Fish. *J Vet Res,* 63**,** 321-332.
PERA, J. B. 2007. Fish survey and carp removal from Model Yacht Pond Centennial Park trust

PERA, J. B. 2008. Fish surveys from ponds in Maluga Park, The Crest, Lansdown, Shortland Brush and Lake Gillawarna Bankstown City Council.
PERA, J. B. 2020. The impact of decaying fish on water quality. WaterNSW.
PERA, J. B., DAVIE, A. W., ROHLFS, A.-M. & MITROVIC, S. M. 2021. Simulating the potential effects of a carp virus fish kill on water quality and phytoplankton in lentic environments %J Marine and Freshwater Research. -.
PERSSON, A. & NILSSON, E. 2007. Foraging behavior of benthic fish as an indicator of ecosystem state in shallow lakes. *Israel Journal of Ecology*

Evolution, 53**,** 407-421.
PESTSMART. 2018. *Carp in Australia* [Online]. PestSmart Connect. Available: https://www.pestsmart.org.au/pestsmart-factsheet-carp [Accessed].

PICK, F. R. & LEAN, D. R. S. 1987. The role of macronutrients (C, N, P) in controlling cyanobacterial dominance in temperate lakes. *New Zealand Journal of Marine and Freshwater Research,* 21**,** 425-434.

PINTO, L., CHANDRASENA, N., PERA, J., HAWKINS, P., ECCLES, D. & SIM, R. 2005. Managing invasive carp (Cyprinus carpio L.) for habitat enhancement at Botany Wetlands, Australia. 15**,** 447-462.

PIR 2023. European carp (includes koi carp). *Government of Australia Department of Primary Industries and Regions*.

POKOROVA, D., VESELY, T., PIACKOVA, V., RESCHOVA, S. & HULOVA, J. 2005. Current knowledge on koi herpesvirus (KHV): a review. *Veterinarni Medicina,* 50**,** 139-148.

PREMKE, K., FISCHER, P., HEMPEL, M. & ROTHHAUPT, K.-O. J. A. L.-I. J. L. 2010. Ecological studies on the decomposition rate of fish carcasses by benthic organisms in the littoral zone of Lake Constance, Germany. 46**,** 157-168.

PROSSER, I. P., CHIEW, F. H. S. & STAFFORD SMITH, M. 2021. Adapting Water Management to Climate Change in the Murray–Darling Basin, Australia. 13**,** 2504.

PROULX, M., PICK, F. R., MAZUMDER, A., HAMILTON, P. B. & LEAN, D. R. S. 1996. Experimental Evidence for Interactive Impacts of Human Activities on Lake Algal Species Richness. *Oikos,* 76**,** 191-195.

PTACNIK, R., SOLIMINI, A. G., ANDERSEN, T., TAMMINEN, T., BRETTUM, P., LEPISTO, L., WILLEN, E. & REKOLAINEN, S. 2008. Diversity predicts stability and resource use efficiency in natural phytoplankton communities. *Proc Natl Acad Sci U S A,* 105**,** 5134-8.

RAHMAN, M. M., KADOWAKI, S., BALCOMBE, S. R. & WAHAB, M. A. 2010. Common carp (Cyprinus carpio L.) alters its feeding niche in response to changing food resources: direct observations in simulated ponds. *Ecological Research,* 25**,** 303-309.

RAKUS, K., OUYANG, P., BOUTIER, M., RONSMANS, M., RESCHNER, A., VANCSOK, C., JAZOWIECKA-RAKUS, J. & VANDERPLASSCHEN, A. 2013. Cyprinid herpesvirus 3: an interesting virus for applied and fundamental research. *Veterinary research,* 44**,** 85-85.

RANDALL, D. & TSUI, T. K. N. 2002. Ammonia toxicity in fish. *Marine pollution bulletin,* 45**,** 17-23.

RATHLAVATH, S., MISHRA, S., KUMAR, S. & NAYAK, B. B. 2016. Incidence of Arcobacter spp. in fresh seafood from retail markets in Mumbai, India. *Annals of Microbiology,* 66**,** 165-170.

REDONDO-GÓMEZ, D., QUAGGIOTTO, M. M., BAILEY, D. M., EGUÍA, S., MORALES-REYES, Z., LÓPEZ-PASTOR, B. D. L. N., MARTÍN-VEGA, D., MARTÍNEZ-CARRASCO, C., SEBASTIÁN-GONZÁLEZ, E., SÁNCHEZ-ZAPATA, J. A. & MOLEÓN, M. 2022. Comparing scavenging in marine and terrestrial ecosystems: a case study with fish and gull carcasses in a small Mediterranean island. *Basic and Applied Ecology,* 59**,** 92-104.

REID, S. M., HAXTON, T. & GUTOWSKY, L. F. G. 2021. Boat-electrofishing transect location and flow levels: influence on riverine fish monitoring in non-wadeable habitats. *Environmental Monitoring and Assessment,* 193**,** 680.

RICHARDSON, M. J., WHORISKEY, F. G. & ROY, L. H. 1995. Turbidity generation and biological impacts of an exotic fish Carassius auratus, introduced into shallow seasonally anoxic ponds. 47**,** 576-585.

RIEDEL, B., PADOS, T., PRETTEREBNER, K., SCHIEMER, L., STECKBAUER, A., ALEXANDRA, H., ZUSCHIN, M. & STACHOWITSCH, M. 2013. Effect of hypoxia and anoxia on invertebrate behaviour: ecological perspectives from species to community level. *Biogeosciences Discussions,* 10**,** 14333-14438.

ROBERTS, J., CHICK, A., OSWALD, L. & THOMPSON, P. 1995. Effect of carp, <I>Cyprinus carpio</I> L., an exotic benthivorous fish, on aquatic plants and water quality in experimental ponds %J Marine and Freshwater Research. 46**,** 1171-1180.

ROGERS, K. & RALPH, T. J. 2010. *Floodplain Wetland Biota in the Murray-Darling Basin: Water and Habitat Requirements*, CSIRO PUBLISHING.

ROHLFS, A.-M., DAVIE, A. & PERA, J. 2021. Alternative cyanobacteria management approaches. *Water e-Journal,* Vol 6 No 2 2021.
SAADOUN, I. M. K., SCHRADER, K. K. & BLEVINS, W. T. 2001. Environmental and nutritional factors affecting geosmin synthesis by Anabaena SP. *Water Research,* 35**,** 1209-1218.
SAGOVA-MARECKOVA, M., BOENIGK, J., BOUCHEZ, A., CERMAKOVA, K., CHONOVA, T., CORDIER, T., EISENDLE, U., ELERSEK, T., FAZI, S., FLEITUCH, T., FRÜHE, L., GAJDOSOVA, M., GRAUPNER, N., HAEGERBAEUMER, A., KELLY, A. M., KOPECKY, J., LEESE, F., NÕGES, P., ORLIC, S., PANKSEP, K., PAWLOWSKI, J., PETRUSEK, A., PIGGOTT, J. J., RUSCH, J. C., SALIS, R., SCHENK, J., SIMEK, K., STOVICEK, A., STRAND, D. A., VASQUEZ, M. I., VRÅLSTAD, T., ZLATKOVIC, S., ZUPANCIC, M. & STOECK, T. 2021. Expanding ecological assessment by integrating microorganisms into routine freshwater biomonitoring. *Water Research,* 191**,** 116767.
SAKANAKA, A., KUBONIWA, M., SHIMMA, S., ALGHAMDI, S. A., MAYUMI, S., LAMONT, R. J., FUKUSAKI, E. & AMANO, A. 2022. Fusobacterium nucleatum Metabolically Integrates Commensals and Pathogens in Oral Biofilms. *mSystems,* 7**,** e0017022.
SALAM, L. B. 2019. Chapter 5 - Metagenomic insights into the diversity and functions of microbial assemblages in lakes. *In:* BANDH, S. A., SHAFI, S. & SHAMEEM, N. (eds.) *Freshwater Microbiology.* Academic Press.
SAMARASIN, P., MINNS, C. K., SHUTER, B. J., TONN, W. M. & RENNIE, M. D. 2015. Fish diversity and biomass in northern Canadian lakes: northern lakes are more diverse and have greater biomass than expected based on species–energy theory. 72**,** 226-237.
SANG, H. M., FOTEDAR, R. & FILER, K. 2011. Effects of dietary mannan oligosaccharide on the survival, growth, immunity and digestive enzyme activity of freshwater crayfish, Cherax destructor Clark (1936). 17**,** e629-e635.
SANTORI, C., SPENCER, R.-J., THOMPSON, M. B., WHITTINGTON, C. M., BURD, T. H., CURRIE, S. B., FINTER, T. J. & VAN DYKE, J. U. 2020. Scavenging by threatened turtles regulates freshwater ecosystem health during fish kills. *Scientific reports,* 10**,** 14383-14383.
SCHAUS, M., VANNI, M., WISSING, T., BREMIGAN, M., GARVEY, J. & STEIN, R. 1997. Nitrogen and phosphorus excretion by detritivorous gizzard shad in a reservoir ecosystem. *Limnology Oceanography,* 42**,** 1386-1397.
SCHINDLER, D. W. 1977. Evolution of phosphorus limitation in lakes. *Science,* 195**,** 260-2.
SCHINDLER, D. W. 1998. Whole-Ecosystem Experiments: Replication Versus Realism: The Need for Ecosystem-Scale Experiments. *Ecosystems,* 1**,** 323-334.
SCHNEIDER, J. C. 1998. Fate of Dead Fish in a Small Lake. 140 %J The American Midland Naturalist**,** 192-196, 5.
SCHOENEBECK, C. W., BROWN, M. L., CHIPPSS, S. R. & GERMAN, D. R. 2012. Nutrient and algal responses to winterkilled fish-derived nutrient subsidies in eutrophic lakes. *Lake and Reservoir Management,* 28**,** 189-199.
SCIENCE, A. A. O. 2019. Investigation of the causes of mass fish kills in the Menindee Region NSW over the summer of 2018–2019. Report of the Australian Academy of Science, Canberra, ACT, Australia.
SÉBERT, P., VETTIER, A., AMÉRAND, A. & MOISAN, C. 2009. High Pressure Resistance and Adaptation of European Eels. *In:* VAN DEN THILLART, G., DUFOUR, S. & RANKIN, J. C. (eds.) *Spawning Migration of the European Eel: Reproduction Index, a Useful Tool for Conservation Management.* Dordrecht: Springer Netherlands.
SEREDA, J., J. HUDSON, J., TAYLOR, W. & DEMERS, E. 2008. Fish as sources and sinks of nutrients in lakes. *Freshwater Biology,* 53**,** 278-289.
SERGEANT, C. J., BELLMORE, J. R., MCCONNELL, C. & MOORE, J. W. 2017. High salmon density and low discharge create periodic hypoxia in coastal rivers. 8**,** e01846.
SHADE, A., PETER, H., ALLISON, S., BAHO, D., BERGA, M., BÜRGMANN, H., HUBER, D., LANGENHEDER, S., LENNON, J., MARTINY, J., MATULICH, K., SCHMIDT, T. & HANDELSMAN, J.

2012. Fundamentals of Microbial Community Resistance and Resilience. *Frontiers in Microbiology,* 3**,** 417.
SHARSHAR, K. & AZAB, E. A. 2008. Studies on diseased freshwater prawn Macrobrachium rosenbergii infected with Vibrio vulnificus. *Pakistan journal of biological sciences : PJBS,* 11**,** 2092-2100.
SHEARER, K. & MULLEY, J. 1978. The introduction and distribution of the carp, Cyprinus carpio Linnaeus, in Australia. *Marine Freshwater Research,* 29**,** 551-563.
SHELDON, F., BARMA, D., BAUMGARTNER, L. J., BOND, N., MITROVIC, S. M. & VERTESSY, R. 2022. Assessment of the causes and solutions to the significant 2018–19 fish deaths in the Lower Darling River, New South Wales, Australia %J Marine and Freshwater Research. 73**,** 147-158.
SIBBING, F. A. 1988. Specializations and limitations in the utilization of food resources by the carp, Cyprinus carpio: a study of oral food processing. *Environmental Biology of Fishes,* 22**,** 161-178.
SIMPSON, K. & DAY, N. 1998. *Field guide to the birds of Australia / Simpson [editor] & Day [illustrator] ; with Peter Trusler,* London, Christopher Helm.
SITTA, K. A., REED, M., MORTENSEN, R., DOLL, C., CALLAHAN, T. & GREENFIELD, D. I. 2018. The influences of nitrogen form and zooplankton grazing on phytoplankton assemblages in two coastal southeastern systems. 63**,** 2523-2544.
SLOANE, R. 1984. Distribution, abundance, growth and food of freshwater eels (<I>Anguilla</I> spp.) in the Douglas River, Tasmania %J Marine and Freshwater Research. 35**,** 325-339.
SMALL, K., KOPF, R., WATTS, R. & HOWITT, J. 2014. Hypoxia, Blackwater and Fish Kills: Experimental Lethal Oxygen Thresholds in Juvenile Predatory Lowland River Fishes. *PloS one,* 9**,** e94524.
SMITH, A., MCNIVEN, I. J., ROSE, D., BROWN, S., JOHNSTON, C. & CROCKER, S. 2019. Indigenous Knowledge and Resource Management as World Heritage Values: Budj Bim Cultural Landscape, Australia. *Archaeologies,* 15**,** 285-313.
SMITH, B. 2005. The state of the art: a synopsis of information on common carp (Cyprinus carpio) in Australia. *Final Tech. Rpt, SARDI Aquatic Sci. Res. Rpt. Ser.*
SMITH, J. 2004. Fish and Company Smell After Three Days: Increasing Capture Rates of Carrion-Eating Varanid Lizards. *Herpetological Review,* 35**,** 41-43.
SMITH, V. H. 1983. Low Nitrogen to Phosphorus Ratios Favor Dominance by Blue-Green Algae in Lake Phytoplankton. 221**,** 669-671.
SMITH, V. H., TILMAN, G. D. & NEKOLA, J. C. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution,* 100**,** 179-196.
SNELLING, W. J., MATSUDA, M., MOORE, J. E. & DOOLEY, J. S. G. 2006. Under the Microscope: Arcobacter. *Letters in Applied Microbiology,* 42**,** 7-14.
SOBIERAJ, J. & METELSKI, D. 2023. Insights into Toxic Prymnesium parvum Blooms as a Cause of the Ecological Disaster on the Odra River. 15**,** 403.
SOKSAWATMAEKHIN, W., KURAISHI, A., SAKATA, K., KASHIWAGI, K. & IGARASHI, K. 2004. Excretion and uptake of cadaverine by CadB and its physiological functions in Escherichia coli. 51**,** 1401-1412.
SORENSEN, P., BAJER, P. & HEADRICK, M. 2015. Development and implementation of a sustainable strategy to control common carp in the Purgatory Creek Chain of Lakes.
SOUNDMETRICS 2008. Dual-Frequency Identification Sonar DIDSON - Operation Handbook. *Bellevue WA: Sound Metrics Corporation.*
STAIGHT, K. 2018. Will the carp virus help or hinder Australian water's native species. ABC.
STENSMYR, MARCUS C., DWECK, HANY K. M., FARHAN, A., IBBA, I., STRUTZ, A., MUKUNDA, L., LINZ, J., GRABE, V., STECK, K., LAVISTA-LLANOS, S., WICHER, D., SACHSE, S., KNADEN, M., BECHER, PAUL G., SEKI, Y. & HANSSON, BILL S. 2012. A Conserved Dedicated Olfactory Circuit for Detecting Harmful Microbes in Drosophila. *Cell,* 151**,** 1345-1357.
STEPHENS, F., RAIDAL, S. & JONES, B. 2004. Haematopoietic necrosis in a goldfish (Carassius auratus) associated with an agent morphologically similar to herpesvirus. *Australian Veterinary Journal,* 82**,** 167-169.

STERNER, R. W. & GEORGE, N. B. 2000. Carbon, Nitrogen, and Phosphorus Stoichiometry of Cyprinid Fishes. *Ecology,* 81**,** 127-140.
STEWARD, A., BLESSING, J., CLIFFORD, S., MARSHALL, J. & NEGUS, P. 2019. Ecological impacts of invasive common carp in Australian dryland rivers. *Aquatic Conservation Marine and Freshwater Ecosystems,* 29.
STOTT, P. 2016. How climate change affects extreme weather events. 352**,** 1517-1518.
STUART, I. 2019. *A national estimate of carp biomass for Australia / Ivor Stuart, Ben Fanson, Jarod Lyon, Jerom Stocks, Shane Brooks, Andrew Norris, Leigh Thwaites, Matt Beitzel, Michael Hutchison, Qifeng Ye, John Koehn, and Andrew Bennett ; edited by Pam Clunie (ARI),* Heidelberg, Victoria, Arthur Rylah Institute for Environmental Research, Department of Environment, Land, Water and Planning.
STUART, I. & CONALLIN, A. 2018. Control of Globally Invasive Common Carp: An 11-Year Commercial Trial of the Williams' Cage. *North American Journal of Fisheries Management,* 38.
STUART, I. & JONES, M. 2002. *Ecology and Management of Common Carp in the Barmah-Millewa Forest: Final Report of the Point Source Management of Carp Project to Agriculture Fisheries & Forestry Australia*, Arthur Rylah Institute for Environmental Research.
STUART, I. G., FANSON, B. G., LYON, J. P., STOCKS, J., BROOKS, S., NORRIS, A., THWAITES, L., BEITZEL, M., HUTCHISON, M., YE, Q., KOEHN, J. D. & BENNETT, A. F. 2021. Continental threat: How many common carp (Cyprinus carpio) are there in Australia? *Biological Conservation,* 254**,** 108942.
SU, W., LI, T., YU, Q., FENG, T., YANG, J., QU, J., LI, X. & LI, H. 2022. Animal corpse degradation enriches antibiotic resistance genes but remains recalcitrant in drinking water microcosm. *International Biodeterioration & Biodegradation,* 168**,** 105372.
SUGITA, H., MIYAJIMA, C., KOBIKI, Y. & DEGUCHI, Y. 1990. The daily fluctuation and inter-individual variation of the faecal flora of carp, Cyprinus carpio L. *Journal of Fish Biology - J FISH BIOL,* 36**,** 103-105.
SUGITA, H. & MIZUKI, H. 2012. Bacterial diversity of the upper and lower intestines of the common carp (Cyprinus carpio Linnaeus, 1758) reared in an indoor tank. 28**,** 647-648.
SUGITA, H., TOKUYAMA, K. & DEGUCHI, Y. 1985. The Intestinal Microflora of Carp Cyprinus carpio, Grass Carp Ctenopharyngodon idella and Tilapia Sarotherodon niloticus. *NIPPON SUISAN GAKKAISHI,* 51**,** 1325-1329.
SUMIDA, P. Y. G., ALFARO-LUCAS, J. M., SHIMABUKURO, M., KITAZATO, H., PEREZ, J. A. A., SOARES-GOMES, A., TOYOFUKU, T., LIMA, A. O. S., ARA, K. & FUJIWARA, Y. 2016. Deep-sea whale fall fauna from the Atlantic resembles that of the Pacific Ocean. *Scientific Reports,* 6**,** 22139.
SUNARTO, A., RUKYANI, A. & ITAMI, T. 2005. Indonesian Experience on the Outbreak of Koi Herpesvirus in Koi and Carp (Cyprinus carpio). *Bulletin of Fisheries Research Agency,* 2**,** 15-21.
SWIREPIK, J. 1999. *Physical disturbance of Potamogeton tricarinatus and sediment by carp (Cyprinus carpio) in experimental ponds.* University of Canberra.
TANG, X., GAO, G., QIN, B., ZHU, L., CHAO, J., WANG, J. & YANG, G. 2009. Characterization of Bacterial Communities Associated with Organic Aggregates in a Large, Shallow, Eutrophic Freshwater Lake (Lake Taihu, China). *Microbial Ecology,* 58**,** 307-322.
TÁTRAI, I., PAULOVITS, G., MÁTYÁS, K. & KORPONAI, J. 2003. The role of fish communities in water quality management of a large shallow lake. *International Review of Hydrobiology: A Journal Covering all Aspects of Limnology*

Marine Biology, 88**,** 498-507.
TEELING, H., FUCHS, B. M., BECHER, D., KLOCKOW, C., GARDEBRECHT, A., BENNKE, C. M., KASSABGY, M., HUANG, S., MANN, A. J., WALDMANN, J., WEBER, M., KLINDWORTH, A., OTTO, A., LANGE, J., BERNHARDT, J., REINSCH, C., HECKER, M., PEPLIES, J., BOCKELMANN, F. D., CALLIES, U., GERDTS, G., WICHELS, A., WILTSHIRE, K. H., GLÖCKNER, F. O., SCHWEDER, T. &

AMANN, R. 2012. Substrate-Controlled Succession of Marine Bacterioplankton Populations Induced by a Phytoplankton Bloom. 336**,** 608-611.
TEKILE, A., KIM, I. & KIM, J. 2015. Mini-review on river eutrophication and bottom improvement techniques, with special emphasis on the Nakdong River. *Journal of Environmental Sciences,* 30**,** 113-121.
TEMPERO, G. W. 2013. Assessment of fish populations in Lake Horowhenua, Levin (ERI Report No. 15). Hamilton, New Zealand. The University of Waikato.
TEUFEL, A. G., LI, W., KISS, A. J. & MORGAN-KISS, R. M. 2017. Impact of nitrogen and phosphorus on phytoplankton production and bacterial community structure in two stratified Antarctic lakes: a bioassay approach. *Polar Biology,* 40**,** 1007-1022.
THACKRAY, L. 2022. *NSW dam managers face huge challenge to stop rivers flooding during near-record wet* [Online]. Australian Broadcasting Corporation. Available: https://www.abc.net.au/news/2022-06-03/burrendong-dam-record-rainfall-managing-flooding-risk/101122316 [Accessed 29/7/2023 2023].
THIEM, J. D., BAUMGARTNER, L. J., FANSON, B., SADEKOV, A., TONKIN, Z. & ZAMPATTI, B. P. 2021. Contrasting natal origin and movement history informs recovery pathways for three lowland river species following a mass fish kill %J Marine and Freshwater Research. -.
THIEM, J. D., WOODEN, I. J., BAUMGARTNER, L. J., BUTLER, G. L., TAYLOR, M. D. & WATTS, R. J. 2020. Hypoxic conditions interrupt flood-response movements of three lowland river fish species: Implications for flow restoration in modified landscapes. 13**,** e2197.
THOMAZ, S. M. & CUNHA, E. R. 2010. The role of macrophytes in habitat structuring in aquatic ecosystems: methods of measurement, causes and consequences on animal assemblages' composition and biodiversity. *Acta Limnologica Brasiliensia,* 22**,** 218-236.
THOMAZ, S. M., DIBBLE, E. D., EVANGELISTA, L. R., HIGUTI, J. & BINI, L. M. 2008. Influence of aquatic macrophyte habitat complexity on invertebrate abundance and richness in tropical lagoons. *Freshwater biology,* 53**,** 358-367.
THORBURN, P. J., BIGGS, J. S., WEIER, K. L. & KEATING, B. A. 2003. Nitrate in groundwaters of intensive agricultural areas in coastal Northeastern Australia. *Agriculture, Ecosystems & Environment,* 94**,** 49-58.
THRESHER, R. E., ALLMAN, J. & STREMICK-THOMPSON, L. 2018. Impacts of an invasive virus (CyHV-3) on established invasive populations of common carp (Cyprinus carpio) in North America. *Biological Invasions,* 20**,** 1703-1718.
TILL, A., RYPEL, A. L., BRAY, A. & FEY, S. B. 2019. Fish die-offs are concurrent with thermal extremes in north temperate lakes. *Nature Climate Change,* 9**,** 637-641.
TILZER, M. M. 1990. Specific Properties of Large Lakes. *In:* TILZER, M. M. & SERRUYA, C. (eds.) *Large Lakes: Ecological Structure and Function.* Berlin, Heidelberg: Springer Berlin Heidelberg.
TIMS, A. R., UNMACK, P. J. & HO, S. Y. W. 2021. A fossil-calibrated time-tree of all Australian freshwater fishes. *Mol Phylogenet Evol,* 161**,** 107180.
TWORT, A., RATNAYAKA, D. & BRANDT, M. 2006. *WATER SUPPLY Fifth Edition*, Butterworth-Heinemann.
UCHII, K., KAWABATA, Z., RSQUO & ICHIRO 2009. Biological invasion of a fish pathogen, cyprinid herpesvirus 3, into Japanese freshwater ecosystems. *Japanese Journal of Limnology (Rikusuigaku Zasshi),* 70**,** 267-272.
UNMACK, P. J. 2001. Biogeography of Australian freshwater fishes. 28**,** 1053-1089.
VAN DYCK, S. & STRAHAN, R. 2008. *The mammals of Australia / edited by Steve van Dyck and Ronald Strahan,* Sydney, New Holland Publishers.
VANNI, M. J., BOWLING, A. M., DICKMAN, E. M., HALE, R. S., HIGGINS, K. A., HORGAN, M. J., KNOLL, L. B., RENWICK, W. H. & STEIN, R. A. 2006. Nutrient cycling by fish supports relatively more primary production as lake productivity increases. *Ecology,* 87**,** 1696-1709.

VERTESSY, R., BARMA, D., BAUMGARTNER, L., BOND, N., MITROVIC, S. & SHELDON, F. 2019. Independent Assessment of the 2018?19 fish deaths in the lower Darling. Australia: Murray Darling Basin Authority and Australian Government.

VFA 2010. Noxious aquatis soecies in Victoria. *Victoria Fisheries Authority*.

VILIZZI, L. & TARKAN, A. S. 2015. Experimental evidence for the effects of common carp (Cyprinus carpio L., 1758) on freshwater ecosystems: a narrative review with management directions for Turkish inland waters. *Journal of Limnology Freshwater Fisheries Research,* 1**,** 123-149.

VILLANUEVA, C. M., CORDIER, S., FONT-RIBERA, L., SALAS, L. A. & LEVALLOIS, P. 2015. Overview of Disinfection By-products and Associated Health Effects. *Curr Environ Health Rep,* 2**,** 107-15.

WALTER, M., RECKNAGEL, F., CARPENTER, C. & BORMANS, M. 2001. Predicting eutrophication effects in the Burrinjuck Reservoir (Australia) by means of the deterministic model SALMO and the recurrent neural network model ANNA. *Ecological Modelling,* 146**,** 97-113.

WALTZEK, T. B., KELLEY, G. O., STONE, D. M., WAY, K., HANSON, L., FUKUDA, H., HIRONO, I., AOKI, T., DAVISON, A. J. & HEDRICK, R. P. 2005. Koi herpesvirus represents a third cyprinid herpesvirus (CyHV-3) in the family Herpesviridae. 86**,** 1659-1667.

WANG, H., SMITH, H. L., KUANG, Y. & ELSER, J. J. 2007. Dynamics of Stoichiometric Bacteria-Algae Interactions in the Epilimnion. 68**,** 503-522.

WANG, Z. & LI, R. 2015. Effects of light and temperature on the odor production of 2-methylisoborneol-producing Pseudanabaena sp. and geosmin-producing Anabaena ucrainica (cyanobacteria). *Biochemical Systematics and Ecology,* 58**,** 219-226.

WANNER, A. G., NENNEMAN, P. M. & KAEMINGK, A. M. 2009. Common carp abundance, biomass, and removal from Dewey and Clear lakes on the Valentine National Wildlife Refuge: Does trapping and removing carp payoff? : University of Nebraska.

WATERNSW 2023. Algae Alerts NSW map.

WEBER, M. J. & BROWN, M. L. 2009. Effects of Common Carp on Aquatic Ecosystems 80 Years after "Carp as a Dominant": Ecological Insights for Fisheries Management. *Reviews in Fisheries Science,* 17**,** 524-537.

WEBER, M. J. & BROWN, M. L. 2016. Effects of resource pulses on nutrient availability, ecosystem productivity, and temporal variability following a stochastic disturbance in eutrophic glacial lakes. *Hydrobiologia,* 771**,** 165-177.

WEISS, P., SCHWEITZER, B., AMANN, R. & SIMON, M. 1996. Identification in situ and dynamics of bacteria on limnetic organic aggregates (lake snow). 62**,** 1998-2005.

WEST, P. 2018. *Guide to Introduced Pest Animals of Australia*.

WHITMORE, J. H. 2003. *Experimental fish taphonomy with a comparison to fossil fishes.* Ph.D., Loma Linda University.

WHITWORTH, K. L., BALDWIN, D. S. & KERR, J. L. 2012. Drought, floods and water quality: Drivers of a severe hypoxic blackwater event in a major river system (the southern Murray–Darling Basin, Australia). *Journal of Hydrology,* 450-451**,** 190-198.

WILLIAMS, A. E., MOSS, B. & EATON, J. 2002. Fish induced macrophyte loss in shallow lakes: top–down and bottom–up processes in mesocosm experiments. *Freshwater biology,* 47**,** 2216-2232.

WILLIAMS, M. & SCHULZ, D. 2023. 2023 Menindee Mass Fish Kill, How it happened. A desktop investigation unravelling the operational decisions that preceded the death of 20 million fish.

WIPFLI, M. S., HUDSON, J. & CAOUETTE, J. 1998. Influence of salmon carcasses on stream productivity: response of biofilm and benthic macroinvertebrates in southeastern Alaska, U.S.A. *Canadian Journal of Fisheries and Aquatic Sciences,* 55**,** 1503-1511.

WOOLLARD, P., VESTJENS, W. & MACLEAN, L. 1978. The Ecology of the Eastern Water Rat Hydromys Chrysogaster at Griffith, N.s.w.: Food and Feeding Habits. %J Wildlife Research. 5**,** 59-73.

WU, H., LI, Y., ZHANG, W., NIU, L., GAO, Y., HUI, C. & BERTILSSON, S. 2021. Hydrostatic pressure influence activity and assembly of bacterial communities in reservoir sediments. 66**,** 1049-1059.

WU, J. T. & JÜTTNER, F. 1988. Effect of Environmental Factors on Geosmin Production by Fischerella Muscicola. *Water Science and Technology,* 20**,** 143-148.

XIA, L. C., STEELE, J. A., CRAM, J. A., CARDON, Z. G., SIMMONS, S. L., VALLINO, J. J., FUHRMAN, J. A. & SUN, F. 2011. Extended local similarity analysis (eLSA) of microbial community and other time series data with replicates. *BMC Syst Biol,* 5 Suppl 2**,** S15.

YACHI, S. & LOREAU, M. 1999. Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *Proceedings of the National Academy of Sciences,* 96**,** 1463.

YANG, C., HOU, X., WU, D., CHANG, W., ZHANG, X., DAI, X., DU, H., ZHANG, X., IGARASHI, Y. & LUO, F. 2020. The characteristics and algicidal mechanisms of cyanobactericidal bacteria, a review. *World Journal of Microbiology and Biotechnology,* 36**,** 188.

YOUN, S. J., KIM, H. N., YU, S. J. & BYEON, M.-S. 2020. Cyanobacterial occurrence and geosmin dynamics in Paldang Lake watershed, South Korea. 34**,** 634-643.

YOUNG, W. F., HORTH, H., CRANE, R., OGDEN, T. & ARNOTT, M. 1996. Taste and odour threshold concentrations of potential potable water contaminants. *Water Research,* 30**,** 331-340.

YUAN, X., WANG, Y., JI, P., WU, P., SHEFFIELD, J. & OTKIN, J. A. 2023. A global transition to flash droughts under climate change. 380**,** 187-191.

ZACHARIAH, M., PHILIP, S., PINTO, I., VAHLBERG, M., SINGH, R., OTTO, F., BARNES, C. & KIMUTAI, J. 2023. Extreme heat in North America, Europe and China in July 2023 made much more likely by climate change. Grantham institute for Climate Change.

ZAMBRANO, L., SCHEFFER, M. & MARTÍNEZ-RAMOS, M. 2001. Catastrophic response of lakes to benthivorous fish introduction. *Oikos,* 94**,** 344-350.

ZAROUBI, L., OZUGERGIN, I., MASTRONARDI, K., IMFELD, A., LAW, C., GÉLINAS, Y., PIEKNY, A. & FINDLAY, B. L. 2022. The Ubiquitous Soil Terpene Geosmin Acts as a Warning Chemical. *Appl Environ Microbiol,* 88**,** e0009322.

ZASTEPA, A., COMTE, J. & CREVECOEUR, S. 2023. Prevalence and ecological features of deep chlorophyll layers in Lake of the Woods, a complex hydrological system with strong trophic, physical, and chemical gradients. *Journal of Great Lakes Research,* 49**,** 122-133.