Interactions between freshwater mussels and non-native species

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Freshwater mussel (*Echyridella menziesii*)

Tuhinga whakarāpopoto

He tāpaetanga te urutomokanga koiora ki ngā panonitanga pūnaha hauropi ao whānui, ā, he pāhikahika, he kaha ake hoki te pāpātanga i te wai māori. Ka kino kē atu tēnei tukanga i ngā pūnaha whakakē, pēnei i te whakahuawai kurawai e pai ai te taiao mō te whakaranea i ngā momo rāwaho. Ko tētahi o ngā tino mōreareatanga nā ngā momo rāwaho, ko te urunga mai o ngā pāhekohekotanga tauhou e pāpā ana ki ngā momo e whai piringa whirinaki ana, ā, he whāiti ngā matea nōhanga i te wā o te tūātupu tōmua. He rauropi whakapiripiri noho takere te kākahi (Bilvalvia: Unionida). Ā, ka whai piringa whirinaki i ētahi rauropi papa pērā i te ika hei whakatutuki i te tūātupu pirinoa ā-waho. Ko ngā piringa torongū (glochidia) ka whanake i runga i ngā ika hei papa pai mōna, ā, ka makere iho i te wā o te pūhouhou ka tau ai ki ngā papa parakiwai. Ki te whakatōhenehenetia nuitia ēnei pāhekohekotanga, ka korehāhā i ngā rohe paetata ki te kore e whakakapia ki, hei tauira, te ika rāwaho. Ā, ki te mimiti rānei te āhei ki ngā nōhanga whakatupu ora tōtika. (hei tauira, ngā nōhanga tipuwai rāwaho). Mā ngā momo rāwaho pea e ōheke nei te haotanga, nā konā, e tuari tītaha nei te rahinga taupori o ngā kākahi kātua. Nō reira, he tāpaetanga mātauranga tēnei tuhinga whakapae o ngā pāhekohekotaga i waenganui i ngā kākahi me ngā momo rāwaho i roto i ngā pūnaha wai māori whakakē. Ā, he tāpae pārongo hoki ki te āwhina i ngā whakahaeretanga o ngā momo me ngā kurawai mō te whāomoomo kākahi.

Ka whakatakororia ngā whakaputanga o tēnei tuhinga whakapae hei rārangi upoko, kua tāngia, kua tukuna, kua whakaritea rānei mō ngā hautaka pūtaiao. Ko te tāhū kōrero (Upoko 1), he whakahoropaki i ngā arohaehaetanga tatau ao whānui o ngā pāhekohekotanga i waenganui i ngā momo rāwaho me ngā kākahi. Ka mutu, ka kitea he mōreareatanga pea nā ngā ika rāwaho me ngā tipuwai rāwaho ki ngā kākahi o Aotearoa (Upoko 2). Āpiti atu, i whakamātauria ētahi ika rāwaho e toru (brown bullhead catfish*, Ameiurus nebulosus;* rudd*, Scardinius erythrophthalmus; and* goldfish*, Carassius auratus*), ā, kāore i tika te rahi hauropi o ngā torongū kākahi e whakanake ana, tēnā i tana noho ki ngā ika (*Gobiomorphus cotidianus*) e mōhiotia ana he rauropi papa tika mō ngā kākahi o Aotearoa (*Echyridella*

menziesii; Upoko 3). E mea ana tēnei kitenga, nā te huringa ki ētahi hapori ika, e ekea ana e ngā momo rāwaho, e raru ai pea te tūātupu o ngā kākahi i te wā o te torongū.

I tētahi rūritanga rohe i Kararāpiro, i te pito raho iho o te whakahuawai kurawai o Waikato awa, e mātotoru ana ngā nōhanga tipuwai rāwaho (*Ceratophyllum demersum* and *Egeria densa*), hāora-kore ana, kikino ana te taiao, ā, he taiao mōrearea rawa atu tēnei mō te kākahi (Upoko 4). I tēnei wāhi, he kikino te taiao i waenganui i te papa parakiwai me te wai o runga tonu, e pātata ana ki ngā pareparenga. Ā, i whakahaerehia mā te whakamatua i te nui o te wai me te rere o te wai i te kurawai, mā te whakamatua hoki i ngā tipuwai ki ngā patu otaota i ngā wāhi o raro iho o te wai. Nō muri mai, ka whakahaerehia tētahi rūritanga rohe anō e whānui kē atu ai ngā putanga o te Upoko 4, ā, i kitea, ko ngā pāpātanga o ngā tipuwai rāwaho, kei te āhua tonu o ngā momo tipuwai me ngā āhuatanga whānui o te mātai arowai. Nā konei, e tuari tītaha nei te rahinga taupori o ngā kākahi kātua i ngā wāhi hōhonu, tēnā i ngā wāhi pāpaku e kitea nei ngā haotanga (Upoko 5).

Ka whakaemihia mai ngā kitenga me ngā otinga o mua ki te whakaatu, ka pēhea ngā urutomokanga o ngā tipuwai, o ngā ika (o ngā ika rāwaho anake) i ētahi tūāhua whakapae huhua, ka pēhea rānei ngā mea e rua, e whakararu ai i te haotanga o te *E. menziesii* (Upoko 6). Nā tēnei tātari whakapae, ka kitea te hira o te whaiwhakaaro ki ngā whakawehi a ngā ika me ngā tipuwai rāwaho, me ngā pāpātanga ki te tēnā wāhanga, ki tēnā wāhanga o te huringa ora o te kākahi me te whakahaerehia, te whāomoomotia hoki ōna. Nā te roa o te oranga o te kākahi, mā te āhukahuka i ngā pāpātanga o ngā momo rāwaho e tuari tītaha nei te rahinga taupori o ngā kākahi kātua, ka haumanu anō ai pea ēnei tukanga e hāpai ana i te haotanga mai i mua i te korehāhā. Nā te whakaaotanga, me te whao pūngaotanga, e āki ana i te kanorite haeretanga me te mimiti haeretanga o ngā ratonga pūnaha rauropi e whai pānga ana. I tēnei horopaki, e whai tikanga nui ana te haepapa o ngā whakahaeretanga ki te ārai me te whakamauru i ngā pāpātanga o ngā urutomokanga koira ki runga ki ngā momo marore whai piringa whirinaki i ngā pūnaha hauropi wai māori haere ake nei.

Abstract

Biological invasions contribute to ecosystem change globally, with a disproportionate and intensified impact in freshwaters. This process is exacerbated in modified systems such as hydrogeneration reservoirs that promote favourable conditions for non-native species proliferation. One of the major threats from non-native species is the introduction of novel interactions that may be particularly impactful on species in affiliate (dependent) relationships and that have narrow habitat requirements during early life-stages. Freshwater mussels (Bivalvia: Unionida) are sessile benthic organisms in affiliate relationships with host fish on which they complete their ectoparasitic life-stage. Attached larvae (glochidia) transform on suitable fish hosts before dropping off as juveniles on surficial sediments. Significant disruption to such interactions may lead to local extinction if affiliate partners are unable to be replaced (i.e., by non-native fish) or the availability of critical life-supporting habitats is reduced (e.g., by non-native macrophytes). Non-native species may play a role in reducing recruitment leading to the adult-skewed mussel population size-structures commonly observed. Accordingly, this thesis contributes knowledge of the interactions between unionid mussels and non-native species in modified freshwater ecosystems, and provides information to assist in species and reservoir management for unionid mussel conservation.

The thesis outputs are presented as chapters that have been published in, submitted to, or prepared for scientific journals. A general introduction (Chapter 1) provides context for a global meta-analysis of non-native species and unionid mussel interactions that highlighted non-native fish and macrophytes as potential threats to New Zealand mussels (Chapter 2). Accordingly, a laboratory experiment on three non-native fish (brown bullhead catfish, *Ameiurus nebulosus*; rudd, *Scardinius erythrophthalmus*; and goldfish, *Carassius auratus*) found mussel glochidia were not transformed in ecologically viable numbers compared to a known host fish (*Gobiomorphus cotidianus*) for a New Zealand unionid (*Echyridella menziesii*; Chapter 3). This finding suggested that shifts towards fish

communities dominated by non-native species have potential to disrupt the obligate glochidial life-stage of unionid mussels.

Dense beds of non-native macrophytes (*Ceratophyllum demersum* and *Egeria densa*) were found to produce adverse anoxic and hypoxic conditions potentially fatal to mussels in a field survey of Karāpiro, the most downstream in the Waikato River hydrogeneration reservoir chain (Chapter 4). Here, adverse conditions at the sediment-water interface in littoral zones were mediated by reservoir management of water-level and water-flow, and by macrophyte control via herbicide application in the lower-lacustrine section. A subsequent field survey extended the Chapter 4 results to show that effects of non-native macrophytes at the sediment-water interface depended on macrophyte species and overarching hydrology, whereby adult-skewed mussel population size-structures were present in the lower-lacustrine of Karāpiro but not in the upper-riverine section where recruitment was occurring (Chapter 5).

The final chapter combined previous findings to show how various hypothetical scenarios of fish and macrophyte invasions could operate separately (non-native fish only) or in combination to disrupt *E. menziesii* recruitment (Chapter 6). This hypothetical analysis highlighted the importance of considering the threats of both non-native fish and macrophytes, which operate primarily on different stages of the unionid life-cycle, in freshwater mussel conservation and management. Due to the long life-span of unionids, recognition of non-native species impacts contributing to adult-skewed mussel population size-structures may provide an opportunity to restore disrupted mechanisms supporting their recruitment before local extinction occurs. Globalisation and energy demand facilitate continued biotic homogenisation and loss of associated ecosystem services. In this context, the role of management in preventing and mitigating the impacts of biological invasions on sensitive species with affiliate relationships will become increasingly important in freshwater ecosystems in the future.

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Preface

The main body of this thesis comprises six chapters; Chapters 2-5 were prepared as individual papers that have been submitted to peer-reviewed scientific journals. Accordingly, there is some repetition of methodological details and referencing, caption, and journal styles may vary between chapters.

Together with Chapter 6, the thesis forms a coherent portfolio of work that makes an original contribution to the chosen thesis topic. The work in this thesis was undertaken with supervision from Associate Professor Kevin Collier (The University of Waikato), Associate Professor Ian Duggan (The University of Waikato) and Dr Sue Clearwater (Department of Conservation).

Co-authors for each chapter are listed below. All co-authors reviewed relevant chapters, and provided advice where necessary.

Chapter 2 has been published as “Interactions between Unionida and non-native species: a global meta-analysis” in Aquatic Conservation: Marine and Freshwater Ecosystems, pages 1438-1451. Authors: T Moore, K Collier, and I Duggan (2019).

Chapter 3 has been published as “Non-native fish as glochidial sinks: elucidating disruption pathways for *Echyridella menziesii* recruitment in Hydrobiologia, https://doi.org/10.1007/s10750-019-04035-w. Authors T Moore and S Clearwater (2019).

Chapter 4 has been published as “Invasive macrophytes induce context-specific effects on oxygen, pH, and temperature in a hydropeaking reservoir” in River Research and Applications, https://doi.org/10.1002/ rra.3674. Authors T Moore, S Clearwater, I Duggan, and K Collier (2020).

Chapter 5 has been submitted to a relevant scientific journal as “Hydrology-mediated impacts of invasive macrophytes on freshwater mussels (*Echyridella menziesii*: Unionida) in a New Zealand hydropeaking reservoir”. Authors T Moore, S Clearwater, I Duggan, and K Collier.

Chapter 6 has been prepared in-part for submission to a relevant scientific journal as “Modelled impacts of non-native species on *Echyridella menziesii* recruitment”. Authors T Moore, S Clearwater, I Duggan and K Collier. Other parts of this chapter summarise pertinent information from the other chapters and provide a general discussion on the how this thesis contributes to the knowledge of non-native species and mussel interactions.

Chapter 1

General introduction

1.1 Biological invasions

Biological invasions consist of species that have a competitive advantage after natural obstacles to proliferation are removed, which may allow them to establish and rapidly spread in novel areas to become dominant in recipient ecosystems (Valéry et al. 2008). Invasions by non-native species (defined here as those that do not occur naturally in a particular realm) are globally recognised as one of the key threats contributing to accelerating biodiversity loss over recent decades (Sala et al. 2000, Dudgeon et al. 2006). Since freshwater ecosystems represent habitat for 10 % of all known species and are hotspots of biological invasions, they are especially vulnerable to non-native species impacts (Strayer and Dudgeon 2010). In particular, freshwater invertebrates face numerous conservation challenges due to extinctions, limited scientific knowledge, few representatives with legal status, and a low societal value linked to insufficient conservation expenditure (Strayer 2006). With enhanced global connectivity, resulting in added pressure on the interaction of high freshwater biodiversity values and human use of water resources, the threat of non-native species to lotic and lentic communities has accelerated (Johnson et al. 2008, Havel et al. 2015), leading to general impacts ranging from predation and habitat-modification to disruption of ecological processes altering food-web interactions and life-history linkages (Fei et al. 2014, Gallardo et al. 2016). However, other impacts may become apparent with the expected increase in future rates of biological invasions in line with globalisation and climate change (Malmqvist and Rundle 2002), especially from unnoticed cryptic invasions and/or impacts on closely associated species (Morais and Reichard 2017).

Invasive species can be particularly detrimental to affiliate (dependent) species in ecologically-balanced relationships that have co-evolved, whereby non-native species provide an unsuitable novel partner or indirectly manipulate existing species' links (Poos et al. 2010, Douda et al. 2013). Affiliate relationships that involve multiple suitable partners may have

redundancy when some generalist links are compromised by non-native species (Prior et al. 2015). However, for native species specialising in limited affiliate partners, such as a life-stage with an obligate relationship, the potential magnitude of non-native species effects can be exacerbated (Morais and Reichard 2017). Accordingly, there is a pressing need to understand the impacts of non-native species on specialised affiliate relationships, not only for species' conservation and targeted management, but also to identify the onset of impacts in newly invaded freshwaters.

New Zealand is a global hotspot for species' invasion and a global exemplar of how non-native species affect native species that have evolved in the absence of their impacts (Leprieur et al. 2008). Non-native vertebrates such as fish are widespread in New Zealand, with 33 % (21) of the extant fish community introduced, leading to a dramatic shift in aquatic communities' abundance and biomass towards non-native fish dominance (Collier et al. 2016, Duggan and Collier, 2018). The general effect mechanisms of non-native fish in lentic systems include: 1) bioturbation that reduces water clarity and redistributes nutrients to the water column; 2) degradation of habitat at the surface-water interface through mobilisation of sediment and consumption of plant material; and 3) top-down and/or bottom-up control of other trophic levels (Duggan and Collier, 2018). Combined, these direct and indirect mechanisms have potential to result in trophic cascades induced by non-native species, some of which are considered 'ecosystem engineers' (Gozlan et al. 2010). For example, the common carp (*Cyprinus carpio*) modifies the sediment-water interface through its foraging behaviour, which uproots plants and resuspends sediment, preventing plant growth and phytoplankton biomass, as well as altering the diversity and abundance of macroinvertebrates (Miller and Crowl 2006).

Another example of an invasive freshwater group with substantial impacts on native New Zealand ecosystems is non-native macrophytes, which comprise a total of 89 introduced species that have established since the 1850s, primarily through the aquarium trade (Champion 2014). Due to their massive biomass that often forms monocultures, non-native macrophytes displace native vegetation, especially in shallow lake areas where they can reach the surface (Hofstra et al. 2018). In these situations, non-native

macrophytes can also be considered ‘ecosystem engineers’, as well as ‘foundation species’, since they modify habitat, and dominate in abundance and influence on lentic ecosystems (Ramus et al. 2017, Wood and Freeman 2017, Emery-Butcher et al. 2020). Shifts towards communities dominated by non-native macrophytes can substantially modify environmental conditions at the sediment-water interface, such as silt accumulation, toxic ion release, and anoxia or hypoxia (Bunch et al. 2010, Andersen et al. 2017, Vilas et al. 2017). These impacts can become more pronounced at the end of summer following peak macrophyte biomass accumulation, in areas of low-water exchange, and during macrophyte senescence (Godshalk and Wetzel 1978, Madsen et al. 2001, Zohary and Ostrovsky 2011, Torma and Wu 2019). Therefore, sessile benthic organisms with early life stages developing during the summer period have potential to be sensitive to their impacts (Andersen et al. 2017).

1.2 Freshwater mussels and non-native species interactions

An order of particularly vulnerable sessile benthic organisms with affiliate species relationships is freshwater mussels (Bivalvia: Unionida). Unionid mussels use host fish to complete their ectoparasitic life-stage through attachment of larvae (glochidia) which transform into juveniles (Denic et al. 2015, Modesto et al. 2017). In New Zealand, three extant freshwater mussel species are recognised: *Echyridella menziesii*, *E. aucklandica*, and *E. onekaka* (Marshall et al. 2014). Of these, *E. menziesii* is the most widely distributed and abundant species, found in particularly high densities in Waikato lakes, North Island (James 1985, Phillips 2007, Marshall et al. 2014). *Echyridella menziesii* is a host generalist and there are many observations of fish species with attached glochidia in the field (e.g., *Gobiomorphus cotidianus*, *G. huttoni*, *G. gobiodes, Anguilla dieffenbachii* and *A. australis*, *Galaxias brevipinnis* (all native), and *Oncorhynchus mykiss* (non-native); Clearwater et al. 2014; Hanrahan 2019). *E. menziesii* glochidia have been successfully transformed into juveniles in laboratory experiments on a subset of these fish species: *Gobiomorphus cotidianus, Gobiomorphus huttoni*, *Galaxias brevipinnis, Galaxias vulgaris, O. mykiss*, *A. dieffenbachii* and *A. australis* (Clearwater et al. 2014b, Brown 2017; M. Melchoir pers.

comm). Despite this range of potential hosts, adult-skewed mussel population size-structures have been observed in the North Island and provide an indicator of reduced or failed recruitment (Roper and Hickey 1994). This has led in-part to the current conservation status of *E. menziesii* being designated as 'At Risk, Declining' (Grainger et al. 2018), a status that is supported by Māori oral history and anecdotal evidence documenting the loss of *E. menziesii* populations from New Zealand lakes and rivers (Rainforth 2008, Clearwater et al. 2013).

Outside of the extensively documented impacts of non-unionid bivalves, such as zebra and quagga mussels (*Dreissena polymorpha* and *D. bugensis*, respectively; Sousa et al. 2009, Karatayev et al. 2014), only 12 % of Unionoida species' evaluations by the International Union of Conservation of Nature Red List recognise the impacts of non-native species (IUCN 2018). Non-native fish appear likely to directly disrupt the co-evolved obligate ectoparasitic life stage of unionids, whereas non-native macrophytes may produce adverse environmental conditions detrimental to juvenile mussel survival (Bauer & Wächtler 2012; Berg et al. 2008). Other animal groups, such as invasive predators, may consume mussels as specialist molluscivores (e.g., muskrats (*Ondatra zibethicus*): Diggins and Stewart 2000, Owen et al. 2011) or opportunistically when mussels are exposed after floods or during droughts. Combined with other non-native groups of zooplankton, diatoms, and cyanobacteria, multiple invaders may facilitate mutual establishment in an 'invasion meltdown', further amplifying their effects (Simberloff and Von Holle 1999, Šlapanský et al. 2016).

Moore et al. (2019; see Chapter 2) reviewed interactions between unionid mussels and non-native species since then (2019) recent literature has underscored the need to recognize non-native species impacts on unionids, especially from invasive macrophytes that act as 'ecosystem engineers' and 'foundation species' (Emery-Butcher et al. 2020, Gagnon et al. 2020). Furthermore, dietary overlap between the invasive fish *Hypophthalmichthys molitrix* (Cyprinidae) and the unionid *Lampsilis siliquoidea* has highlighted a competitive pathway potentially resulting in reduced mussel growth (Tristano et al. 2019). Additionally, Bradshaw-Wilson et al. (2019) documented predation by the invasive fish *Neogobius melanostomus*

(Gobiidae) demonstrating the growing threats from invasive mussel consumers, and Pearson and Duggan (2020) investigated the potential of a non-native zooplankton (*Daphnia pulex*) to compete for agal resources with *E. menziesii*, although limited supporting evidence was found.

1.3 Interactions with flow regulation

Freshwater mussels can occur in high numbers in lakes and rivers modified for hydrogeneration through the construction of dams that can increase the vulnerability of upstream waterbodies to invasion by non-native fish and macrophytes (Gallardo et al. 2016). As the number of dams continues to increase globally (Zarfl et al. 2014), context-specific effects on hydrology (e.g., daily water-level fluctuations from hydropeaking) are accelerating the spread of non-native species in hydrolake littoral zones (Zhao et al. 2012, Shivers et al. 2018), as well as directly affecting resident native species. For example, a recent study in a hydropeaking reservoir built on the Navasota River, Texas, North America, found mussel community composition shifted towards species favouring more stable habitats post-impoundment (Khan et al. 2020).

Altered hydrologies may impact unionids by exposing mussel beds to desiccation or predation during low water-levels, or by exacerbating adverse water-quality conditions caused by increased lake residence times that restrict re-oxygenation of stagnant waters (Torma and Wu 2019). Furthermore, since invasive macrophytes often proliferate in these flow-regulated systems, control measures such as vegetation dredging or herbicide application may also impact unionid populations through physical removal or indirectly through prolonged anoxic and hypoxic events related to macrophyte decomposition (Aldridge 2000, Greer et al. 2016, Waltham and Fixler 2017). Therefore, flow regulation may be an important context-specific factor to consider for mussel conservation that mediates non-native macrophyte and fish interactions.

The Waikato River system consists of a highly regulated chain of eight hydrogeneration reservoirs, the most downstream of which (Karāpiro) was the focal field site of this thesis (Chapter 4 – Moore et al. 2020; Chapter 5). For the purposes of this study, Karāpiro was divided into two sections with

contrasting hydrologies and different dominant non-native macrophyte species: the lower-lacustrine section is subject to variable water levels and supports *Ceratophyllum demersum* beds, while the upper-riverine section experiences variable flows with macrophyte beds dominated by *Egeria densa* (Clayton et al. 2009). Accordingly, Karāpiro enabled a comparison of how variable flow-hydrologies and non-native macrophyte species interact to promote adverse environmental conditions at the sediment-water interface, and how these conditions influenced mussel population size-structure and density. This hydroreservoir is also highly-invaded by non-native fish species and so provided the opportunity to explore scenarios involving coupled effects of non-native macrophyte and fish interactions on freshwater mussel populations.

1.4 Aim and objectives

This thesis aims to contribute knowledge of unionid mussel and non-native species interactions in modified freshwater environments that will assist with species management and conservation. The first objective was to identify known and likely interactions between Unionoida and non-native species, with particular reference to New Zealand, through a global meta-analysis of published literature to review the current state of knowledge and information gaps. In the context of this review, which highlighted the potential threat of non-native fishes as unsuitable mussel-hosts elsewhere, the second objective was to determine host suitability of selected non-native fish for *E. menziesii* glochidia to test if shifts from fish communities dominated by native species to communities dominated by non-native species could contribute to reduced mussel recruitment. The third objective focussed on effects of invasive macrophytes on water quality and benthic habitat in a hydropeaking reservoir, and how these factors interacted to affect freshwater mussels. The final objective was to understand the relative contribution of known interactions of non-native fish and non-native macrophytes leading to reduced *E. menziesii* population recruitment, then broadly apply these insights in the context of the overseas literature to inform the importance of non-native species in freshwater mussel conservation in flow-regulated environments. My overarching hypothesis is that non-native species proliferation adversely affects the density and size-

structure of *E. menziesii* populations through (i) disruption of the obligate parasitic larval stage of mussels with host fish, and (ii) promotion of detrimental environmental conditions at the sediment-water interface beneath macrophyte beds which are likely to disproportionally affect the juvenile life stage of mussels.

1.5 Thesis overview

To address the objectives above, the following four chapters have been published in, or submitted to, peer-reviewed scientific journals.

Chapter 2 presents a global meta-analysis of literature examining interactions between Unionoida and non-native species. The search identified major non-native groups that had known and probable interactions with unionids, then applied this knowledge to the New Zealand context for development of future research directions. Of particular relevance to the chapters that follow were host interactions with non-native fish and the effects of invasive macrophytes.

Chapter 3 examines the ability of non-native fish to disrupt the obligate ectoparasitic life-stage of *E. menziesii* using a laboratory experiment. Here I quantified glochidial attachment and juvenile metamorphosis rates on three non-native fish to compare with a known native host. All fish species used in this experiment were known to occur in the main study site (Karāpiro) where mussels were also collected.

In Chapter 4, a field survey was used to investigate the water quality conditions at different depths associated with non-native macrophyte beds in littoral zones, and how these effects were influenced by hydrogeneration management operations and macrophyte spraying which occurred unexpectedly during the study. The two non-native macrophyte species studied dominated in different sections of Karāpiro with contrasting hydrologies; *Ceratophylum demersum* and *Egeria densa* dominated in the lower-lacustrine (variable water level) and upper-riverine (variable water flow) sections of the lake, respectively.

Chapter 5 extends the work in Chapter 4 by examining interactions between non-native macrophytes, physicochemical conditions at the sediment-water

interface and within surficial sediments, and overarching hydrology on the adult *E. menziesii* population in the littoral zones of Karāpiro. I used structural equation models to test how mussel population size structure, biomass and density varied inside and outside dense macrophytes in the lower-lacustrine and upper-riverine sections of Karāpiro, with a particular focus on evidence of recruitment, and the direct and indirect mechanisms that may explain these relationships.

Finally, Chapter 6 combines the findings of previous chapters to model how various hypothetical scenarios of non-native fish and non-native macrophyte dominance potentially disrupt *E. menziesii* recruitment. This part of the chapter will be developed for a future publication. Furthermore, this chapter also provides a synthesis of the key findings from the preceding chapters, identifies the main conclusions in relation to management implications for non-native species threats and freshwater mussel conservation, and discusses future research directions.

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Chapter 2

Interactions between Unionida and non-native species: a global meta-analysis

2.1 Abstract

Understanding the multiple agents of decline is important for the conservation of globally threatened Unionida (Class Bivalvia), but threats from non-native species have received limited attention outside of non-unionid bivalves. To address this gap, a global meta-analysis was conducted aimed at identifying known interactions and mechanisms of impact and informing potential effect pathways for the New Zealand unionid fauna. The main non-native groups identified as interacting with unionids were fish (38% of published studies), macrophytes (33%), and vertebrate predators (30%), with ~70% of interactions leading to adverse impacts on mussels. Most studies used field surveys (~50%) and were conducted in rivers (~50%). Impacts occurred across the unionid life cycle (adult, glochidia, host, and juvenile), and primarily affected processes that determine the transitions between life-cycle stages (fertilization, infestation, settlement, and maturation). The impacts of non-native macrophytes and fish were predicted to be greater for transitional stages than the impact of vertebrate predators, which mostly affected adult mussels. New Zealand Unionida are most likely to be affected by interactions with non-native species in lowland lakes and waterways, where connectivity for diadromous native fish hosts and high bioinvasion potential intersect.

2.2 Introduction

The order Unionida (Bivalvia) represents 72% of the global diversity of freshwater bivalves (Lopes-Lima et al., 2018). They are distributed across all continents, except in glaciated and desert areas, with diversity hotspots in the United States of America, Central America, the Indian subcontinent, and Southeast Asia (Bogan, 2008; Graf & Cummings, 2007; Lopes-Lima et al., 2018; Lydeard et al., 2004). The largest Unionida superfamily (Unionidae) likely originated from Southeast and East Asia during the

Jurassic age, and has an extraordinary diversity and unique life-cycle that defines the unionid group (Bolotov & Kondakov et al., 2017). To reproduce, unionid mussels must attach larvae (glochidia) to an often narrow range of fish hosts, before transformation into juveniles (Barnhart, Haag, & Roston, 2008; Berg, Levine, Stoeckel, & Lang, 2008). Host fish serve as agents of unionid mussel dispersal, as well as providing energy and nutrients for growth of encysted glochidia (Denic, Taeubert, & Geist, 2015). Although unionid mussels occur in most freshwater habitats, highest diversity and biomass are found within medium to large rivers, typically in dense multispecies beds that contribute the majority of benthic invertebrate biomass (Strayer et al., 2004). When occurring in high abundances, freshwater mussels can have important ecosystem functions, sometimes acting as ecosystem engineers (Boeker, Lueders, Mueller, Pander, & Geist, 2016; Vaughn, 2018). Since unionid mussels are relatively long-lived (most lifespans range between 15 and 40 years in North America (Haag, 2012) and nearly 200 years for the European freshwater pearl mussel (*Margritifera margaritifera*) (Bauer, 1992)), and some species function as ecological indicators (Atkinson, Christian, Spooner, & Vaughn, 2014), umbrella, and flagship species, they are important targets for aquatic conservation efforts (Geist, 2010, 2011).

Functions that unionid mussels perform can be categorised into regulating, supporting, provisioning, and cultural ecosystem services (Vaughn, 2018). For example, mussel biofiltration regulates water quality by removing various particles (e.g., phytoplankton, zooplankton, bacteria, and suspended/re-suspended algae) from the water column and interstitial sediments (Raikow & Hamilton, 2001; Vaughn, Nichols, & Spooner, 2008). Mussel biofiltration is extremely resilient across a wide range of suspended solids concentrations (Lummer, Auerswald, & Geist, 2016), and in high densities unionids can even deplete phytoplankton biomass sufficiently to markedly improve water quality and cause ‘biological oligotrophication’ (Chowdhury, Zieritz, & Aldridge, 2016; Ogilvie & Mitchell, 1995; Welker & Walz, 1998). Supporting services by mussels include nutrient cycling and storage, which couples benthic and pelagic ecosystem compartments though biodeposition of filtered material excreted as faeces or

pseudofaeces (e.g., for algae and heterotrophic bacteria), and accumulation of nutrients in their tissues (Atkinson & Vaughn, 2015; Vaughn et al., 2008). These processes promote retention of nitrogen (N) and phosphorus (P) within the freshwater ecosystem and assimilation into the food web, rather than propagation downstream towards marine environments where they may remain bioavailable and have the potential to contribute to eutrophication (Paerl, 2009; Vaughn, 2018). Hoellein, Zarnoch, Bruesewitz, and DeMartini (2017) calculated that the maximum potential quantities of N removed by two unionid mussel species (*Lasmigona complanata* and *Pyganodon grandis*, in estimated populations of 610,000 and 170,000 individuals, respectively) in the East Branch DuPage River, North America, was equivalent to a waste water treatment plant costing US$266,638 per year.

Mussel aggregations also function to increase aquatic biodiversity by providing or modifying habitat for algae and macroinvertebrates, respectively, which then support higher trophic levels and adjacent ecosystems (Aldridge, Fayle, & Jackson, 2007; Vaughn, 2018; Vaughn et al., 2008). For example, Allen, Vaughn, Kelly, Cooper, and Engel (2012) found unionids likely altered the mussel-derived N:P ratios that determined benthic algal community structure; in turn, this algal shift (towards diatom dominance) significantly increased the emergence rate of grazing aquatic insects linked to spider abundance in the riparian zone. Unionids also influence links from terrestrial to freshwater ecosystems, as shown by Smith, Aldridge, and Tanentzap (2018) who found mussel density was substantially stronger in determining geochemical sediment composition and associated littoral organism abundance (e.g., zooplankton and benthic algae) than terrestrial organic matter inputs. Finally, mussel provisioning and cultural values demonstrate the socio-cultural connections people have with freshwater environments. For instance, in New Zealand, freshwater mussels (primarily *Echyridella menziesii*) were part of the historical indigenous Māori diet, as well as integrated within their belief system where all things are interconnected through whakapapa (genealogy) (Hamilton, 1908; Hiroa, 1921; Rainforth, 2008; Watt, 1969).

In New Zealand, three extant species of freshwater mussel (Unionida: Hyriidae) are recognised based on recent DNA sequence data; *E. menziesii*, *E. onekaka*, and *E. aucklandica* (Marshall, Fenwick, & Ritchie, 2014). These endemic unionid species belong to the Hyriidae family, which is only found in the Southern Hemisphere (other countries include Australia, New Guinea, and South America (Graf, Jones, Geneva, Pfeiffer, & Klunzinger, 2015)). The most widely distributed and abundant species is *E. menziesii,* which is found throughout the North and South Islands, with the other species having sparse and/or localised distributions (James, 1985; Marshall et al., 2014; Phillips, 2007). New Zealand freshwater mussels are relatively large-bodied (20 g of wet flesh weight (Clearwater, Thompson, & Hickey, 2013)), and *E. menziesii* has been reported to live up to 55 years (Grimmond, 1968; James, 1985; Roper & Hickey, 1994). New Zealand freshwater mussels perform similar functions to unionid mussels elsewhere, in terms of filtration, biodeposition and nutrient excretion rates (Collier, Clearwater, Neijenhuis, & Wood, 2017; Cyr, Collier, Clearwater, Hicks, & Stewart, 2016)).

Among threatened freshwater animal groups, the Unionida mussels are the most imperilled, having undergone severe global declines in diversity and biomass over the last century (Haag & Williams, 2013; Lopes-Lima et al., 2016; Walker, Jones, & Klunzinger, 2014; Zieritz et al., 2017). As with other aquatic invertebrates facing biodiversity losses, Unionida are grossly under-represented in conservation status assessments, with few species targeted for management efforts (Collier, Probert, & Jeffries, 2016). At present, the IUCN Red List includes 536 Unionida species, with 32 categorised as Extinct or Extinct In The Wild, 167 Critically Endangered, Endangered, or Vulnerable (together representing 31% of evaluated species), and 89 as Data Deficient (IUCN, 2018). In New Zealand, all three extant mussel species are considered Nationally Threatened or At Risk (Grainger et al. 2014). The concern over declines in unionid mussel distribution and population abundance is further supported by the commonly-observed, adult-skewed size structure, which may be the result of insufficient juvenile recruitment to sustain populations over the long term (Araujo & Ramos, 2000; Bailey & Green, 1989; Green, 1980; Harriger, Moerke, & Badra, 2009; Hastie & Toy, 2008; James, 1985).

The greatest global threats to freshwater bivalves as assessed by the IUCN Red List were pollution and natural system modification, which accounted for 42% and 20% of records, respectively (Lopes-Lima et al., 2018). Urban development, exploitation, agriculture, climate change, mining, and non-native species also play a role (together representing less than 10% of records). Lopes-Lima et al. (2018) showed the relative percentages of recorded threats was generally similar across the global ecoregions they examined (Afrotropical, Australasian, Indotropical, Nearctic, Neotropical, and Palaearctic). However, pertinent to this global meta-analysis, Australasia has a higher proportion of agricultural related-threats resulting primarily from water diversion and extraction (Lopes-Lima et al., 2018), with eutrophication of particular concern in New Zealand along with loss of connectivity for diadromous host fish species. No significant impacts from non-unionid bivalves or overharvesting have so far been identified in Australasia (for a comprehensive list of impacts see Table 3 in Walker et al. (2014)).

Outside of the extensively-documented effects of non-unionid bivalves such as zebra mussels (*Dreissena polymorpha*) and quagga mussels (*D. bugensis*), the threat of non-native species on unionid mussels has received limited attention (Karatayev, Burlakova, & Padilla, 2014; Sousa, Gutiérrez, & Aldridge, 2009). Non-native species (defined here as species that do not occur naturally in a particular country) that modify habitat, are directly related to the Unionida life-cycle, or are consumers of freshwater mussels require particular attention, since there is evidence to suggest they may be particularly important drivers of unionid populations (Lopes-Lima et al., 2016). In fact, these threats may be underrepresented, as the IUCN Red List only recognised impacts of non-native species in 12% of Unionida species evaluations (IUCN, 2018). The long life span and co-evolved reproductive associations with specific fish hosts makes unionid mussels susceptible to potentially strong effects from non-native species invasion. Specifically, life-cycle disruption appears likely from non-native fish during the mussel obligate ectoparasitic stage, while non-native macrophytes and non-aquatic predators may adversely affect the adult sessile stage (Bauer & Wächtler, 2012; Berg et al., 2008). Lowland lakes and rivers, where

freshwater mussels can occur in high numbers, are often hotspots for human-assisted invasion, particularly in modified freshwaters such as hydroelectric reservoirs where environmental conditions promote non-native species dispersal and establishment (Collier, Leathwick, & Rowe, 2016; Früh, Stoll, & Haase, 2012; Havel, Kovalenko, Thomaz, Amalfitano, & Kats, 2015).

In the future, interactions between non-native species and freshwater mussels are likely to increase due to global biotic homogenisation (Douda et al., 2013) and climate change (Spooner, Xenopoulos, Schneider, & Woolnough, 2011). Given this impending issue, and the significant role dense mussel populations play in freshwater ecosystem processes, it is timely to evaluate evidence for the poorly-documented impacts of species invasions, and consider implications for New Zealand which is considered a freshwater invasion hot-spot (Leprieur, Beauchard, Blanchet, Oberdorff, & Brosse, 2008). Accordingly, a global meta-analysis was conducted to: 1) identify confirmed and known probable interactions between Unionida and non-native species; 2) propose mechanisms by which non-native species' groups potentially influence unionid life-stages; 3) determine knowledge gaps and directions for future research; and 4) evaluate the implications of this analysis for the New Zealand unionid mussel fauna.

2.3 Methods

Three searches were conducted of publications that examined interactions between Unionida and non-native species using the Web of Science database search engine (search date: 20.10.17). The first search aimed to identify all literature relating to freshwater mussels and non-native species interactions, and was performed on article title and topic by crossing the following keywords: [freshwater* OR lake* OR stream* OR river* OR pond*] AND [union* OR bivalve* OR glochid* OR mussel* OR naiad* OR clam*] AND [inva* OR exotic* OR nonindigenous* OR non-indigenous* OR pest* OR alien* OR nonnative* OR non-native* OR native* OR affiliate OR host-parasite]. This search returned 1422 articles published from 1967 to October 2017.

As the vast majority of literature returned in the first search investigated various impacts of non-unionid mussels, most notably non-native *D. polymorpha* and *Corbicula fluminea*, a second independent search was conducted on the Web of Science to distinguish only interactions between Unionida and non-unionids by appending with the keywords: AND [zebra OR dreiss* OR polymorpha OR corbicula OR quagga OR limnoperna* OR golden* OR sinano* OR Dreissena-polymorpha]. This search was conducted to determine the proportion of the literature that investigated interactions between non-unionids and Unionida. However, since non-unionid and Unionida interactions have been reviewed extensively elsewhere (e.g., Fei, Phillips, & Shouse, 2014; Lopes-Lima et al., 2016; Nobles & Zhang, 2011; Sousa et al., 2009; Sousa, Novais, Costa, & Strayer, 2014), this literature was excluded from the meta-analysis. Finally, a third independent search that excluded non-unionid mussels was conducted to represent interactions between unionids and all other non-native species; the analysis was performed by replacing the appended search term above from 'AND' to 'NOT'. These three searches were conducted independently on the Web of Science database rather than nested to ensure wider capture of relevant articles. For this reason, the totals of searches two (1141 articles) and three (315 articles) exceed the total articles retrieved in search one (1422 articles).

Search outputs were summarised using the package 'bibliometrix' v1.7 (Aria & Cuccurullo, 2017) to compare the number of articles published over time (Table 7-1 in Appendix 7.7.1). Each abstract was examined to determine its relevance to the motivating question using the following criteria: 1) the freshwater mussel species, or the dominant species in a mussel assemblage, must be native and from the Order Unionida; and 2) non-native species must be a habitat modifier, directly involved in the unionid life-cycle, or a consumer of freshwater mussels. Articles that were not excluded based on their abstract or title were read in full. The cited literature of selected articles, and topic themes connecting relevant papers (e.g., parasitology and mussel microhabitat studies), were examined to identify other potentially relevant articles not found from the Web of Science searches. Due to the limited number of relevant articles available, studies

documenting both qualitative and quantitative results were included, as well as data collected from unpublished sources. If selected articles on the interactions between Unionida and a non-native species group numbered at least ten publications (i.e., fish, macrophyte, and predators), they were analysed and presented in summary tables (Tables 7-2, 7-3, and 7-4 in Appendix 7.1.2). This article threshold was selected to provide some confidence in general inferences made. Rejected non-native species groups (i.e. <10 articles) that had interactions with Unionida were zooplankton, diatoms, and Cyanobacteria.

The following attributes for each species group were collected; freshwater mussel species, life-stage and response to the non-native species, the non-native species involved, method (if any) used to determine the significance of effects, effect direction (positive, negative, neutral, or unknown), study type, ecosystem, and country. The attribute “significance of effects” reflected the authors’ inferences that ranged in strength from observational (i.e., where effects are inferred without statistical support), to correlative with statistical support, through to experimental effects with statistical support. Additional attributes were collected specific to each non-native species group. For macrophytes, the dominant native unionid species and non-native macrophyte species were recorded, along with information on plant habitat traits (floating, submerged, or emergent). For fish, typical habitat (benthic or pelagic) was recorded along with whether unionid mussels were host generalists or host specialists in terms of glochidial attachment. For unionid consumers, the predator name and type (freshwater or terrestrial) were recorded.

2.4 Results

2.4.1 **Literature search**

Articles returned from the literature search related to interactions between Unionida and non-native species were largely made up of the same list of publications as that returned from the refined search on interactions between unionid and non-unionid mussel species only (Table 7-1 in Appendix 7.1.1). In comparison, articles returned from the literature search related to Unionida and non-native species excluding non-unionid mussels

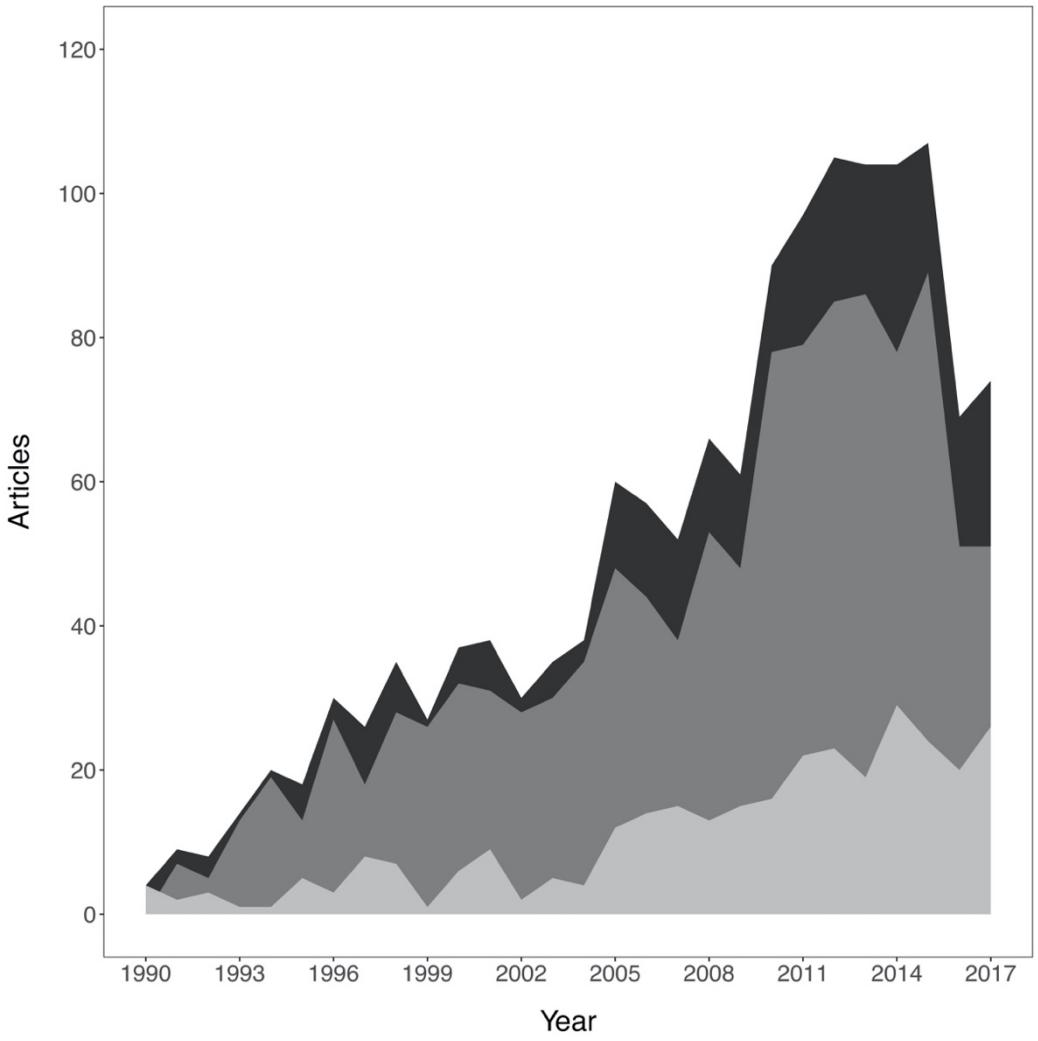

Figure 2-1: Comparison of three independent searches identifying literature relating to interactions between Unionida and (1) non-native species (black); (2) non-unionid freshwater mussels (dark grey) and; (3) non-native species other than non-unionid mussels (light grey). Where the sum of articles from the latter two searches does not equal all non-native species interactions in a given year, this indicates overlap in articles between the three separate searches. See text for details of search criteria.

identified only 315 articles and a slightly lower annual increase in publication rate (10.4% per annum compared to 13.5% and 15.7% for the other searches, respectively). All searches returned articles predominantly from North America (~60% of literature), and there was a noticeable increase in the number of articles published per year from 2002 (Figure 2-1) following invasion of the Great Lakes by dreissenid mussels (Scholesser & Schmuckal, 2012).

Articles comprised ~50% field surveys, and ~25% each for laboratory experimental and observational studies. Rivers were the most commonly studied ecosystem at ~50% of articles, with lakes comprising ~25%; the

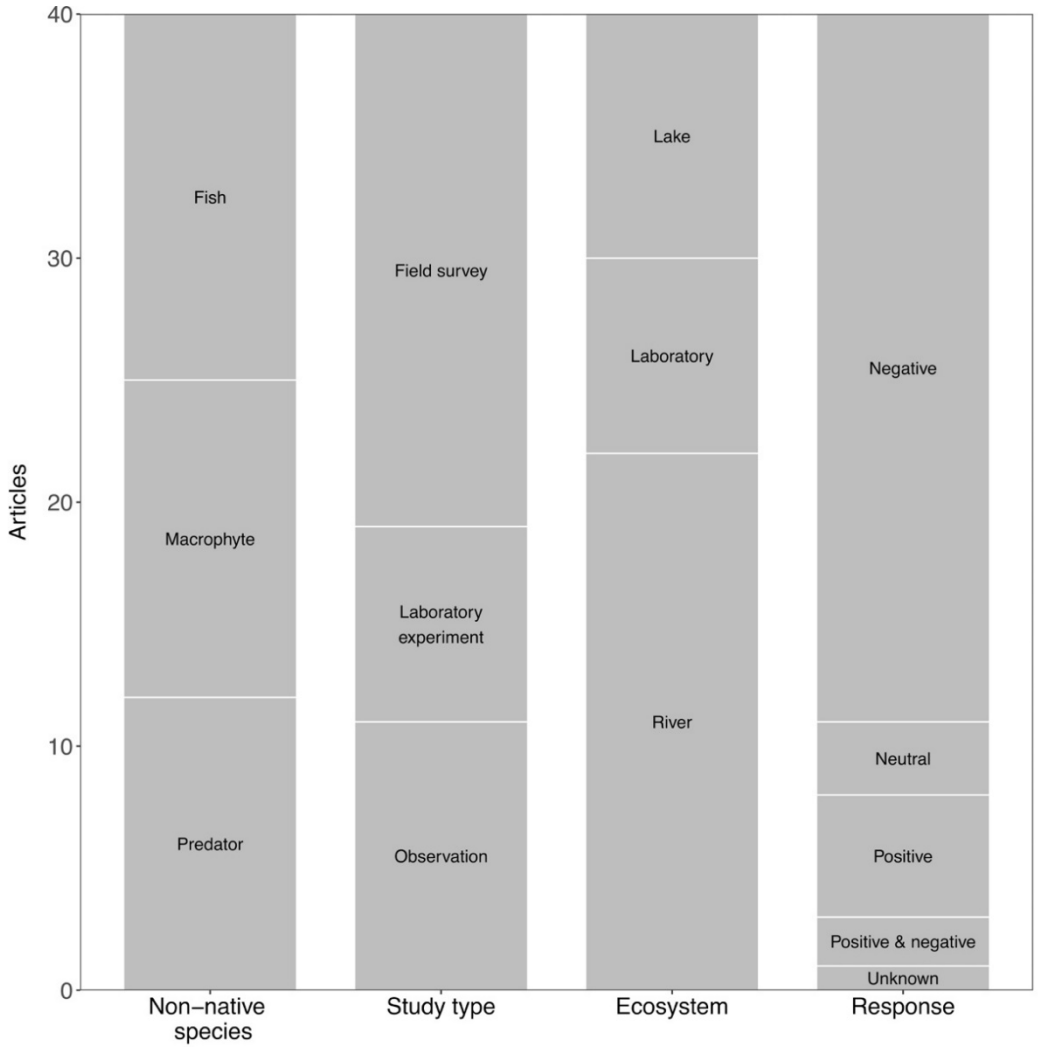

Figure 2-2: Summary histogram showing number of articles reviewed in the global meta-analysis partitioned by non-native species group, study type, ecosystem, and response.

remainder of studies was conducted in laboratories. A negative response between non-native species and Unionida was documented in ~70% of articles, while ~13% showed positive responses, ~8% were neutral, with other categories totalling ~9 % (Figure 2-2).

2.4.2 **Fish**

The selected literature identified 15 articles that focused on assessing the suitability of non-native fish as hosts for freshwater unionid glochidia across eight countries (Table 7-2 in Appendix 7.1.2). Notably, six articles conducted field surveys to identify the prevalence and mean intensity (infestation rate) of glochidia on non-native fish species to provide information on their suitability; hosts found in the field were termed ‘ecological hosts’ (Levine, Lang, & Berg, 2012). The remaining eight studies conducted laboratory

experiments to assess the suitability of non-native fish as 'physiological hosts' by determining glochidia transformation or metamorphosis rates into juvenile mussels. One study by Salonen, Marjomäki, and Taskinen (2016) used both laboratory experiments and field surveys to asses fish host suitability. All unionid species assessed were host generalists (except *Lampsilis cardium*; Watters & O'Dee 1998). Across all studies, 136 laboratory experiments were conducted to assess non-native fish host suitability. The Cyprinidae family and *Neogobius* genus were well represented in trials testing host suitability of the Unionidae genera *Anodonta* and *Unio*. Tested fish species were predominantly benthic dwellers or feeders.

Interactions between Unionida mussels and non-native fish species were mostly negative (n=9), such that glochidia failed to attach or had a very low transformation rate in the laboratory, or had lower prevalence or mean intensity of glochidia in the field compared to native hosts, although some studies also found both negative and positive responses (n=2) for different fish species. Positive effect directions (i.e., transformation rates were approximately equal or exceeded native hosts) were only found for one study (Watters & O'Dee, 1998), and for two studies effect direction was not determined (Araujo & Ramos, 2000; Zhokhov, Pugacheva, & Molodozhnikova, 2017). Only three studies based inferences on statistically significant differences, and most results were based on comparisons of non-native host suitability relative to native hosts.

2.4.3 **Macrophytes**

A total of 13 studies identified interactions between Unionida and non-native macrophyte species from five countries in lake (n=7) and river (n=6) ecosystems. All studies involved a field survey to assess the response of the adult freshwater mussel life-stage (although Hastie, Boon, & Young, 2000 also identified juveniles) in relation to submerged (n=10), floating (n=1; Lopes-Lima et al., 2016), or emergent (n=2; Burlakova & Karatayev, 2007; Hastie et al., 2003) macrophyte plant forms. The predominant mussel response was measured as density per m^2 (n=9). Studies that recorded assemblages of unionid mussels were usually dominated by one unionid

species, and in diverse macrophyte beds the dominant macrophyte species was always non-native (Table 7-3 in Appendix 7.1.2).

Interactions between unionid mussels and non-native plant species were mostly negative (n=7), with evidence provided from statistical analysis or observation of pronounced declines (e.g., a 'considerable decrease') in mussels within macrophyte beds (Sorrell, Phillips, Wells, & Sykes, 2007). Often, strong negative relationships were reported between Unionida density and non-native macrophyte bed density in lake ecosystems (Burlakova & Karatayev, 2007; James, 1985; Lopes-Lima et al., 2016; Sorrell et al., 2007). Where effect direction was positive (n=3), the statistical evidence was weak (i.e., Weatherhead & James, 2001), or based on observation (n=2); all of these studies were in river ecosystems (Nobes, 1980; Salmon & Green, 1983). Three studies had a neutral effect direction, where the relationship was not statistically significant, although all displayed weak positive relationships between unionid density and non-native macrophyte cover (Butterworth, 2008; Hastie et al., 2000; Lodge, 2012).

2.4.4 **Predators**

In total, 12 articles were identified that observed predation of at least 10 species of native adult freshwater mussels by non-native species spanning eight countries from lake (n=3) and river (n=8) ecosystems; Parisi and Gandolfi (1974) observed predation in both rivers and lakes (Table 7-4 in Appendix 7.1.2). Most studies were observational, with only two articles documenting a quantitative response (Saarinen & Taskinen, 2003; Xuan et al., 2015). The effect direction of predation in all studies was negative, although hypothesised to be weak in some cases (Cosgrove, Hastie, & Sime, 2007; Xuan et al., 2015). The non-native mammalian predators involved were rats (n = 5; *Rattus norvegicus*, *Hydromys chrysogaster*, and other *Rattus* spp.), the feral hog (n=3; *Sus scrofa*), American mink (*Mustela vison*), muskrat (*Ondatra zibethicus*), and red fox (*Vulpes vulpes*). A non-native amphibian, *Lithobates catesbeianus*, was also recorded as a freshwater mussel predator in China (Xuan et al., 2015).

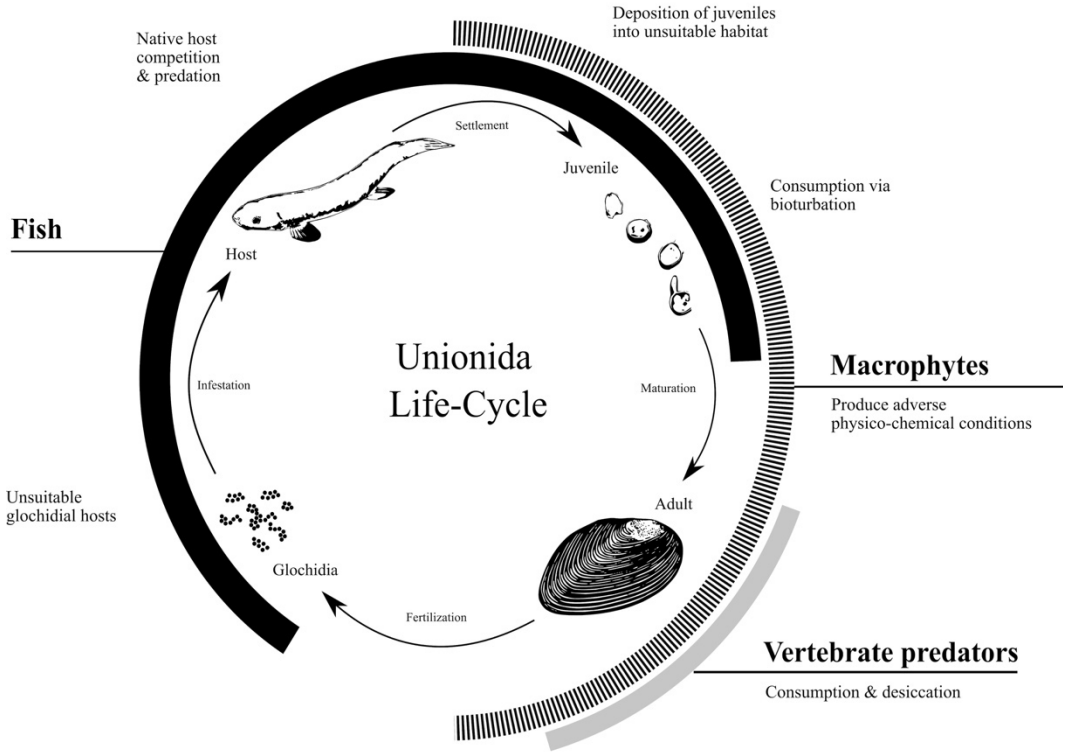

Figure 2-3: Conceptual stage-based diagram of the hypothesised interactions revealed in this global meta-analysis. Non-native species, grouped into macrophytes, fish, and predators, are predicted to interact with unionid freshwater mussels across different life-stages (adult, glochidia, host, or juvenile) and processes that determine the transition from one life-stage to another (fertilisation, infestation, settlement, and maturation). Predicted effect magnitude (thicker lines indicate stronger interactions) is depicted; i.e., fish are predicted to have the strongest impact, then macrophytes, and finally predators. Effect mechanisms are labelled.

2.5 Discussion

The global meta-analysis has identified major threats to different stages of the Unionida life cycle (adult, glochidia, host, and juvenile) through interactions with non-native macrophytes, fish, and non-aquatic predators. Recorded interactions were mostly negative (~70%) and occurred through mechanisms that affected fertilisation, infestation, settlement, and maturation. The conceptual model developed from this meta-analysis highlighted host suitability, competition and predation, along with juvenile habitat suitability and incidental and targeted predation, as key effect pathways on unionid mussels induced by non-native species (Figure 2-3).

Articles returned from the literature searches were geographically biased towards North America, a trend shared with other literature reviews involving unionid and non-native species (Modesto et al., 2017; Sousa et al., 2014). This was unsurprising since North America has a large number of unionid species (~ 300), of which most are threatened (Haag & Williams, 2013). Although selected articles for this analysis were more evenly distributed at a global scale (i.e., across North America, Europe, and Australasia), unionid diversity hotspots in the Indian subcontinent (Lopes-Lima et al., 2018) and Southeast Asia (Bolotov, Vikhrev, et al., 2017) remain underrepresented. Only nine articles, all involving observation or terrestrial predation, were recovered for New Zealand from the literature searches. This lack of global representation inhibits a generalised understanding of the interactions between unionid and non-native species groups (Modesto et al., 2017).

The comparison between the third independent search (n=315) and selected articles (n=40) only found a small overlap in identical publications, which indicated both information collection methods were required to capture knowledge related to unionids and non-native species interactions. Across freshwater ecosystems and study types, the availability of information for non-native species was broader for fish than macrophytes and vertebrate predators, a focus most likely reflecting the direct role fish hosts have in the unionid life-cycle (Berg et al., 2008). Overall, studies including statistical support were the most useful in determining interactions between unionid and non-native species. Nonetheless, a large proportion of these studies reported summary statistics only, limiting the inferences that could be made.

2.5.1 **Fish**

The majority of non-native fish species were not suitable hosts for glochidia of native unionids, suggesting that this group of mussels has not adapted to shifts towards non-native fish dominated communities (Modesto et al., 2017; Poos, Dextrase, Schwalb, & Ackerman, 2010). However, contrary to expectations, a few non-native fish had equal or higher transformation rates than native hosts in laboratory trials (Huber & Geist, 2017; Mierzejewska et al., 2014; Watters & O'Dee, 1998), although host identification using

laboratory experiments does not necessarily validate host suitability in the field or other places where fish interactions occur (Levine et al., 2012). This finding demonstrates the value of studies using multiple methods to assess suitability, including both standardized laboratory studies and field experiments (Taeubert, Gum, & Geist, 2013). For example, Salonen et al. (2016) used experimental trials, cage experiments and field surveys to provide multiple lines of evidence to confirm that non-native brook trout (*Salvelinus fontinalis*) were poor hosts of the European freshwater pearl mussel (*M. margaritifera*). Overall, negative non-native fish interactions were identified in the meta-analysis, highlighting a need for future research to address the effects of reduced recruitment at the mussel population scale.

Several likely mechanisms support the negative interactions with non-native fish hosts identified in selected articles. These include, incompatible physiology (e.g., immune response to glochidia), differentiation between fish and mussel ecological niches, and the long time required for co-evolutionary adaptations to develop (Berg et al., 2008; Mierzejewska et al., 2014). Moore & Clearwater (2019) (Chapter 3) found a combination of these mechanisms may have prevented glochidial transformation on non-native brown bullhead catfish (*Ameiurus nebulosus*) after successful glochidia attachment in laboratory experiments (Figure 2-4). This finding substantiates the ability of non-native fish to act as glochidial sinks, reducing the reproductive capacity available for suitable native host species (Tremblay, Morris, & Ackerman, 2016). Nonetheless, if some non-native fish species can serve as suitable mussel hosts, they may provide unexpected benefits where native fish hosts have been displaced (Araujo, Bragado, & Ramos, 2000), and thus provide a novel dispersal vector (Sakai et al., 2001). Ultimately, the effect direction of fish-mussel interactions is context- and species-dependent, with recent evidence suggesting non-native fish species with geographically distinct lineages (Reichard et al., 2015) and previous glochidial exposure (Donrovich et al., 2017) may also mediate host-mussel interactions.

Directions for future research focused on conservation of the mussel-fish host relationship have been thoroughly reviewed by Modesto et al. (2017). Pertinent to this review, and of particular relevance to New Zealand where

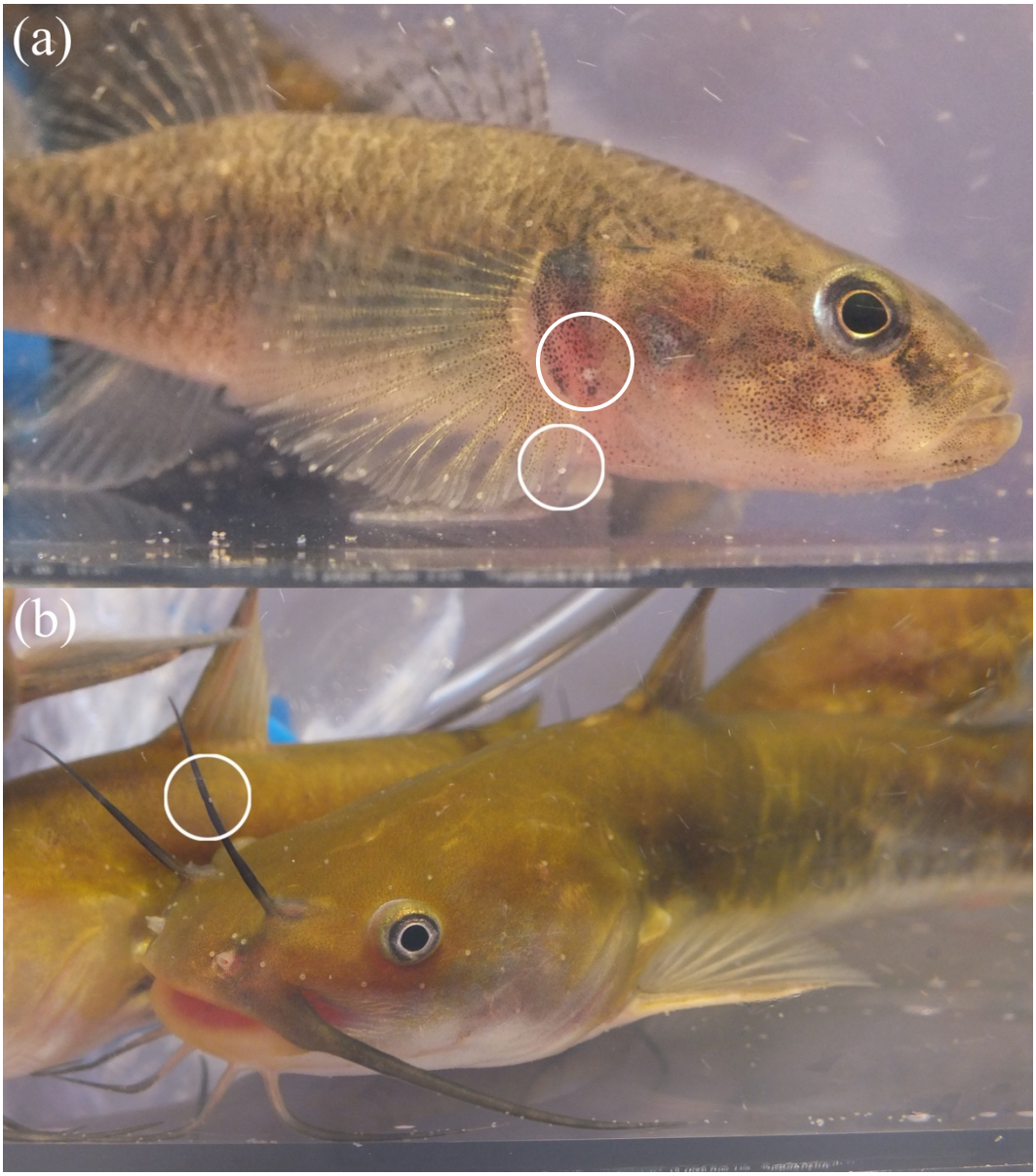

Figure 2-4: A) known native fish host, the common bully (*Gobiomophus cotidianus*), of the New Zealand freshwater mussel, *Echyridella menziesii*. Glochidia shown are attached to the edge of fins and operculum. B) non-native brown bullhead catfish (*Ameiurus nebulosus*) with glochidia attached to fins and sensory organs (not shown), and barbs (Moore and Clearwater, 2019) (Chapter 3).

a large proportion of the native fish fauna is diadromous, is the need to consider fish-passage connectivity when developing mussel conservation and invasive species management strategies, particularly in relation to unionid source populations (Benson, Close, Stewart, & Lymbery, 2018; Bódis, Tóth, & Sousa, 2016). While fish barriers may restrict native host-fish movement and thus mussel dispersal, as well as impacting the recruitment

of obligate diadromous fish-hosts that require access to the sea (Clavero, Hermoso, & Cao, 2015; Vaughn, 2012), they may also prevent spread of potentially unsuitable non-native fish hosts that would reduce unionid recruitment through the mechanisms highlighted above. Furthermore, barriers to fish migration may provide time for co-evolutionary adaptions to develop enabling mussels to successfully parasitize non-native fish, although fish may also evolve counter adaptations (Douda et al., 2017; Douda et al., 2013). An interesting direction for future research is the influence of marginal/poor hosts on mussel recruitment at the population scale, and if this changes over time where native fish hosts are excluded.

2.5.2 **Macrophytes**

Comparison across studies was limited in the meta-analysis as macrophyte density/coverage was measured in multiple ways (e.g., presence/absence, percent coverage, biomass, density) and involved multiple species. Nevertheless, the strength of non-native macrophyte impacts appears to be mediated by the size and density of the macrophyte bed, rate of water exchange, and natural seasonal and diurnal variations (Caraco & Cole, 2002; Turner, Cholak, & Groner, 2010; Wilcock, Champion, Nagels, & Croker, 1999), which can lead to both positive and negative effects on unionids depending on the context. In two Texas, USA, impoundments, adult unionid density was negatively correlated ($r = -0.49$) with percent coverage of *Myriophyllum spicatum* (50% cover) and *Neluumbo lutea* (60% cover), while a third lake with 10% cover of mainly non-native *Chara* sp. had no correlation with unionid density (Burlakova & Karatayev, 2007). Similarly, in New Zealand, high adult mussel density below, and low densities within, dense beds of *Ranunculus trichophyllus* and *Elodea canadensis* have been reported (Cyr, Phillips, & Butterworth, 2017; James, 1985; Weatherhead & James, 2001).

Adult mussels may avoid physiochemical impacts from non-native macrophytes by dispersing away from macrophyte beds, or if they are unable to relocate, by responding with fitness trade-offs; e.g., reduction in anti-predator traits or biomass (Burlakova & Karatayev, 2007; Wright, Byers, Koukoumaftsis, & Gribben, 2012). The juvenile mussel life-stage was predicted to be more sensitive to mortality through non-native macrophyte-

induced changes, since they are thought to live within sediments where they undertake pedal-feeding on fine particulate organic matter (Yeager, Cherry, & Neves, 1994). This is consistent with Geist & Auerswald (2007) who found redox potential of flowing water at the substrate surface, as well as 5 and 10 cm into the sediments, differed markedly at sites without recruitment of the European freshwater pearl mussel (*M. margaritifera*). Additionally, aquatic weed (*Ranunculus* spp.) in the River Spey in northern Scotland had determinantal effects on *M. margaritifera* by trapping mussels in roots and smothering them with fine sediments (Laughton, Cosgrove, Hastie, & Sime, 2008). Despite the higher likelihood of adverse physicochemical conditions during summer, coinciding with the release and transformation of freshwater mussel glochidia on fish hosts (Haag, 2012), studies that addressed interactions of larval mussels and non-native macrophytes were not encountered. However, a *Ranunculus* species native to the United Kingdom, but not in the River Spey where it was recently introduced, was found associated with dead juvenile *M. margaritifera* (Sime, 2014), suggesting dense beds of non-native macrophytes could act as sinks for juvenile mussel recruitment. Furthermore, avoidance by fish of macrophyte beds due to adverse environmental conditions will reduce encounter rates between mussels releasing glochidia and potential fish hosts (Schultz & Dibble, 2012).

Macrophytes have been identified as an important driver for sediment dynamics and hyporheic exchanges in streams (Braun, Auerswald, & Geist, 2012) which can in turn govern mussel distribution patterns. Another mechanism by which macrophytes may adversely affect mussels is through mass senescence at the end of summer (e.g., non-native *Myriophyllum aquaticum*, *Elodea canadensis* and *Egeria densa*, all of which are widespread in New Zealand), which can result in accumulation of dead organic matter and consequent reduction in redox potential and dissolved oxygen concentrations (Lopes-Lima et al., 2016). This effect has been observed for swan mussel (*Anodonta cygnea*) populations in three small lakes, which experienced high mortality from mass die-off of a water hyacinth (*Eichhornia crassipes*) on the Iberian Peninsula (Lopes-Lima et al., 2016). Furthermore, accumulation of organic matter or prolific macrophyte

growth can block waterways, leading to management actions such as dredging that can cause mortality of freshwater mussels (Aldridge, 2000; Greer, Hicks, Crow, & Closs, 2016).

The meta-analysis has highlighted the need for further research on interactions between non-native macrophytes and freshwater mussels in the following areas: 1) quantifying adverse physicochemical conditions produced at the sediment-water interface and standardising their effect as a measurement of macrophyte density; 2) conducting ecotoxicological trials of these adverse physicochemical conditions (e.g., anoxia) in the laboratory to isolate mechanisms of impact; and 3) examining responses of juvenile mussels as these are predicted to be particularly susceptible to adverse non-native macrophyte impacts on sediment composition and chemistry.

2.5.3 **Predators**

Unionid predation by non-native vertebrates was prevalent across mussel species, freshwater ecosystem types, and countries, indicating common behavioural strategies for native mussel consumption in geographically distinct regions. If non-native predators are known to exploit mussels in their native range this is not unexpected. However, yet unknown but likely predators of freshwater mussels may be common, since generalist diets are typical of successful non-native species (Allen et al. 2013). Consequently, the diverse diets of vertebrate predators are predicted to have weak and rare impacts on unionid populations, as their feeding strategy is often opportunistic and mediated by access to mussel beds (Cosgrove, Hastie, & Sime., 2007). This observation is consistent with the lead author's observations of broken *E. menziesii* shells with *Rattus* spp. predation marks alongside a shallow beach of a hydroelectricity reservoir in northern New Zealand (Figure 2-5). Indeed, all New Zealand articles involving mussel predation were exclusively related to *Rattus* spp. (Beveridge & Daniel, 1965; O'Donnell, Weston, & Monks, 2017; Theobald & Coad, 2002). On the other hand, more specialised mollusc predators such as the muskrat (*O. zibethicus*) are likely to have stronger interactions with unionids, since they are known to affect unionid population composition, size and age structure in their native North American range (Burlakova & Karatayev, 2007; Diggins & Stewart, 2000; Owen, McGregor, Cobbs, & Alexander Jr, 2011).

Potential mechanisms of predation impacts were direct consumption of adult unionid mussels by fish, direct competition with or predation on indigenous fish hosts, and unintentional consumption or disturbance of juvenile mussels through bioturbation of bottom sediments (Fei et al., 2014; Poos et al., 2010). Mortality as a result of predation occurred after failed consumption via desiccation following transfer to the terrestrial environment (Skyrienė & Paulauskas, 2012), as has been observed along some New Zealand streams (Moore, pers. obs.). Only one non-lethal interaction emerged, where mussel burrowing depth was deeper for species with thinner shells that were more susceptible to predation (Saarinen & Taskinen, 2003). Published evidence of direct non-native fish predation on native unionids was not found in the articles reviewed, although this may occur indirectly on juvenile mussels through benthic feeding activities. Similarly, no evidence of predation was found for the introduced round goby (*Neogobius melanostomus*) in North America (Poos et al., 2010). This was interesting, since the introduced round goby is one of the few fish species known to consume molluscs, although only predation on non-native species (*D. polymorpha* and *C. fluminea*) has been documented (Brandner, Auerswald, Cerwenka, Schliewen, & Geist, 2012). Nonetheless, the introduced round goby has potential to directly impact native unionid mussels and in particular juveniles. However, predation from various predator groups on abundant non-native mussels has been commonly reported (Kipp, Ricciardi, & Ramcharan, 2012; Ruetz, Reneski, & Uzarski, 2012).

Apart from committed mussel predators, such as *Rattus* spp. and muskrats, consumption was mediated by ease of access to mussel beds. *Rattus* spp. were able to dive to collect mussels and consume them on shore or in rat dens (Beveridge & Daniel, 1965; O'Donnell et al., 2017; Theobald & Coad, 2002). Indeed, accumulation of shells as a result of rat predation is a factor recorded in surveys of mussels in New Zealand streams (see Caitlin et al., 2017). In contrast, feral hog predation was restricted to small, shallow streams, which indicated strong interactions can only occur in low flows or tributaries (Kaller, Hudson III, Achberger, & Kelso, 2007; Williams & Benson, 2004; Zengel & Conner, 2008). Equally, high flows related to storm events

Figure 2-5: Predation of the New Zealand freshwater mussel, *Echyridella menziesii*. Characteristic, angular tooth mark of a *Rattus* spp. marked by a circle (C. M. King, University of Waikato, pers comm, 21 March, 2018).

can strand mussels onshore where they may be consumed; e.g., as suggested by the red fox, *V. vulpes,* in Australia (Walker, 1981), although mussels would have died from desiccation anyway.

Future research on the interactions between non-native predators and freshwater mussels would benefit from quantitative studies in the following areas: 1) identification of species known to consume freshwater mussels in their native range that have potential to be introduced into a non-native range (e.g., North American river otters (Toweill, 1974)); 2) studies of non-native species that are not regarded as predators, but are potentially capable of consuming freshwater mussels if the opportunity arises, such as the small Asian mongoose (*Herpestes javanicus*; (Vilella, 1998)) and crab-eating macaque (*Macaca fascicularis aurea*; (Gumert & Malaivijitnond, 2012)); and 3) investigation of how flow alteration mediated by climate-change will influence the frequency and occurrence of opportunistic freshwater mussel predation.

2.5.4 Implications for New Zealand Unionida

In New Zealand, the interactions of most concern between non-native species and freshwater mussels are impacts resulting from shifts towards non-native fish communities (Collier et al. 2016). Although introduced brown trout (*Salmo trutta*) has been established as a suitable host of *E. menziesii*, recent research on catfish (*A. nebulosus*), rudd (*Scardinius erythrophthalmus*), and goldfish (*Carassius auratus*) has found juveniles were not produced in ecologically significant numbers (Moore & Clearwater, 2019) (Chapter 3). One of the mechanisms leading to poor juvenile production of non-native fish is a limited number of sites available for glochidial attachment, meaning coarse fish with large scales in New Zealand might also be poor hosts. Furthermore, the bioturbation of bottom sediments by feeding common carp (*Cyprinus carpio*), goldfish and catfish may indirectly consume juvenile freshwater mussels, and prevent the recruitment of populations where these non-native fish occur in high numbers.

Other than non-native fish species, the major threats to mussels in New Zealand freshwater ecosystems include flow alteration, loss of connectivity, physical barriers, habitat degradation, poor water quality, and climate change (Gerbeaux, Champion, & Dunn, 2016). Although of pressing concern, these threats can also promote habitat conditions favourable for non-native species establishment and spread (Johnson, Olden, & Vander Zanden, 2008). For example, Lake Karāpiro, a New Zealand lake formed above a dam for hydroelectricity generation, contains a large number of non-native macrophyte beds such as *Ceratophyllum demersum* (Chapman, 1996; Chapman, Brown, Hill, & Carr, 1974) and a fish community dominated by non-native species (Jellyman & Harding, 2012). Consequently, the *E. menziesii* population in this lake has had to respond to the combined potential impacts of hydrological alterations and multiple non-native species. Such co-existence of high densities of non-native species and native mussels highlights the need for studies investigating their interactions, as well as research that aims to understand the general ecology of New Zealand Unionida to predict non-native species impacts. Overall, a precautionary approach in controlling the spread and establishment of non-

native species in New Zealand would be the most effective current strategy for unionid conservation efforts.

In the future, non-native threats to New Zealand Unionida may include those not currently prevalent in the literature, such as dense growths of diatoms (Kilroy, Larned, & Biggs, 2009), severe toxic blooms of Cyanobacteria, some of which may be non-native (Clearwater et al., 2014), and non-native zooplankton which may compete for planktonic food resources. Unlike non-native species of vertebrates or macrophytes, control methods for algae are much more challenging, with eradication post-establishment nearly impossible (Duggan and Collier, 2018). For instance, the non-native diatom *Didymosphenia geminata* has invaded much of New Zealand's South Island where it creates dense mats that could smother benthic habitat, inhibiting the ability of mussels to suspension feed, disperse and interact with fish hosts (Kilroy, Larned, & Biggs, 2009). Although the impacts of non-native freshwater mussel introductions are of concern, as an island nation, New Zealand is well placed to prevent such incursions through border controls (Smith & Dodgshun, 2008). Accordingly, protection of freshwater mussel populations from non-native species' impacts in New Zealand should focus on control of macro-organisms and prevention of the establishment and spread of non-native algae.

2.6 Conclusions

Based on the findings of this meta-analysis, a conceptual framework was developed to assess the potential for interactions between unionid mussels and non-native species that depicts the effect mechanisms and magnitude during different unionid mussel life stages (Figure 2-3). Fish are predicted to have the strongest impact on Unionida, as they may compete with native fish hosts. Primary producers such as macrophytes are most likely to interact with juvenile mussels, as they strongly affect sediment conditions and water flow. Finally, non-native predators are predicted to affect adult unionid populations, but impacts are considered weak as interactions may be infrequent and often opportunistic in nature. The interactions of different non-native species groups are predicted to overlap to exacerbate effects at certain life-stages (Figure 2-3). Where these interactions occur, an effect

bottleneck may prevent the development of juveniles, or adversely influence subsequent life-stages. This may potentially contribute to a long-term decline of the unionid mussel population, even if other impacts can be recovered from or exert weak effects.

While the mechanisms identified may have broad application, the limited geographic spread of the research carried out to date limits generalisations that can be made. Studies of macrophyte interactions and impacts of non-native fish, in particular outside of North America, were highlighted as key directions for future research. The need for such research is most pressing in lowland lakes and waterways, where the risk of non-native species invasion and interactions with native fish hosts are more likely due to close proximity to human population centres, notably for island nations such as New Zealand where proximity to the sea affects the recruitment of native diadromous fish species (Compton, De Winton, Leathwick, & Wadhwa, 2012; Leathwick et al., 2016). The long life-spans of freshwater unionids may present opportunities for freshwater managers to aid recovery and mitigate adverse effects of non-native species on mussel recruitment through early intervention. Future studies determining the causes of unionid mussel population decline should also assess the risk of non-native species interactions at different life stages. Research accounting for the cumulative effects of these interactions with other pressures at the population- or basin-scale remains to be developed.

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Chapter 3

Non-native fish as glochidial sinks: elucidating disruption pathways for *Echyridella menziesii* recruitment

3.1 Abstract

A potential mechanism of global decline in freshwater mussel (Unionida: Bivalvia) abundance and diversity is disruption of their obligate parasitic life-cycle by non-native fish species, which may introduce novel interaction pathways that threaten unionid recruitment. We assessed three non-native fish (brown bullhead catfish, *Ameiurus nebulosus*; rudd, *Scardinius erythrophthalmus*; and goldfish, *Carassius auratus*) as glochidial hosts for the New Zealand freshwater mussel *Echyridella menziesii* to test the hypotheses that (i) non-native fish will have lower glochidial attachment rates than a native fish (the common bully *Gobiomorphus cotidianus*), and (ii) encystment rate will be lower on non-native species. We found that the non-native fish had significantly lower total glochidial attachment than the native control fish after infestation and did not produce ecologically significant quantities of juvenile mussels. This research supports the general assumption that non-native species are less suitable hosts of native freshwater mussels. However, confirming our findings in the field will indicate if removing non-native fish or enhancing native fish populations is recommended for conservation of *E. menziesii* populations in New Zealand.

3.2 Introduction

Freshwater mussel (Bivalvia: Unionida) abundance and diversity globally has declined severely over the last century, with 40% of species classified by the International Union for Conservation of Nature as Near Threatened, Threatened, or Extinct (Lopes-Lima et al., 2018). In New Zealand, data available on freshwater mussels (Unionida: Hyriidae) support this trend, with *Echyridella menziesii* (Gray 1843) and *E. aucklandica* (Gray 1843) classified as At Risk and Threatened, respectively, and *E. onekaka*

(Fenwick & Marshall, 2006) as Data Deficient in a recent conservation status assessment (Grainger et al., 2018). Consequently, the important ecosystem functions and services mussels provide in dense beds may be impaired, resulting in profound effects that may encompass individuals to ecosystems (Walker et al., 2014; Vaughn, 2018). For example, mussel biofiltration can remove suspended solids across a wide range of concentrations to markedly improve water quality (Ogilvie & Mitchell, 1995; Welker & Walz, 1998; Lummer et al., 2016). This ability also means that mussels can cycle and store nutrients long-term, rather than (for example) nutrients remaining bioavailable to phytoplankton and causing adverse algal blooms typical of eutrophication (Paerl, 2009; Strayer, 2013). *Echyridella menziesii* filtration (0.02–1.3 l mussel^{-1} h^{-1}) and nutrient excretion (4–50 µg N mussel^{-1} h^{-1}) rates are similar to those of European and North American mussels, and provide a substantial source of nutrients that is important to consider in nutrient budget models (Cyr et al., 2016). Furthermore, mussels are considered indicators of freshwater health (Atkinson et al., 2014), ecosystem engineers because of their ability to modify habitat (Aldridge et al., 2007), and umbrella, flagship, and keystone species that are important targets for conservation efforts (Geist, 2011).

Freshwater mussel distribution is limited by a unique co-evolved relationship with fish that defines the unionid group (Modesto et al., 2018). In order to complete their life-cycle, freshwater mussels must attach larvae (glochidia) to suitable fish tissues (e.g., gills and fins) to encyst and transform into juveniles (Barnhart et al., 2008). Successful glochidial attachment is dependent on initial contact with host fish, which in turn is influenced by microhabitat preferences, behaviour, and abundance, the distinct infestation strategy of a particular mussel species, and suitable ecosystem conditions for both fish and mussels (Barnhart et al., 2008; Donrovich et al., 2017). Successful completion of the encystment stage requires host fish to have suitable chemical and nutrient characteristics for mussel development. Also, glochidia must be resistant to the host-fish immune system that may cause “sloughing off” before transformation (Jansen et al., 2001). Mussel–fish relationships vary in their degree of host specificity, ranging from mussels that infest a single fish host to a generalized strategy

where multiple fish species are capable of producing viable mussel juveniles (Barnhart et al., 2008). *Echyridella menziesii* is considered a host generalist: many fish species have been found with attached glochidia in the field (e.g., *Gobiomorphus cotidianus* (McDowall, 1975), *Anguilla dieffenbachii* (Gray, 1842) and *A. australis* (Richardson, 1841), *Galaxias brevipinnis* (Günther, 1866), *G. gobiodes* (Valenciennes, 1837) (all native), and *Oncorhynchus mykiss* (Walbaum, 1792)(non-native); Clearwater et al., 2014 and papers cited therein), and glochidia have been observed to transform into juveniles on seven species in laboratory trials [*G. cotidianus*, *G. brevipinnis*, and *O. mykiss*, Clearwater et al. unpublished data 2012; *Galaxias fasciatus* (Gray, 1842), *Galaxias vulgaris* (Stokell, 1949), A. *dieffenbachii* and *A. australis* (Brown et al., 2017)]. However, despite this broad reproductive strategy, adult-skewed size structures have often been observed in *E. menziesii* populations (James, 1985; Roper & Hickey, 1994). This is of concern, as lack of juvenile size-classes in a mussel population may indicate recruitment failure, an observation also recorded worldwide for other unionid mussels (Bailey & Green, 1989; Araujo et al., 2000; Hastie & Toy, 2008; Harriger et al., 2009).

A top research priority for freshwater mussel conservation is to identify host fish, understand their conservation status, and determine threats to their mussel relationship (Modesto et al., 2018; Ferreira-Rodríguez et al., 2019). Although multiple threats impact freshwater mussels, including agricultural pollution and habitat modification (Walker et al. 2014; Lopes-Lima et al., 2018), the role of non-native species may be under-represented in unionid mussel threat assessments (Moore et al., 2019). Human-mediated global biotic homogenization has resulted in a shift towards freshwater communities increasingly dominated by non-native species (Olden, 2006; Rahel, 2007; Tricarico et al., 2016). In New Zealand, non-native fish are more frequently occurring with freshwater mussels in lowland lakes and rivers (Rowe & Wilding, 2012; Collier et al., 2016), and have the potential to disrupt the obligate glochidial larval stage of the unionid life-cycle (Berg et al., 2008; Poos et al., 2010). This can occur directly by providing an unsuitable host in the mussel–fish relationship (Douda et al., 2013; Salonen et al., 2016; Šlapanský et al., 2016), and indirectly through competition and

predation of native host-fish populations (Poos et al., 2010). Non-native fish may also interact according to the 'Enemy Release Hypothesis' (Torchin et al., 2003), whereby comparatively lower infestation rates on introduced fish reduce the associated physiological cost of glochidial development to the fish [e.g., inhibited respiration, reduced movement, and higher mortality (Meyers & Millemann, 1977; Taeubert & Geist, 2013; Thomas et al., 2014)], thereby conferring a competitive advantage to non-native species (Salonen et al., 2016). In addition, non-native fish can act as glochidial sinks and reduce the number of larvae available to infest suitable native hosts (Tremblay et al., 2016). This mechanism, where glochidia attach or encyst but do not transform into juveniles, may be particularly important when non-native fish species are abundant in an ecosystem.

The aim of this study was to determine the suitability of three widespread non-native fish as glochidial hosts for the New Zealand freshwater mussel, *E. menziesii*. Laboratory infestations were conducted to test the hypotheses that (i) non-native fish will have lower glochidial attachment rates than a native fish (the common bully *G. cotidianus*) in accordance with the 'Enemy Release Hypothesis,' and (ii) encystment rate will be lower on non-native species (glochidial sinks) and, as a consequence, they will not produce ecologically significant quantities of juvenile mussels. Non-native brown bullhead (*Ameiurus nebulosus* (Lesueur, 1819); hereafter catfish), rudd (*Scardinius erythrophthalmus* (Linnaeus, 1758)), and goldfish (*Carassius auratus* (Linnaeus, 1758)) were selected for infestation experiments due to their distributional overlap with *E. menziesii* populations and habitat use (e.g., benthic or littoral feeding) that increases the likelihood of freshwater mussel larvae encounters in the field (Collier & Grainger, 2015; Collier et al., 2016).

3.3 Methods

3.3.1 **Glochidia preparation**

Echyridella menziesii were collected by snorkelling in Lake Karāpiro, northern New Zealand (37°56′51″S, 175°38′54″E) in 1.0 to 1.9 m water depth and temperature of 18–20°C. Mussels were gently prised open (~ 10 mm) using a rounded knife and females with enlarged and

orange/brownish marsupia (or brood pouches) were selected for laboratory trials on three occasions between December 2017 and March 2018. Approximately 30 mussels with 'ripe' brood pouches were transported to the laboratory wrapped in damp towels inside an ice cooler to reduce stress-induced glochidial release (ASTM, 2006). Mussels were then transferred to a 100-l tank filled with aerated, dechlorinated tap water in a constant-temperature room set at 20°C with a 16:8-h light:dark cycle to simulate conditions at time of capture, and allowed to acclimate over two days. Ammonia concentrations (API® Ammonia Test Kit) and water temperature were monitored daily and water was exchanged if ammonia exceeded 0.5 mg l^{-1}.

After acclimation and gentle cleaning of loosely adhered material from mussel shells, glochidial release was stimulated by placing individual mussels in 0.5-l glass beakers of dechlorinated water and allowing water temperatures to increase gradually to approximately 23°C. A sub-sample of the 29,000–50,000 glochidia released by multiple females for each batch was assessed for viability by exposing 100–150 glochidia to 1.5 ml of brine solution (80–100 ppt of concentrated oceanic seawater). The numbers of closed and unclosed glochidia were counted before and within 1 min of brine exposure. Only glochidia that closed after brine exposure were considered to be viable (Wang et al., 2007). Batches of glochidia with > 90% viability were pooled and diluted to produce a solution with ~ 2000 viable glochidia l^{-1} for infestation, following Dodd et al. (2005): catfish trial ~ 2280 glochidia l^{-1} (total 22 l, four mussels); rudd trial ~ 2130 glochidia l^{-1} (total 16 l, four mussels); and goldfish trial ~ 2090 glochidia l^{-1} (total 14 l, three mussels).

3.3.2 **Fish collection**

To exclude the possibility of an acquired immune response from previous glochidia exposure, fish were collected from sites not known to support extant *E. menziesii* populations (i.e., living mussels have not recently been collected in the lower Waikato River, including adjacent to the native control fish collection site used in the present study (Collier & Hogg, 2010; Collier et al., 2014), Knighton Lake (Paul & Hamilton, 2008), and Lake Rotoroa). Catfish and goldfish were collected using fyke nets (November 2017) and backpack electric fishing (March 2018), respectively, from Knighton Lake on

The University of Waikato campus (37°47′09″S, 175°18′54″E), while rudd were captured using an electric fishing boat (January 2018) from urban Lake Rotoroa (37°47′53″S, 175°16′29″E), and common bullies using a seine net (November 2017, January 2018, and March 2018) in the lower Waikato River near Hamilton City (37°48′24″S, 175°18′22″E). The targeted length for all fish was ~ 100 mm to ensure optimal holding conditions in experimental tanks. Fish species were acclimated separately for at least one week in a constant-temperature room (16:8-h light:dark, 20°C) in 120-l tanks (0.03–0.15 fish per l) containing dechlorinated tap water adjusted to 3-5 ppt saline solution by addition of natural seawater to reduce disease risk. Each tank had an aerator and a recirculating pump with a biofilter. Water quality was monitored daily and water was exchanged if ammonia concentration exceeded 0.5 mg l^{-1}. Once fish were readily consuming 5–10% of their body weight per day of frozen chironomids (Advanced Hatchery Technology, Inc.) and considered to be in good condition (i.e., no external evidence of disease or fin damage), glochidial infestation was performed.

3.3.3 **Infestation**

Infestation was conducted on eight non-native fish of the same species (catfish, rudd, or goldfish) and four control fish individuals (native common bullies) for each laboratory trial (Figure 7-1 in Appendix 7.2.1). To start a laboratory trial, fish were exposed for 15 min to a homogenous glochidial suspension in three batches of four individuals separated by species [i.e., two batches of four non-native fish and one batch of four native fish (Dodd et al., 2005)]. The single infestation bath (3-l tank) was vigorously aerated to keep glochidia in suspension and the glochidial solution was renewed for each successive infestation. After infestation, fish were transferred to a water bath without glochidia for another 15 min to remove loosely attached or non-attached larvae. Individual fish were then randomly assigned to separate 10-l, self-cleaning tanks (Pentair Aquatic Eco-Systems; PC90 tanks, LID90I-4 lids, and BAF10.01-4 baffles) with filters (150-µm mesh) receiving the outflow of each flow-through tank to collect detached glochidia or transformed juvenile mussels. The tanks were supplied with internally recirculating dechlorinated water (20°C) from a sump with biofiltration (Fluval 206 canister filter). A single rectangular shelter for the fish was

provided in each flow-through tank. In addition, a bulk exposure of non-native fish (10 catfish, 5 rudd, and 4 goldfish) was performed using the same methods to provide ancillary data on the glochidia transformation progress to assess internal structures for infestation. These fish were held in the same conditions post-infestation as for acclimation.

Water flow through the fish tanks was maintained at ~ 0.5 l min^{-1} using a pump (Hailea HX-6830), to promote self-cleaning of the tanks. Temperature and ammonia were measured daily, and fish were fed every other day for the three-week duration of laboratory trials. Temperature averages differed slightly among trials: catfish 20.6 ± 0.9 SD; rudd 21.6 ± 0.2 SD; and goldfish 21.3 ± 0.6 SD. Each day, flow-through tanks were flushed for 20 min with a high flow of water (i.e., > 3 l min^{-1}) to ensure any glochidia retained in tanks were removed. The goldfish experiment was terminated at day 19 due to fungal infection (cf 21 days for the catfish and rudd trials): fish mortality occurred from day 14, by which point almost all glochidia had been lost from goldfish and native control fish continued to excyst juveniles until day 18. At the end of the trials, fish were euthanized by anesthetic overdose (> 175 mg l^{-1} AQUI-S for 20 min) and dissected to assess if larvae were still encysted.

Detached glochidia were considered alive based on valve movement and juveniles on valve movement and/or active pedal movement (Steingraeber et al., 2007) by examination in a Bogorov tray under a stereomicroscope at × 40 magnification (LEICA M80). Any closed glochidia or inactive juveniles were held for at least a week after collection and observed daily to positively confirm their status as alive or dead (see “Results” for further detail). The number of attached glochidia was defined as the sum of lost glochidia (detached, dead and alive) and excysted juveniles. Most excysted juveniles survived for at least a week post-trial, although the earliest juveniles to excyst took a few days to activate their gape response and/or move the foot muscle. In contrast, three days from when the first juveniles were produced, the juveniles that excysted subsequently were immediately and constantly active and therefore easy to classify. Laboratory trials were considered complete once the rate of juvenile mussels extracted from positive control tanks plateaued (Figure 3-1).

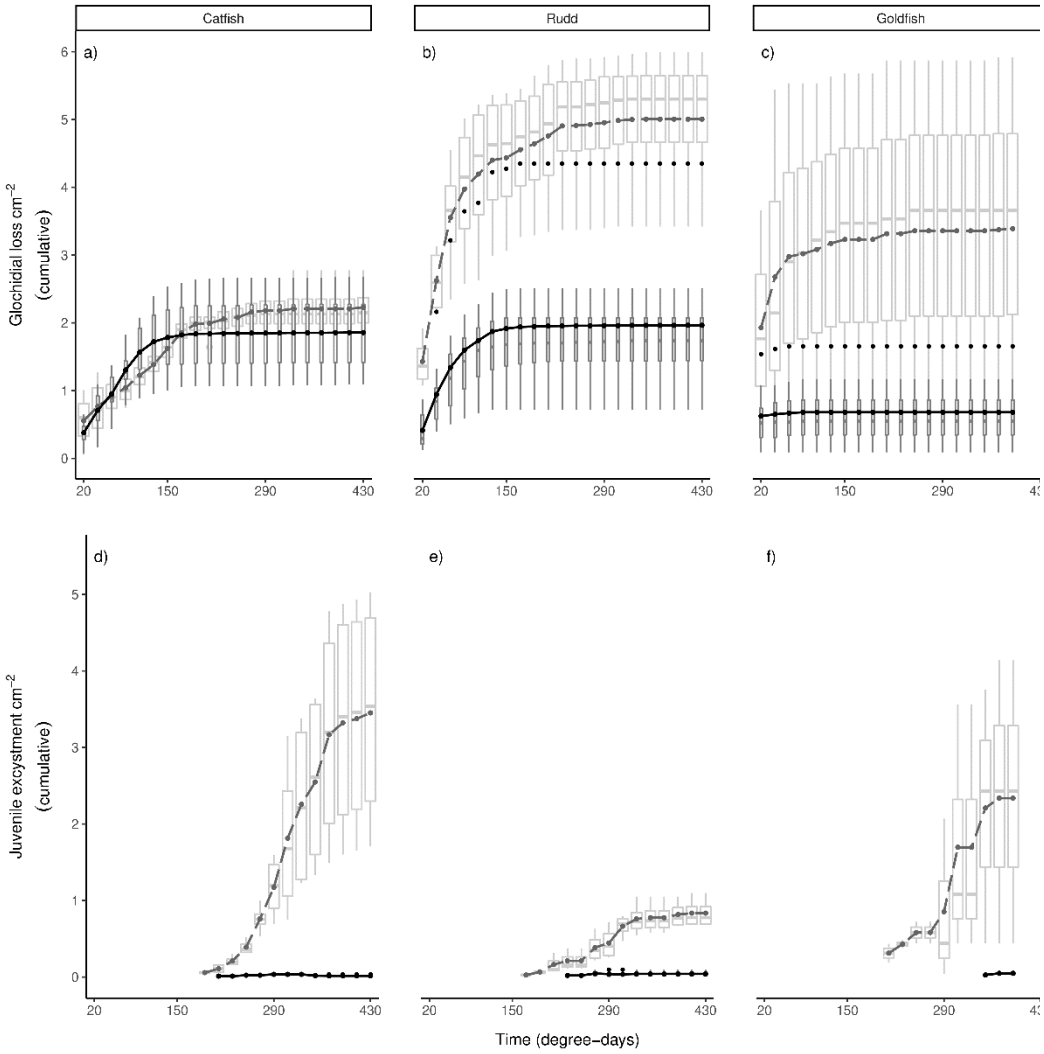

Figure 3-1: Comparisons between non-native catfish, rudd, and goldfish (solid black line and thin boxplots) and native control fish (dashed gray line and thick boxplots) for (a), (b), (c) glochidial loss and (d), (e), (f) juvenile excystment, per unit fish surface area. Data are presented cumulatively over time in degree-days (the product of daily water temperatures and number of days) with boxplots: mean [dashed gray line or solid black line linking boxplots]; median [gray line inside boxplot]; interquartile range [box]; min/max [whiskers]; and outliers [> 1.5 × interquartile range, black dots] displayed.

3.3.4 **Glochidia attachment sites**

Catfish from the bulk exposure tank were sacrificed and examined periodically from day three onwards throughout the experiment to assess glochidia attachment sites. This examination enabled us to determine whether glochidia were attached to internal structures, but was not completed during the other non-native fish trials as no external glochidia were observed (see “Results”). The position and number of glochidia on external (i.e., the dorsal, adipose, pectoral, pelvic, anal, and caudal fins, lips, snout, operculum cover, and skin) and internal fish structures (mouth and

gills) was recorded using the stereomicroscope at × 40 magnification. All fish from flow-through tanks were assessed using the same methods after each laboratory trial. Glochidial attachment sites, and fish body length, wet-weight, surface area, and fin surface area were measured according to O'Shea et al. (2006). Surface area and fin edge measurements were calculated by scanning 1 cm^{-2} grid paper with fish body and fin outlines into Inkscape (version 0.92.3), visually drawing paths around fish-part outlines, and calculating area and length using the measure path tool (Bah, 2011).

3.3.5 **Statistical analysis**

All analyses were conducted in the R statistical software package version 3.5.0 (R Core Team, 2018). Glochidial loss and juvenile excystment were standardized by fish surface area and reported cumulatively across degree-days (dd) (i.e., the product of daily water temperatures and number of days) (Taeubert et al., 2014). To determine differences between non-native and native control fish in glochidia attachment, loss, and excystment as juvenile mussels, non-parametric Wilcoxon signed-rank tests were performed for each trial since *t* test assumptions were not met. Differences between trials for native control fish, and between non-native fish species standardized by native control fish (i.e., non-native fish individuals divided by the mean of control fish in each respective trial), were tested using Kruskal–Wallis rank sum tests. Wilcoxon signed-rank tests corrected for multiple tests were used to determine differences between groups.

To assess the importance of sources of variation among experiments (i.e., fish surface area, fin surface area, fin edge length, length, weight, and temperature) in determining glochidial loss and juvenile excystment within native control fish, an information-theoretic approach (Burnham & Anderson, 2002) was applied using the R package INLA (Rue et al., 2009) with forward model selection and the Deviance Information Criterion (DIC) to compare models with different sets of covariates (Thogmartin & Knutson, 2007; Zuur et al., 2017). This method estimates posterior values by using numerical integrations for fixed effects and Laplace integral approximation to random effects (for more details see Rue et al., 2009). Effect direction was identified from the posterior mean and 95% credible intervals, where explanatory variables with 95% credible intervals exclusive

of zero were considered important (Zuur et al., 2017). An AR1 (autoregressive model of order 1) trend for regularly spaced time-series data and Poisson error distribution were selected to account for temporal dependency (i.e., measurements on a given day were influenced by data from previous time periods: Spearman rank coefficients for temporal dependency were 0.52 for glochidial loss and 0.91 for juvenile excystment) and count data, respectively (Blangiardo & Cameletti, 2015; Zuur et al., 2017). A model with random intercept and random slope was selected (Zuur et al., 2017) to account for dependency among observations taken from the same fish, and variability among fish. All continuous explanatory variables were centered using the "scale function" (Becker et al., 1988), and defaults were used for regression parameters (Gaussian distribution) and hyperparameters (diffuse priors) (Rue et al., 2009). Model validation followed a normality check, and inspection of residuals against fitted values and explanatory variables for homogeneity of variance (Zuur et al., 2017). The 95% credible intervals were inspected for the best model subset to assess the importance of each explanatory variable in the model (Zuur et al., 2017). R-codes for models are available in Appendix 7.2.2.

3.4 Results

3.4.1 **Infestation**

Glochidia viability prior to infestation ranged from 88% (catfish trial) to 96% (goldfish trial). During infestation, common bullies resided on the infestation tank bottom and took cover behind the aerators, which increased the probability of contact with suspended glochidia. Catfish exhibited similar behaviors; however, rudd and goldfish were mainly active in midwater positions of the infestation tank. Fish surface area varied between species: common bullies were on average 27.1 cm^2 ± 8.8 SD (combined across trials), with non-native catfish, rudd, and goldfish larger than native controls on average (Table 3-1).

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Table 3-1: Native and non-native fish species body size parameters and number of individuals used in each trial.

Fish species	Wet-weight $\bar{x} \pm$ SD (g)	Length $\bar{x} \pm$ SD (mm)	Surface area $\bar{x} \pm$ SD (cm^2)	Fin surface area $\bar{x} \pm$ SD (cm^2)	Fin edges $\bar{x} \pm$ SD (cm)	No. of fish
Gobiomorphus cotidianus (common bully) – catfish trial	3.6 ± 0.7	66.3 ± 3.5	21.2 ± 3.2	5.1 ± 1.8	20.7 ± 1.2	4
Gobiomorphus cotidianus (common bully) – rudd trial	4.2 ± 1.6	68.5 ± 6.6	36.1 ± 6.5	12.3 ± 3.6	27.4 ± 3.0	4
Gobiomorphus cotidianus (common bully) – goldfish trial	2.8 ± 0.5	62.7 ± 5.9	19.6 ± 3.3	3.9 ± 2.0	14.6 ± 7.1	3
*Ameiurus nebulosus ((*brown bullhead catfish)	25.3 ± 7.2	141.9 ± 11.7	107.3 ± 26.2	34.4 ± 8.4	46.0 ± 5.6	8
Scardinius erythrophthalmus (rudd)	7.0 ± 1.2	80.5 ± 5.2	52.7 ± 16.8	15.0 ± 9.2	31.8 ± 5.6	8
Carassius auratus (goldfish)	3.7 ± 1.5	69.0 ± 6.5	28.5 ± 7.1	8.2 ± 7.1	22.9 ± 3.8	8

No. is number; $\bar{x}$ is the mean; SD is standard deviation

3.4.2 Native control fish across trials

Native control fish had glochidia encysted on all fin surfaces, predominantly around the fin edges and on opercula. Glochidia attached to the skin, the snout, and inside the mouth detached quickly after attachment (i.e., less than a day). The average number of total glochidia attached to common bullies was very similar across trials (5.7 cm^{-2}; Figure 3-2a) and average total glochidial loss ranged from 2.2 to 5.0 cm^{-2} (Figure 3-2b). Total glochidia attached and lost per unit fish surface area were not statistically different for common bullies compared across trials (Kruskal–Wallis test, $H = 0.67$, $P = 0.72$ and $H = 4.55$, $P = 0.10$, respectively). In the rudd trial, the average number of juveniles excysted from common bullies was not significantly different (0.8 cm^{-2}) from bullies in the catfish and goldfish trials (3.5 and 2.3 cm^{-2}, respectively; Figure 3-2c) ($H = 5.05$, $P = 0.08$). Variation in the glochidial loss of common bullies increased across trials from December 2017 to March 2018, but this was not evident for juvenile excystment where the lowest variation was observed in the rudd trial (Figure 3.1). No larvae were found encysted on native control fish at the end of trials indicating all had developed into juveniles and/or detached. Glochidial loss started to plateau at 190–200 dd for common bullies in the catfish and rudd trials, and earlier in the goldfish trial at 40–50 dd (Figure 3-1). However, excystment of juveniles from native control fish occurred over a similar time frame in all trials (i.e., between 170 to 433 dd) (Table 3-2). Duration to peak juvenile excystment from common bully varied between trials, peaking earliest in the rudd trial compared to goldfish and catfish trials (Table 3-2).

The best subset model that predicted glochidial loss of common bully across trials included a positive effect of temperature and fin surface area (Table 3-3). The 95% credible interval of temperature and fin surface area was strictly positive and exclusive of zero, which indicates importance in the model. All measures of glochidial attachment sites on fish (length, weight, surface area, fin surface area, and fin edges) produced models with similar evidence ratios (i.e., within 0.1 of each other) and therefore the best subset model was only slightly better at predicting glochidial loss than other subsets (Table 3-3). Juvenile excystment from native control fish was predicted in the best model subset by temperature and fish surface area, with the 95%

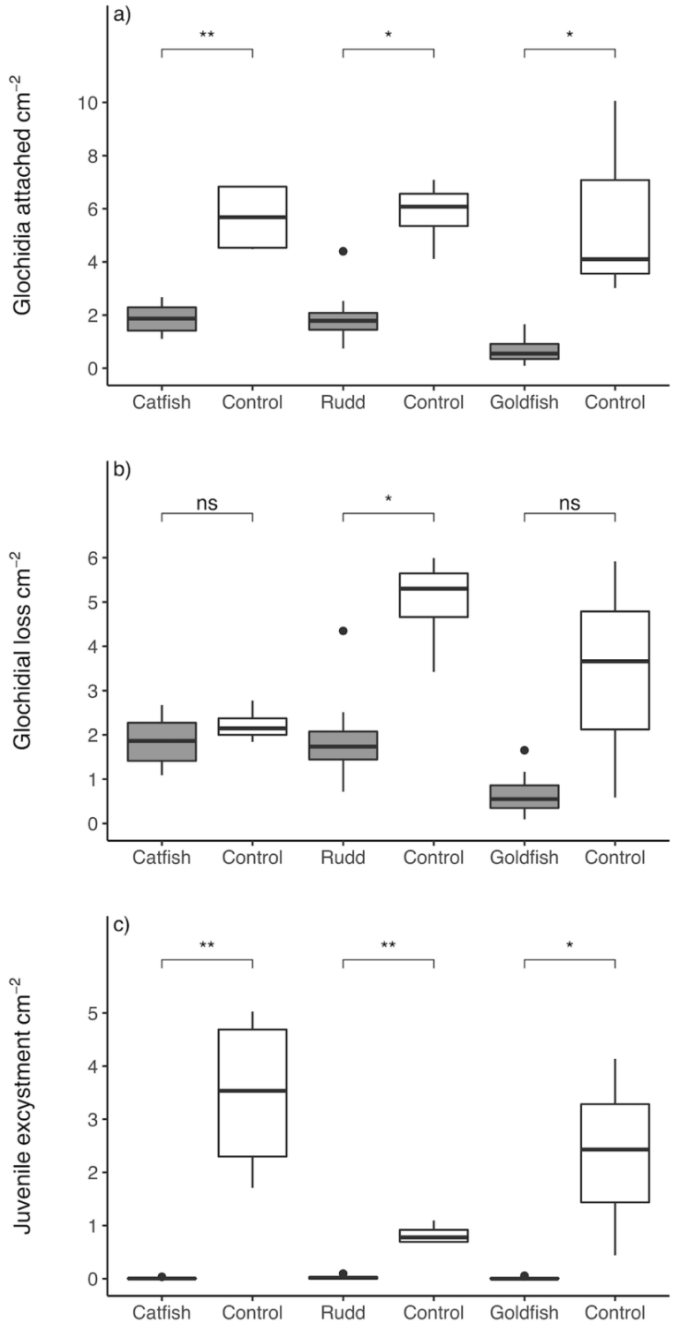

Figure 3-2: Differences between non-native catfish, rudd, and goldfish and corresponding native control fish for (a) total glochidia attached by fish surface area, (b) total glochidial loss by fish surface area, and (c) total juveniles excysted by fish surface area. Boxplots show median [black line inside boxplot]; interquartile range [box]; min/max [whiskers]; and outliers [> 1.5 x interquartile range, black dots]. Statistical significance of comparisons (Wilcoxon signed-rank tests) indicated above plots: $^{**}P < 0.01$, $^{*}P < 0.05$; $nsP > 0.05$ with brackets showing the comparison.

credible intervals of both variables strictly positive and exclusive of zero. The best subset model that predicted juvenile excystment of native control fish was markedly better than other subset models based on evidence ratios (Table 3-3).

Table 3-2: Summary statistics for native control fish (bullies) and non-native catfish, rudd, and goldfish for attached glochidia, glochidial loss, and juvenile excystment per fish.

Fish species		Catfish	Control	Rudd	Control	Goldfish	Control
Glochidial attachment	$\bar{x} \pm$ SD	190.4 ± 41.8	136.3 ± 53.9	101.0 ± 47.3	211.5 ± 68.5	19.6 ± 13.7	113.7 ± 82.6
Glochidial loss	$\bar{x} \pm$ SD	189.6 ± 41.8	52.3 ± 13.1	99.9 ± 47.3	180.8 ± 57.5	19.3 ± 13.4	64.7 ± 55.3
Start-end of loss	dd	19-412	19-432	21-432	21-366	22-82	22-405
Peak	dd	19	19	42	42	22	22
Juvenile excystment	$\bar{x} \pm$ SD	0.8 ± 1.0	84.0 ± 47.3	1.1 ± 1.4	30.8 ± 11.8	0.4 ± 0.7	49.0 ± 39.7
Start-end of loss	dd	202-370	181-433	237-345	172-432	363-385	210-384
Peak	dd	366	388	370	280	276	323

No. is number; $\bar{x}$ is the mean; SD is standard deviation; the attribute “start-end” of loss or excystment is presented as degree-days (dd) (i.e., the product of daily water temperatures and number of days); “peak” indicates the highest observed rate of glochidial loss or juveniles excysted during this period

3.4.3 **Non-native fish trials**

Encystment locations for catfish included the gills and barbels, as well as dorsal, pectoral, anal, and caudal fins. Dissection of catfish at days three, five, and seven found between 2 and 36 attached glochidia per fish, compared to day 11 when only a few glochidia were found encysted, open, and dead but still attached to catfish tissues. No glochidia were observed attached to external structures of rudd and goldfish, although rapid gaping and gulping occurred during infestation, thereby providing access to fish internal structures. Dissection of fish at the end of trials found no juveniles encysted on internal tissues. The number of glochidia lost per fish surface area almost equalled the number attached by surface area for all non-native species (Table 3-2).

Total number of glochidia attached per unit fish surface area was significantly lower for all non-native species than for corresponding native fish controls (catfish trial, 1.9 cm^{-2}; rudd trial, 2.0 cm^{-2}; goldfish trial, 0.7 cm^{-2}; native control fish 5.7 cm^{-2}) (Figure 3-2a). Differences in total glochidial loss per fish surface area was significant for rudd compared to control fish (Wilcoxon signed-rank test, $P < 0.05$), but not for catfish and goldfish ($P = 0.55$ and $P = 0.13$, respectively) (Figure 3-2b). All non-native fish species excysted fewer juveniles per fish (average 0.4–1.1 per fish) than their respective native control fish (average 31–84 per fish), and over a shorter number of degree-days (Table 3-2; Figure 3-2c).

Significantly fewer glochidia attached to goldfish when normalized by fish surface area (on average 0.7 cm^{-2}) compared to catfish (1.8 cm^{-2}) or rudd (1.9 cm^{-2}) (Kruskal–Wallis sum of ranks test, $H = 10.93$, $P < 0.01$; Figure 3-2a). Total juvenile excystment per fish surface area did not differ between non-native fish (range 0–0.1 cm^{-2}) (Kruskal–Wallis sum of ranks test, $H = 2.04$, $P = 0.36$) (Figure 3-2c). Rudd excysted the most juveniles (average 1.1 per fish) (Table 3-2), although no statistical difference was found for the percentage of juveniles excysted from initially attached glochidia between non-native fish species (2.0% compared to 1.1% and 0.4% for rudd and catfish, respectively) (Kruskal–Wallis sum of ranks test, $H = 1.68$, $P = 0.43$).

Table 3-3: Model selection results for glochidial loss and juvenile excystment from common bullies across trials.

Best subset model	Explanatory variables	*n*	DIC[a]	ΔDIC[b]	ω_i[c]	Evidence ratio[d]
Glochidial loss	**Temp + Fin Surface Area**	**11**	**910.8**	**0.0**	**0.2**	**1.0**
	Temp + Surface Area	11	910.8	0.1	0.2	1.0
	Temp + Weight	11	910.9	0.1	0.2	1.1
	Temp	11	910.9	0.2	0.2	1.1
	Temp + Fin Edges	11	911.0	0.2	0.2	1.1
	Temp + Length	11	911.0	0.2	0.2	1.1
	Null	11	924.4	13.6	0.0	911.4
Juvenile excystment	**Temp + Surface Area**	**11**	**847.9**	**0.0**	**0.6**	**1.0**
	Temp + Fin Surface Area	11	849.9	2.0	0.2	2.7
	Temp + Fin Edges	11	853.0	5.1	0.0	12.9
	Temp	11	853.9	6.0	0.0	20.1
	Temp + Weight	11	854.3	6.4	0.0	24.5
	Temp + Length	11	855.8	7.9	0.0	52.2
	Null	11	863.9	16.0	0.0	2995.9

The Null model is included for comparison and includes temporal autocorrelation, random slope, and random intercept, but not explanatory variables; n = number of fish; [a]DIC is Deviance Information Criterion; [b]ΔDIC is the difference between the model of interest and the best model; [c]ω_i is the model weight; and [d]Evidence ratio is the model weight of the best model divided by the weight for the model of interest. See text for details.

Since glochidial attachment, loss, and excystment varied between trials for the native control fish (see above), these variables were standardized by the corresponding control to make comparisons among catfish, rudd, and goldfish (Figure 3-3). After accounting for native control fish, glochidial attachment and juvenile excystment were not significantly different between non-native fish species (Figure 3-3a, c). However, control-standardized glochidial loss of non-native fish was significantly different (Figure 3-3b). Standardized glochidial loss was lowest for goldfish compared to catfish and rudd, and goldfish produced a higher relative number of juveniles per fish surface area when “corrected” for controls, but rates for all species were low (< 1% of control fish) (Table 3-2; Figure 3-3c).

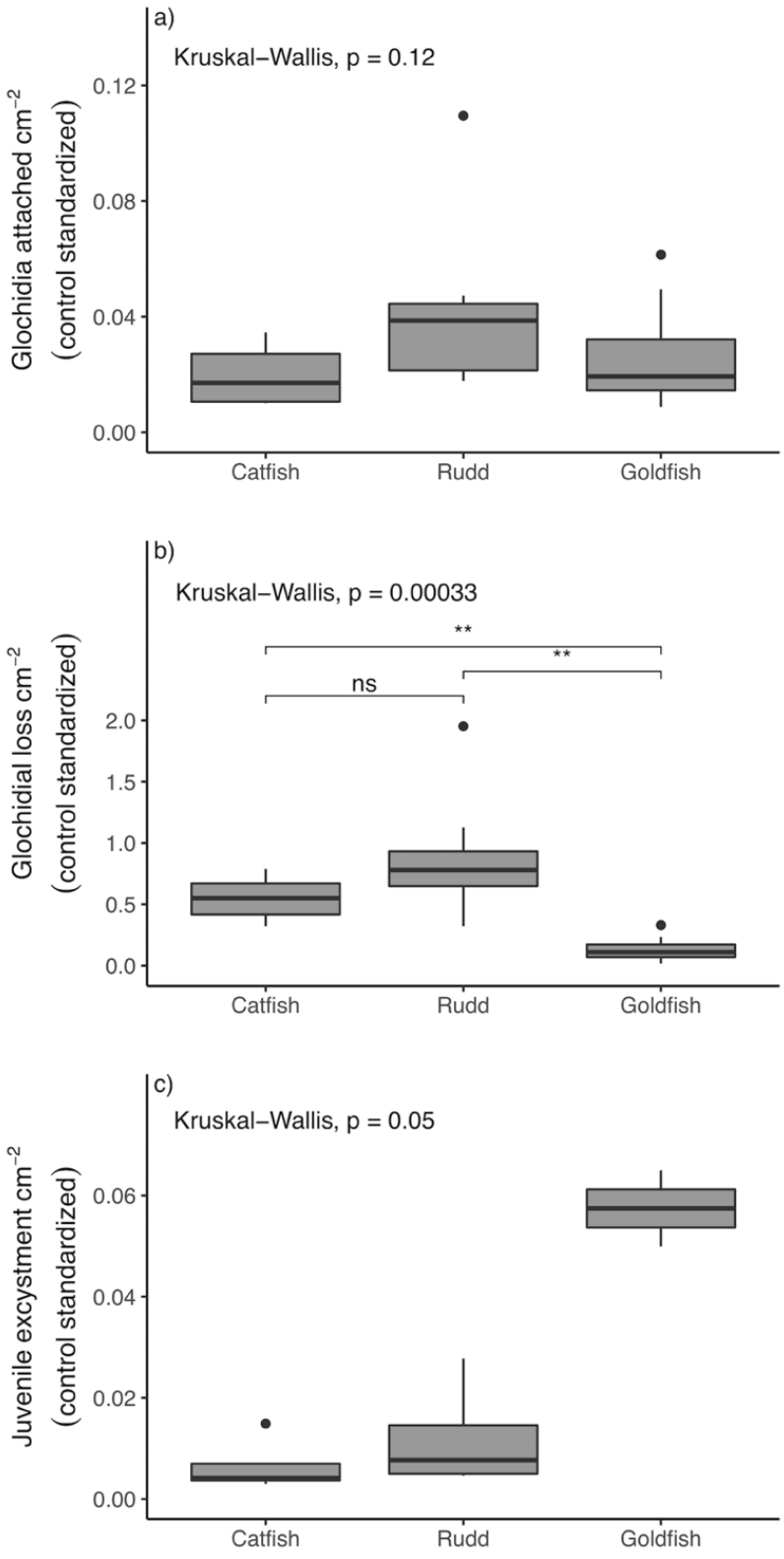

Figure 3-3: Non-native catfish, rudd, and goldfish values standardized by native control fish for (a) glochidia attached, (b) glochidial loss, and (c) juvenile excystment per fish surface area. Boxplots show median [black line inside boxplot]; interquartile range [box]; min/max [whiskers]; and outliers [> 1.5 x interquartile range, black dots]. For statistically significant Kruskal–Wallis rank sum tests, pairwise comparisons (Wilcoxon signed-rank tests) corrected for multiple tests are indicated above plots: $^{**}P < 0.01$, $^{*}P < 0.05$; *nsP* > 0.05, with brackets showing the comparison.

3.5 Discussion

This study recorded for the first time the host–larval relationship between *E. menziesii* glochidia and non-native catfish, rudd, and goldfish in New Zealand by comparing glochidial attachment, glochidial loss, and juvenile excystment with a known native host, the common bully. The results support the 'Enemy Release Hypothesis,' where total glochidial attachment after infestation was substantially lower on non-native than native control fish, which might offer a competitive advantage to non-native fish species by reducing the associated physiological cost of glochidial development. Additionally, catfish, rudd, and goldfish did not produce ecologically significant quantities of juveniles which detached earlier than native controls (indicating lower juvenile quality). Therefore, non-native fish species have potential to act as glochidial sinks when they co-occur in abundance with mussel populations.

3.5.1 **Variation in infestation of native fish**

Glochidial attachment on native control fish was similar between trials, indicating consistency in the assessment of glochidial viability and fish-stress behaviors that determine glochidial exposure (e.g., ventilation rate and position in infestation tank) (Mikheev et al. 2014). However, aspects of glochidial fitness other than attachment ability and excystment were not examined, and likely contribute to variability between trials and individuals. For example, multiple *E. menziesii* females were used to provide glochidia for each trial and it is not known if a single female can be fertilized by multiple males, which may introduce variability resulting from differences in paternal fitness (Christian et al. 2007; Ferguson et al. 2013). On the other hand, the fish immune system plays a large role in protection against glochidia attachment/encystment, and consists of innate and adaptive components (Lieschke and Trede 2009). The adaptive immunity component was likely excluded from this study by collecting fish from areas not known to support mussel populations, thus indicating variation in the innate immunity component (general defense mechanisms always present to respond to foreign substances) as potentially important in explaining differences between native control fish (Donrovich et al. 2017).

Native control common bully glochidial loss and juvenile excystment were not significantly different, which indicated uniformity between trials (although this result may partly be due to the small sample sizes used and the large variation between individuals). The important predictors positively related to glochidial loss and juvenile excystment for native control fish were temperature for both models, and fin surface area or fish surface area for loss and excystment, respectively. The positive effect of temperature on glochidial loss and juvenile excystment from host fish recorded in this study between 20 - 21°C extends the range glochidial development is known to occur in this mussel population, which is likely to be adapted to the natural water temperature range of 18-20°C found in late summer (Cyr et al. 2016). Glochidial loss was predicted only marginally better when including co-variates that represented different glochidial attachment sites, whereas prediction of juvenile excystment was greatly improved when fish surface area was included. This may be explained by different processes driving the outcomes (e.g., glochidial quality for loss and availability of attachment sites for juvenile excystment).

3.5.2 **Role of non-native fish in mussel recruitment**

In their native range, goldfish, catfish, and rudd can be suitable hosts for native freshwater mussels: goldfish host the Chinese pond mussel (*Sinanodonta woodiana*) (85.4 ± 3.8% metamorphosis; Douda et al. 2017); rudd host the European thick shelled river mussel (*Unio crassus*) and duck mussel (*Anodonta anatina*) (mean metamorphosis 74.7% and 65.6–73.4%, respectively; Douda 2015; Douda et al. 2012, 2013); and catfish are recorded hosts (non-quantitively) of seven North American species (FMHD 2017). As invasive species with potential to be mussel hosts, goldfish have been studied more frequently than catfish and rudd (Table 3-4), and are predominantly poor hosts (0.001–15.4% metamorphosis (Douda et al. 2013; Watters et al. 2005; Watters and O'Dee 1998)).

Table 3-4: Summary table of fish–mussel interactions in the native or non-native range of different fish species (spp.) and determination of host suitability for goldfish, rudd, and catfish. N = No; Y = Yes

Fish spp.	Mussel spp.	Interaction – fish range	Evidence (laboratory trials)	Suitable host?	Citation
Goldfish (*Carassius auratus*)	*Alathyria jacksoni* *Velesunio ambiguus*	Non-native	Glochidia generally detached within 2-3 hours. In a few instances it appeared detachment occurred during the initial stages of encystment	N	(Walker 1981)
	Velesunio ambiguus	Non-native	No glochidia attached	N	(Hiscock 1951)
	Lampsilis cardium *Utterbackia imbecillis*	Non-native	17.5 glochidia attached per fish, 0% metamorphosis. 8.7 glochidia attached per fish, 15.4% metamorphosis	Y/N	(Watters and O'Dee 1998)
	Villosa iris	Non-native	Goldfish expressed humoral defense factor specific to glochidial antigens after infestation with glochidia	N	(O'Connell and Neves 1999)
	Tritogonia verrucosa	Non-native	One trial, two fish, 1-5 days to rejection, 0% metamorphosis	N	(Hove et al. 2011)
	Quadrula fragosa	Non-native	One trial, one fish, 1-3 days to rejection, 0% metamorphosis	N	(Hove et al. 2012)
	Westralunio carteri	Non-native	26 exposed individuals, glochidia attachment may have occurred briefly, 0% metamorphosis	N	(Klunzinger et al. 2012)
	Anodonta anatina	Non-native	15 Fish 79.5 ± 6.4 Fish length 22.6 ± 0.4°C 82.9 Mean number of attached glochidia per fish 0.1 Mean number of juveniles per fish 0.001 Metamorphosis rate (%) 6 days to metamorphosis	N	(Douda et al. 2013)

Fish spp.	Mussel spp.	Interaction – fish range	Evidence (laboratory trials)	Suitable host?	Citation
Goldfish (*Carassius auratus*)	*Lasmigona costata*	Non-native	10% metamorphosis (all @ 20°C)	Y/N	(Watters et al. 2005)
	Plethobasus cyphyus		No metamorphosis		
	Pleurobema cordatum		No metamorphosis		
	Pleurobema sintoxia		No metamorphosis		
	Pyganodon grandis		9% metamorphosis		
	Strophitus undulatus		No metamorphosis		
	Margaritifera auricularia	Non-native	10 experiments, attachment only observed in one experiment, no encystment or metamorphosis	N	(Lopez and Altaba 2005)
Rudd (*Scardinius erythrophthalmus*)	*Margaritifera auricularia*	Non-native	5 experiments, no attachment, encystment or metamorphosis occurred	N	(Lopez and Altaba 2005)
Catfish (*Ameiurus nebulosus*)	*Tritogonia verrucosa*	Native	3 trials, glochidia growth observed in 2 trials but no metamorphosis	N	(Hove et al. 2011)
	Lampsilis s. claibornensis	Native	No juveniles	Y/N	(Keller and Ruessler 1997)
	Megalonaias nervosa		No juveniles		
	Villosa lienosa		16 Juveniles (non-quantitative)		

Previous studies suggest that goldfish resistance may result from the thick mucus produced by their epithelial cells which can slough to detach glochidia within 2–3 h (Walker, 1981). Furthermore, goldfish may produce humoral defense factors specific to glochidial antigens (O'Connell & Neves, 1999), as well as develop delayed and 'irregular' cyst formation (Rogers-Lowery & Dimock, 2006). In contrast, Roberts & Barnhart (1999) found higher metamorphosis rates on another Cyprinidae, the golden shiner (*Notemigonus crysoleucas* (Hildebrand & Towers, 1928)), in trials conducted at a range of temperatures (i.e., 67, 62, and 42% metamorphosis at 10, 15, and 21°C, respectively). This possibly resulted from host immunosuppression, which may occur through multiple mechanisms. For example, the stress response hormone cortisol (which causes immunosuppression) can increase the number of attached glochidia (42%) and metamorphosis success (28%) by host fish when artificially elevated through intraperitoneal injection (Dubansky et al., 2011). In the present study, all fish were acclimated to laboratory conditions prior to infestation, making stress-induced immunosuppression unlikely.

For the 'Enemy Release Hypothesis' (Torchin et al., 2003) to be fully supported, a physiological cost must be associated with glochidial development on the fish host (Horký et al., 2014; Slavik et al., 2017). For example, non-native brook trout (*Salvelinus fontinalis* (Mitchill, 1814); non-host) were more abundant than native brown trout (*Salmo trutta* (Linnaeus, 1758); host) in streams containing the freshwater pearl mussel (*Margaritifera margaritifera* (Linnaeus, 1758)) (Salonen et al., 2016), which develop on fish from 8 to 12 months and induce a respiratory cost, reduced swimming ability, and higher mortality (Meyers & Millemann, 1977; Taeubert & Geist, 2013; Thomas et al., 2014). In contrast, glochidia of *E. menziesii* can develop on suitable host fish between 9 and 22 days (Clearwater et al., 2014), suggesting any costs incurred may be short term. However, the high percentage of viable mussels produced by native controls in this study (~ 30–80%), coupled with the potential for consecutive infestation over the mussel spawning season, may lead to a substantial cost being incurred for individual fish. Evidence that may support interspecific competition between the non-native species used in this study and common bully is sparse:

Collier et al. (2018) found catfish predation of common bullies occurred in 42.9% of individuals and Hicks (2003) suggested a potential for dietary overlap between rudd and common bullies. Nonetheless, if competition occurs, non-native fish species may therefore have an advantage over suitable native fish hosts due to lower infestation rates, and thus indirectly impact *E. menziesii* recruitment, especially in areas where dense mussel beds occur that would normally have high infestation rates on native host fish.

Another mechanism by which the studied non-native species may impact *E. menziesii* through limiting successful unionid recruitment is by acting as a glochidial sink, whereby glochidia are able to attach but not transform (or in low numbers) on unsuitable host fish (Taeubert et al., 2012; Douda et al., 2013; Tremblay et al., 2016). This was the case for the invasive round goby (*Neogobius melanostomus* (Pallas, 1814)), which was determined to be a glochidial sink based on the ratio of glochidial loss to juvenile production in comparison to primary hosts for five native freshwater mussel species in the Laurentian Great Lakes region (Tremblay et al., 2016). Accordingly, based on the results of the present study goldfish should probably be considered "weak" glochidial sinks, since few glochidia were attached and few subsequently lost under laboratory conditions (Figure 3-3). However, rudd and catfish, which can reach large densities and biomass in New Zealand lakes (Collier et al., 2016), are large fish and are therefore stronger candidates to be glochidial sinks, although their attachment rates were markedly lower than for the native bullies.

3.5.3 **Implications for conservation and future directions**

This research supports the assumption that non-native species are generally less suitable hosts of native freshwater mussels (Lopes-Lima et al., 2016; Modesto et al., 2018) and 'biotic homogenization' of freshwater communities is a threat to previously co-evolved and evolutionarily balanced host–parasite relationships (Douda et al., 2013). Exceptions to this generalization may be explained when a fish family has suitable hosts in the native range that are also represented overseas (e.g., Poecilidae and Fundulidae for *Lampsilis cardium* (Rafinesque, 1820)), previous fish contact with unionids to develop similar co-evolutionary adaptations (Watters &

O'Dee, 1998), or a mussel species has highly developed glochidia (with large hooked larvae) that transform before an effective innate immune response is initiated, such as glochidia of the freshwater swan mussel (*Anodonta cygnea* (Linnaeus, 1758)) on grass carp (*Ctenopharyngodon idella* (Cuvier & Valenciennes, 1844)) (Huber & Geist, 2017). Despite the generalist host strategy of *E. menziesii,* the suggestion that other freshwater mussels with broad-host spectrums are also not able to effectively use non-native fish as hosts is supported by this study (see also Douda et al., 2013).

Since non-native fish produced a small number of juvenile mussels in the present study, there may be capacity for *E. menziesii* to adapt and more effectively parasitize newly arrived host resources over an evolutionary time scale. However, at the same time, counter-adaptations against mussel glochidia may be developed by non-native fish species, which reflects uncertainty in the future co-evolutionary development of fish–mussel relationships. This is due to variability that can arise in the same host–parasite interaction between areas of recent and ancient sympatry (Douda et al., 2017), geographically distinct lineages (Reichard et al., 2015), and cross-resistance to glochidia from other mussel species (Donrovich et al., 2017). In addition, despite glochidial excystment occurring on non-native fish, earlier excystment could indicate a lower quality of juveniles that contain lower energetic reserves for development (Marwaha et al., 2017). Earlier development from 'poor hosts' has also been documented for *A. cygnea* (Huber & Geist, 2017) and *A. anatina* (Huber & Geist, 2019), resulting in a limited duration to which glochidia can uptake nutrients from their host, thereby reducing subsequent post-excystment fitness characteristics such as growth rate, size at excystment, and survival (Marwaha et al., 2017).

Adult-skewed size structures observed in freshwater mussel populations in New Zealand and worldwide may be caused in part by recruitment failure resulting from disruption to the unionid life-cycle (James, 1985; Bailey & Green, 1989; Roper & Hickey 1994; Araujo et al., 2000; Hastie & Toy, 2008; Harriger et al., 2009), which might not be immediately apparent due to their relatively long life-span (Haag, 2012). Non-native fish species have high

potential for recruitment disruption through multiple direct and indirect mechanisms, and therefore identifying threat mechanisms to unionid mussels, which are in decline globally, is important to target conservation action (Haag & Williams, 2013; Lopes-Lima et al., 2016; Zieritz et al., 2017). Linking the applicability of laboratory evaluations of host suitability to field-based action has limitations, since assessed suitability may differ in the wild when host and mussel behavior are considered (Mierzejewska et al., 2014). Also, artefacts resulting from potential stress-induced behaviors that would decrease host suitability in laboratory environments are removed in a field study (Levine et al., 2012). Therefore, confirming the observation that goldfish, catfish, and rudd are unsuitable hosts for *E. menziesii* in the field is the next step for future research. This would indicate whether enhancing native fish populations and removing non-native fish is recommended to conserve *E. menziesii* populations in New Zealand.

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Chapter 4

Invasive macrophytes induce context-specific effects on oxygen, pH, and temperature in a hydropeaking reservoir

4.1 Abstract

Dense macrophyte beds are known to produce extreme diurnal oxygen and temperature conditions in shallow lakes, however their influences in managed hydropeaking reservoirs has received limited attention. We measured dissolved oxygen, pH and water temperature in the Lake Karāpiro hydroreservoir, northern New Zealand, across a gradient of proportional water-column height occupied by the invasive macrophytes *Egeria densa* and *Ceratophyllum demersum*, which dominated in the upper-riverine (variable water inflow) and lower-lacustrine (variable water level) sections, respectively. Hypoxia and anoxia events that occurred inside invasive macrophyte beds during their summer peak biomass accumulation period were more pronounced for *C. demersum* than for *E. densa*, and within the bottom 20 % of the water column. In contrast, pH and temperature changed little in relation to proportional macrophyte height. Macrophyte species differences in the production of hypoxia and anoxia events increased when site-specific hydropeaking management covariates (depth, inflows, water level) were accounted for. This association with hydropeaking likely resulted from contrasting hydrodynamics in the lower-lacustrine and upper-riverine lake sections, where oxygen can decrease with higher water levels and lower water inflow rates, respectively. During the course of our study, some macrophyte beds were treated with herbicide, enabling us to document prolonged and sustained hypoxic/anoxic conditions near the bottom following spraying. These results underscore the adverse effects of invasive macrophytes on water physicochemical attributes that sustain aquatic biota, and highlight the context-dependent nature of these effects moderated by reservoir management for hydropeaking and macrophyte control.

4.2 Introduction

Invasive macrophytes readily establish in human-modified environments such as hydrogeneration reservoirs (Havel, Lee, & Vander Zanden, 2005; Johnson, Olden, & Vander Zanden, 2008), where daily water level fluctuations from reservoir management (i.e., hydropeaking) play a critical role in their proliferation in littoral zones (Shivers, Golladay, Waters, Wilde, & Covich, 2018; Zhao, Jiang, Cai, & An, 2012). These beds can accumulate massive biomass over summer in temperate regions (Madsen, Chambers, James, Koch, & Westlake, 2001; Zohary & Ostrovsky, 2011), resulting in reduced native vegetation diversity (Andersen, Kragh, & Sand-Jensen, 2017; Parveen, Asaeda, & Rashid, 2017), changes in community composition of other trophic levels such as benthic invertebrates (Kelly & Hawes, 2005; Kovalenko & Dibble, 2010), and potentially the loss of ecosystem functions and services (Bunn, Davies, Kellaway, & Prosser, 1998; Villamagna & Murphy, 2010). Consequently, invasive macrophytes commonly represent 'foundation species' (Ramus, Silliman, Thomsen, & Long, 2017; Wood & Freeman, 2017) and have been referred to as 'ecosystem engineers' due to their dominance in abundance and influence on lentic ecosystems (Thomaz, Mormul, & Michelan, 2014; Yarrow et al., 2009). A primary mechanism of impact by invasive macrophytes is the production of adverse physicochemical conditions above the sediment-water interface, which has been recorded inside dense beds in shallow lakes (Andersen et al., 2017; Bunch, Allen, & Gwinn, 2010; Vilas, Marti, Adams, Oldham, & Hipsey, 2017). However, examination of the relationship between invasive macrophytes and adverse physicochemical conditions in the context of a hydropeaking reservoir has received limited attention.

Studies of water physicochemical changes in shallow lake and slow-flowing river ecosystems report that dense macrophyte beds promote extreme diurnal variability in dissolved oxygen (i.e., anoxia-supersaturation), which can occur throughout the water column or be more starkly pronounced in bottom waters compared to surface waters (Andersen et al., 2017; Bunch et al., 2010; Caraco & Cole, 2002; Ribaudo et al., 2018; Vilas et al., 2017). These extreme diurnal cycles can be associated with changes in pH (Andersen et al., 2017; Ribaudo et al., 2018) and are driven by high

volumetric rates of daytime photosynthesis and nocturnal respiration (Christensen, Sand-Jensen, & Staehr, 2013; Martinsen, Andersen et al., 2017). Furthermore, invasive macrophytes can facilitate temperature stratification when they reach a threshold of percentage cover in the water column. For example, Vilas et al. (2017) recorded a 10 °C maximum difference between the water surface and lake bottom during the daytime inside *Potamogeton crispus* beds occupying at least 50 % of the water depth. Extreme diel changes in physicochemical conditions present a challenge for the survival of sessile and mobile animals (e.g., unionid mussels), and is expected to drive selection towards species tolerant of high temperature and/or hypoxia (Andersen et al., 2017).

The strength of invasive macrophyte impacts is dependent on their density and the consequent rate of hydrological exchange (Andersen et al., 2017; Vilas et al., 2017). Such impacts can be particularly pronounced at the end of summer when macrophyte senescence results in mass decomposition of organic matter that may consume large quantities of oxygen for prolonged periods (Godshalk & Wetzel, 1978). Although processes operating in shallow lakes may also occur in littoral zones of deep lakes, water level variations due to hydropeaking may further mediate the influence of invasive macrophytes on physicochemical parameters. This is especially so given that dams can create conditions suitable for the proliferation of aquatic plants, but the nature of these conditions varies due to hydropeaking demand and the rate of water level change in inflows (Zhao et al. 2012).

With an increasing number of dams being constructed for hydropower generation globally (Zarfl, Lumsdon, Berlekamp, Tydecks, & Tockner, 2014), and the associated spread of invasive species (Johnson et al. 2008), there is a need to understand the role invasive macrophyte species have on ecologically-relevant physicochemical conditions during their peak biomass accumulation period in hydropeaking reservoirs. Accordingly, a field study was conducted across a gradient of invasive proportional macrophyte height during the austral summer in the most downstream of a series of hydropeaking reservoirs on New Zealand's longest river, the Waikato River. Two invasive macrophyte species, *Egeria densa* and *Ceratophyllum demersum*, dominated the upper-riverine and lower-lacustrine sections of

this hydroreservoir, respectively, enabling a comparison between species where water inflow or water level were expected to generate context-specific effects on macrophyte-mediated physicochemical parameters. The following hypotheses were tested: 1) the magnitude of summer daytime physicochemical conditions will vary spatially in relation to a gradient of invasive macrophyte proportion (i.e., the height of macrophyte canopy expressed as a proportion of the water column depth) and water column-benthic processes, and; 2) hydropeaking effects on physicochemical conditions produced by different macrophyte species in contrasting lake sections will be moderated by site hydrology (i.e., riverine vs lacustrine locations). During the course of our study, some macrophyte beds were treated with herbicide, enabling us to examine treatment effects on physicochemical conditions, notably the diurnal magnitude and duration of bottom-water hypoxia conditions as the macrophytes decayed.

4.3 Materials and methods

4.3.1 **Study site**

Karāpiro (37° 55′ 42.82″ S, 175° 32′ 40.3″ E) is a large, deep (5.4 km^2 surface area; 11 m mean and 30.5 m maximum depths; Lowe & Green, 1987) eutrophic (Livingston, Biggs, & Gifford, 1986) hydropeaking reservoir on the Waikato River. It had a mean water inflow during the study of 262 m^3 s^{-1} (minimum = 208, maximum 320 m^3 s^{-1}) equating to residence times of 3.3, 2.6 and 2.2 days, respectively, assuming full water column mixing and a lake water volume of 60 x 10^6 m^3 (Gibbs et al. 2015). The upper section of Karāpiro is riverine, with highly variable flows controlled by discharge from the upstream Arapuni hydropower station (i.e., mean discharge 271 m^3 s^{-1}, range 0.1-668 m^3 s^{-1} in 2018). In contrast, the lower section closer to the dam is more lacustrine, with a diurnally variable water level related to hydropeaking operations at Karāpiro dam (mean daily water level range of 1.2 m in 2018).

Two invasive macrophyte species are abundant in Karāpiro: *C. demersum* and *E. densa* (Clayton, Wells, & Taumoepeau, 2006; McCarter, de Winton, Clayton, Wells, & Tanner, 1993; Schwarz, Wells, & Clayton, 1999). *Ceratophyllum demersum* dominates the lower-lacustrine section and is

present in almost all shallow littoral areas to 5 m depth (Hofstra & de Winton, 2016), where it forms extensive monospecific beds. These beds develop dense subsurface canopies that displace and exclude native and other non-native vegetation beneath (Coffey & Clayton, 1988). The resulting recreational, cultural, and environmental threats to hydrogeneration, in the lower-lacustrine section has led to annual *C. demersum* control using the herbicide diquat (Hofstra & de Winton, 2016). In the upper-riverine section, *E. densa* dominates littoral zones forming large, dense and monospecific beds that are rooted to the bottom and can withstand faster flows (Clayton, Matheson, & Smith, 2009). Although both *E. densa* and *C. demersum* are found throughout the year, rapid growth occurs in spring: e.g., 2–10 % day^{-1} and 2–8 % day^{-1} of dry biomass, respectively (Eller et al., 2015). Rapid summer growth leads to peak accumulation of biomass in autumn when both species often reach the water surface (Hofstra & de Winton, 2016).

4.3.2 **Measurement of physicochemical parameters**

To understand differences in daytime physicochemical parameters in the water column (i.e., pH, temperature (°C), dissolved oxygen saturation % (hereafter oxygen), and specific conductivity (µS cm^{-1} at 25 °C)) associated with growth of macrophyte beds over the peak accumulation period, field data were collected at four sites in each of the lacustrine (*C. demersum*) and riverine (*E. densa*) sections between 20 November - 7 December 2018 (*C. demersum* only) and January 22 – 30, 2019 (both species) following an initial echo-sound survey and aquatic vegetation mapping (Helminen 2019; for site locations see Figure 4-1; Figure 7-2 in Appendix 7.3.1). At each site, vertical profiles of water-column physicochemical parameters were measured at four points designated in terms of macrophyte proportion (range 0-1) as: “macrophyte-free” (A; $\bar{x}$ ± SD; 0.1 ± 0.3 proportional macrophyte height), “light” (B; 0.3 ± 0.2), “dense-edge” (C; 0.6 ± 0.3) and “dense-bed” (D; 0.7 ± 0.3) (see Figure 4-2b for further explanation). Profiles at these four points were taken across three transects (5-10 m in length depending on depth) located 10 m apart, running perpendicular to the shore on each sampling occasion (Figure 4-2a).

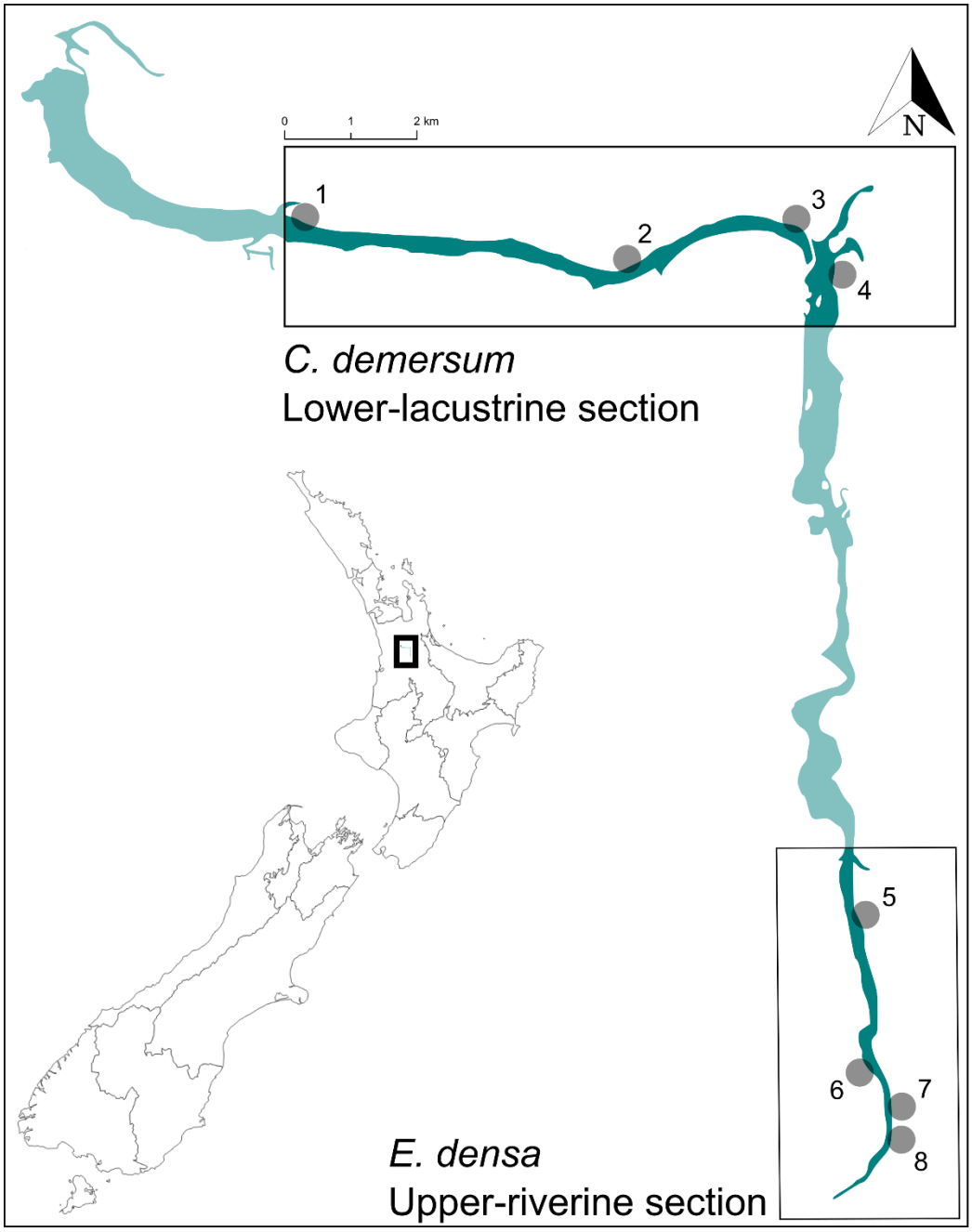

Figure 4-1: Study site locations in *C. demersum* and *E. densa* in the lower-lacustrine and upper-riverine sections of Karāpiro, respectively. Enlarged area is located in the Waikato region, North Island, New Zealand (black box on country outline).

A total of 144 physicochemical water-column profiles was collected to create a spatial dataset spanning a gradient of invasive macrophyte canopy height (i.e., 0-1.7 m for *C. demersum* in November; 0–4.2 m for *C. demersum* and 0–2.1 m for *E. densa* in January). From a boat anchored at two points to prevent movement, physicochemical parameters were measured with a sonde (650 MDS, YSI Incorporated, Yellow Springs, Ohio, United States). Measurement points started at the water surface (referred to as '0 m', but

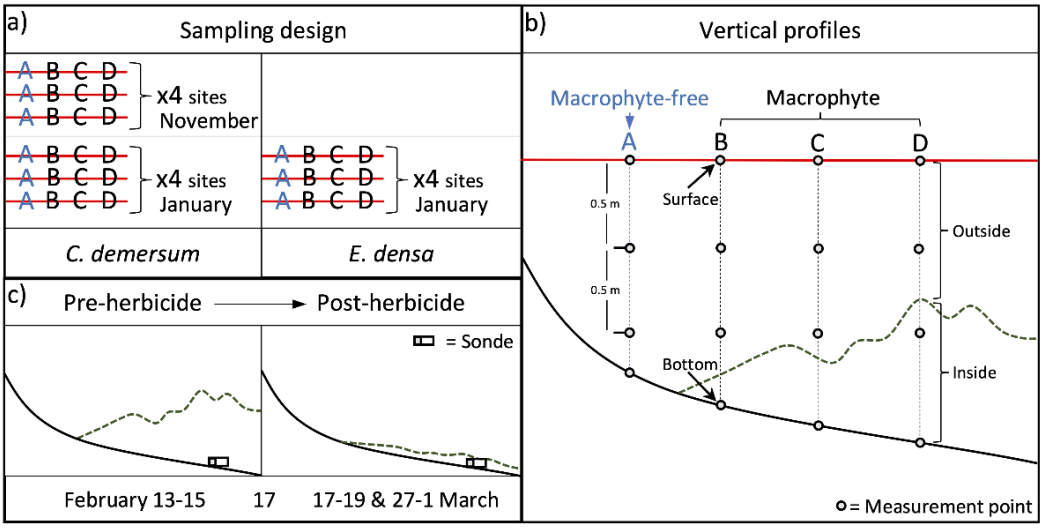

Figure 4-2: Study design showing: a) number of transects (red) perpendicular to the shoreline and location of vertical profiles (A, B, C, D) at 4 sites for each sampling occasion (*C. demersum* -lacustrine November*; C. demersum* -lacustrine January; and *E. densa* -riverine January); b) measurement points (every 0.5 m from water surface including lake bottom) in macrophyte-free (A – blue) and macrophyte (B, C, D – black) vertical profiles, with inside/outside macrophyte bed (green dashed line) labelled; and c) herbicide-impact measurement by 7-day deployment of a sonde in a *C. demersum* bed (dashed green line) and two-day periods selected for analysis.

equivalent to the depth required to submerge sonde probes: i.e., 0.05 m) and then every 0.5 m towards and including the lake bottom (Figure 4-2b). Invasive macrophyte height was measured by lowering the sonde to the subsurface canopy (viewed using a bathyscope) then subtracting the calibrated depth reading from total depth. After each vertical profile, the time (09:30-16:00 h), GPS location (easting, northing to 3-5 m), and water depth (1-4.2 m) were recorded.

To examine diurnal variation in physicochemical parameters associated with *C. demersum*, the sonde was deployed at the lake bottom inside a bed previously used for vertical water profiles (37° 56′ 41.2″ S, 175° 34′ 50.4″ E). During the first deployment (12-19 February), herbicide was unexpectedly applied (as indicated by a spike in specific conductivity (increasing from 234 to 305 µS cm^{-1} at 25°C) across the site as part of annual *C. demersum* macrophyte control, enabling comparison of short-term physicochemical changes before and after herbicide application (Figure 4-2c). The sonde was redeployed at the same site on 26 February for a further 7 days to

assess longer-term changes in physicochemical parameters. All these sonde measurements were collected on the bottom every ten minutes.

4.3.3 **Data preparation**

For analysis of the spatial dataset (within the water column across lake sections), sonde measurements were classified as collected from inside or outside macrophyte beds if sonde depth was ≤ or > macrophyte height, respectively (Figure 4-2b). Specific conductivity (range 158-284 µS cm^{-1} at 25^{o}C) showed no patterns in relation to macrophyte abundance so was not included in the spatial analysis, but it was used in the herbicide impact analysis to detect time of herbicide application and changes associated with *C. demersum* decomposition. For the latter analysis, data collected from the first hour of the seven-day sonde deployments before and after herbicide application were excluded to remove variation related to sonde installation.

To account for variability in physicochemical parameters related to macrophyte metabolism or Karāpiro water level and water inflow fluctuations during the 6.5 hours required to collect the spatial data, the following covarying factors were included in data analyses: (1) measurement time expressed as minutes past midnight on each day; and (2) half-hourly data on water level at the Karāpiro dam and water inflow (January 2018 –May 2019) from the Arapuni dam located directly upstream (data acquired from Mercury New Zealand Limited). The time of measurement was rounded to the nearest half-hour to align with the supplied water level and water inflow data. Karāpiro water level and water inflow were significantly inversely correlated (r = -0.69, p < 0.001).

Data collected from the 'surface' (i.e., sonde probe depth of 0.05 m) and 'bottom' (i.e., sonde probe 0.05 m from the lake bed) in the spatial dataset (Figure 4-2b) were used to examine the strengths of relationships between potential covarying factors and physicochemical parameters, which differed most at these extremes. Linear regression was used to model each relationship, with the physicochemical parameter and potential covarying factor as the response and predictor variables, respectively. All relationships followed linear models (including measurement time; Figure 7-4, in

Appendix 7.3.3) over the 7.5-hour period of daylight that measurements were collected (Table 7-5 in Appendix 7.3.5).

To remove the influence of the covarying factors of measurement time, and associated temporal variations in water depth induced by changes in Karāpiro water level and water inflow, detrending (see below and Figure 7-3, 7-4, and 7-5 in Appendix 7.3.3 for details) was performed prior to examining the relationship between proportional macrophyte height and measured pH, temperature or oxygen. Karāpiro water level and water inflow were both detrended as different relationships with physicochemical parameters were shown for *C. demersum* and *E. densa* sites. To detrend a physicochemical parameter, a correction was applied as follows:

$$x.detrended = x + (\overline{x} - \widehat{y})$$

where *x* is the raw physicochemical parameter and *y* the covarying factor. This was based on methods shown by Weisberg (2005), where a correction (difference between the mean physicochemical variable value ($\overline{x}$) and fitted covarying factor value ($\widehat{y}$)) was applied to the raw physicochemical parameter. For oxygen, resulting detrended values < 0 were recoded to 0 (e.g., anoxic conditions measured in the afternoon could be adjusted to a negative value when accounting for the positive effect of measurement time).

4.3.4 **Statistical analyses**

All data analyses presented were conducted using the R statistical software program v3.5.2 (R Core Team 2019) and presented in "ggplot2" v3.1.0 (Wickham, 2016). The relationship between raw physicochemical parameters and covarying factors collected across the three sampling occasions was explored using Principal Component Analysis (PCA) performed in the "Vegan" community ecology package v2.5-4 (Oksanen, 2015). Prior to performing the PCA, raw physicochemical parameters and covarying factors were centered and scaled (subtracted from sample means and divided by their standard deviate) to standardize measurements on different scales (Sergeant, Starkey, Bartz, Wilson, & Mueter, 2016). Statistical significance and coefficients of determination of physicochemical parameters and covarying factors were tested with permutation tests (999) using the "envfit" function in "Vegan" (Oksanen, 2015).

To examine changes with depth, mean values of raw physicochemical parameters in a vertical profile were binned into five groups of equal size based on proportional depth, and displayed as boxplots. Comparisons of proportional macrophyte height, Karāpiro water level and water inflow, and physicochemical parameters between sampling occasions, sites, and vertical profiles were tested using ANOVA or t-tests if parametric assumptions were met, or if not, their non-parametric equivalents were used (Kruskal-Wallis or Wilcoxon signed-rank tests). To account for multiple pairwise comparisons, Bonferroni corrections were applied for all tests with multiple groups. Proportional data was arcsine transformed prior to analysis (Zar, 1999).

Relationships between detrended physicochemical parameters of temperature, pH, and oxygen (transect mean of vertical profiles) were visualized in a ternary plot (scaled from 0-100) using "ggtern" v3.1.0 (Hamilton & Ferry 2018). To test the relationship between proportional macrophyte height and detrended physicochemical parameters at the lake bottom and water surface for each sampling occasion, linear quantile regressions were performed using the 10^{th}, 50^{th}, and 90^{th} quantiles ("quantreg" v5.38; Koenker et al. 2019). Each quantile regression slope was tested for significance from zero with xy-pair bootstrap standard errors (Koenker, 2019; Parzen, Wei, & Ying, 1994). Quantile regression was chosen since relationships were heteroscedastic, with triangular patterns displayed in physicochemical parameters across the macrophyte proportion gradient. The 10^{th} and 90^{th} percentiles represent the upper and lower boundaries of these relationships and thereby can determine potential high and low limits in the data (Anderson & Jetz, 2005).

To examine the impact of herbicide application on diurnal variation of physicochemical parameters inside a *C. demersum* bed, two-day periods (starting at 09:00 hours; 288 measurements) were selected before (13-15 February), after (17-19 February) and 10-days after (27 February – 1 March) herbicide application (17 February; Figure 4-2c). For each period, the coefficient of variation, and 10^{th}, 50^{th} and 90^{th} percentiles were calculated, with differences between periods in median value and variability tested using Wilcoxon Signed-rank and Levene's tests, respectively. As the

herbicide-impact study was serendipitous, Bayesian structural models on the time-series data were applied to understand the effect of herbicide application compared to a modelled control (i.e., 'counterfactual'; if no herbicide impact had occurred) using the 'CausalImpact' package (Brodersen, Gallusser, Koehler, Remy, & Scott, 2015). This impact analysis generated the modelled control based on the 'before' two-day period for specific conductivity, pH, and oxygen (log x+1) using covarying factors identified in the PCA (i.e., temperature, depth, measurement time, and Karāpiro water level and water inflow) to compare with the "after" two-day periods.

4.4 Results

4.4.1 **Sampling site characteristics**

Water level in the lower-lacustrine section was significantly higher on average in January than November (mean ± SD of vertical profile measurement points: 52.8 ± 0.1 and 52.6 ± 0.1 meters above sea level, respectively; Wilcoxon signed-rank test, $P < 0.001$), with significant differences between sampling sites (November, Kruskal-Wallis, $H = 32.46$, $P < 0.001$; January, Kruskal-Wallis, $H = 44.15$, $P < 0.001$). Water inflow in the upper-riverine section varied by 100 m^3 s^{-1} on average between sampling days (overall mean 269.9 ± 42.9 m^3 s^{-1}; Kruskal-Wallis, $H = 39.09$, $P < 0.001$).

Across sampling occasions, macrophyte-free profile locations were 0.6-0.8 m shallower than locations with macrophytes (transect means 1.2 ± 0.5 and 1.9 ± 0.9 m, respectively; Wilcoxon signed-rank test, $P = 0.016$; Table 4-1). Vertical profile data were collected in significantly deeper water for *C. demersum* than *E. densa* sites in January (site means 1.9 ± 0.7 and 1.1 ± 0.2 m, respectively; Table 4-1) (Wilcoxon signed-rank test; $P = 0.029$).

Ceratophyllum demersum occupied 58 % and 64 % of the water column on average in November and January, respectively, reaching mean heights of 1.3 and 1.4 m (Table 4-1). However, the proportion of water column occupied by *C. demersum* was not significantly different between sampling occasions (site mean arcsine transformed; Wilcoxon signed-rank test, $P = 0.91$). Across *C. demersum* transects (e.g., profile A versus profile C or D),

vertical profile height was significantly different (Kruskal-Wallis, transect means, $H = 24.2$, $P < 0.001$), although A-B and C-D profiles showed non-significant pairwise differences (Wilcoxon signed-rank tests Bonferroni corrected, $P = 0.59$ and $P = 0.39$, respectively). In January, *E. densa* occupied a significantly higher proportion of the water column than *C. demersum* (by 20 %; site mean arcsine transformed; t-test, $P = 0.013$; Table 4-1). As with *C. demersum*, *E. densa* height (mean 1.1 m; Table 4-1) was significantly different across vertical profiles (ANOVA on transect means, $F = 13.72$, $P = 0.003$) except between A-B and C-D profiles (t-tests Bonferroni corrected, $P = 0.063$ and $P = 0.68$, respectively).

Table 4-1: Summary statistics of water depth, macrophyte height, proportion of the water column occupied, measured oxygen, pH, and temperature for *C. demersum* (November 2018 and January 2019) and *E. densa* (January 2019) sites in macrophyte-free and macrophyte-occupied vertical profiles (see Figure 4-2).

Sampling occasion	Profiles (n)			Macrophyte-free (A)				Macrophyte (B-D)					
	M-F	M		Depth (m)	Oxygen (%)	pH	Temp (°C)	Depth (m)	Height (m)	Proportion (0-1)	Oxygen (%)	pH	Temp (°C)
C. demersum November 2018	12	36	$\bar{x}$	1.27	140.43	7.73	19.49	2.10	1.32	0.58	124.95	7.56	19.25
			SD	0.25	13.34	0.33	0.55	0.90	1.08	0.30	40.42	0.39	0.48
			CV	20.06	9.50	4.28	2.80	42.93	81.69	51.92	32.35	5.20	2.49
C. demersum January 2019	9*	39	$\bar{x}$	1.58	141.87	7.59	23.49	2.06	1.44	0.64	111.79	7.15	22.81
			SD	0.47	8.25	0.36	0.47	0.97	1.14	0.30	43.95	0.38	0.53
			CV	29.75	5.82	4.73	1.99	47.08	79.28	46.64	38.51	5.30	2.35
E. densa January 2019	12	36	$\bar{x}$	0.63	125.02	6.82	22.50	1.24	1.09	0.84	112.84	6.79	22.50
			SD	0.12	14.61	0.20	0.25	0.41	0.57	0.29	43.70	0.41	0.35
			CV	18.51	11.68	2.91	1.12	33.19	52.34	34.37	38.73	6.02	1.55

n, number; M-F, macrophyte free; M, macrophyte; SD, standard deviation; CV, coefficient of variation. * Encroachment of *C. demersum* from November to January resulted in three profile locations that were previously vegetation-free to contain macrophyte.

4.4.2 **Temporal and spatial patterns**

The PCA explained 36 % and 22 % of the variation in the spatial dataset across the first and second principle components, which were associated with distinctly different environmental gradients (all vectors $P < 0.001$). PC1 was positively associated with temperature and water level, and negatively with pH and water inflow, whereas PC2 was positively associated with oxygen and negatively with measured depth (Figure 4-3). The measurement time vector appeared on the diagonal in relation to axes 1 and 2. *Ceratophyllum demersum* sampling occasions spread out temporally across the PC1 axis. Within sampling profile locations, macrophyte species spread out spatially across the PC2 axis, with macrophyte-free profiles (A) at the top and dense-bed profiles (D) at the bottom (Figure 4-3).

In macrophyte-free profiles (A), oxygen (range 140-141 %) and pH (range 7.6-7.7) were not significantly different on average (transect mean; t-test; $P = 0.6$ and $P = 0.57$) between sampling occasions for *C. demersum*, but water temperature was significantly warmer by 4.0 ^{o}C from November to January (t-test on transect mean, $P < 0.001$; Table 4-1). Similarly, in vertical profiles with *C. demersum* (B-D; see Figure 4-2), oxygen was not significantly different between sampling occasions (t-test, $P = 0.23$), but lower average values of pH (difference 0.4) and higher temperature (difference 3.5 ^{o}C) were found in January (t-test, $P = 0.034$ and $P < 0.001$, respectively; Table 4-1). Comparison of macrophyte-free (A) and dense-bed (D) profiles indicated oxygen was significantly higher (by 15-30 %) where *C. demersum* was absent (transect mean of vertical profiles A and D; Wilcoxon signed-rank test, $P < 0.001$; Table 4-1). Significantly higher pH (difference range 0.2-0.4 units) and temperature (difference range 0.2-0.7 ^{o}C) values were also found in macrophyte-free profiles at *C. demersum* sites, with a more pronounced difference observed in January (transect mean of vertical profiles A and D; Wilcoxon signed-rank test, $P = 0.023$ and $P < 0.001$, respectively; Table 4-1).

Oxygen was the most variable physicochemical parameter at *C. demersum* sites, with higher coefficients of variation in macrophyte (range 32-39 % CV) than macrophyte-free (range 6-10 % CV) profiles, while temperature and pH were $\leq$ 5 % CV (Table 4-1). Vertical profiles of oxygen (transect mean)

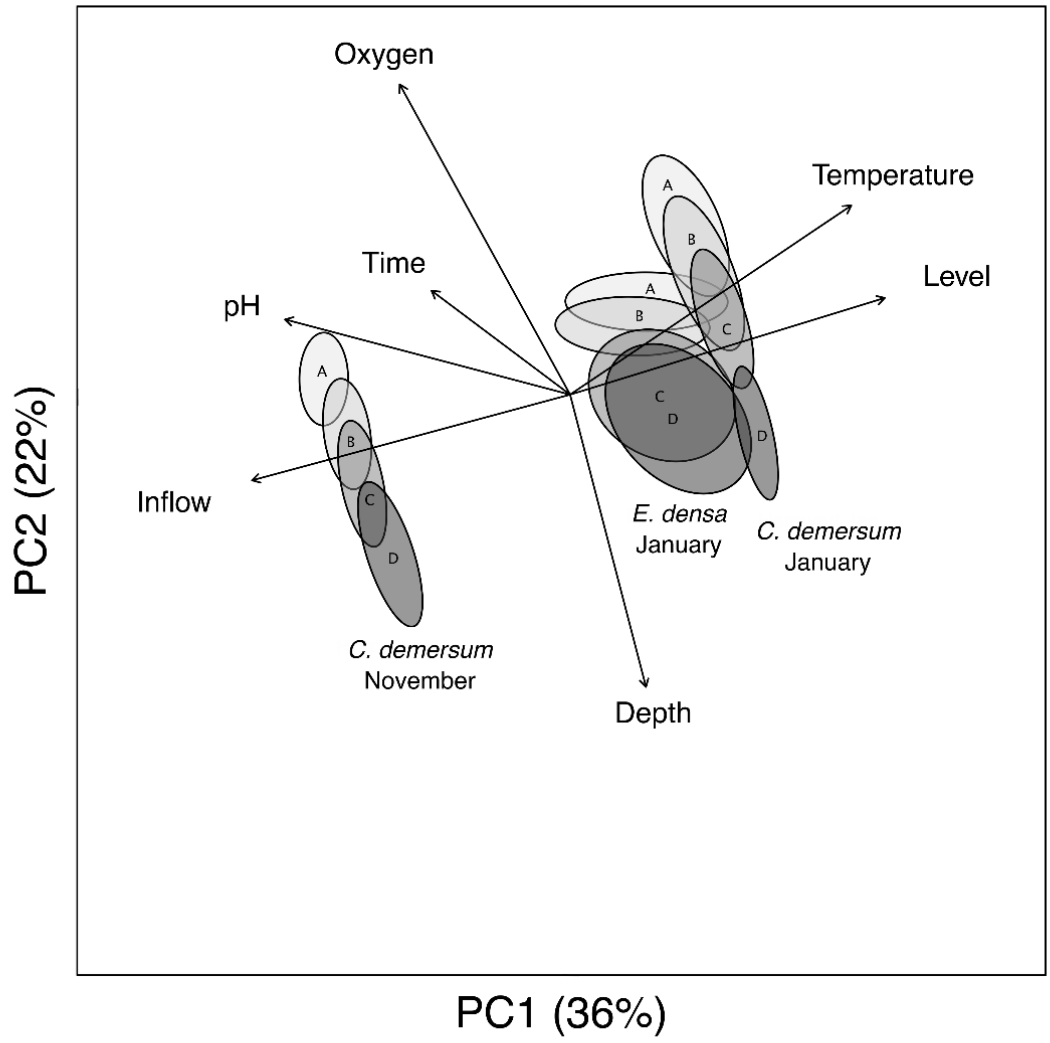

Figure 4-3: Principal component analysis of environmental parameters. Vertical profiles are labelled on ellipses indicating standard error with 95 % confidence intervals. Overlaid environmental vectors were statistically significant at $P < 0.001$.

showed depletion at 20 % of the water depth from the lake bottom, with stronger depletion at higher *C. demersum* proportion (Figure 4-4). Temperature and pH showed no clear patterns with depth across profiles (see Figure 7-6 and 7-7 in Appendix 7.3.4). Comparison of macrophyte-free and dense-bed profiles within 20 % of the lake bottom found oxygen was significantly lower in November but not in January (transect mean of vertical profiles A and D; t-test, $P = 0.01$ and $P = 0.37$).

No significant differences in oxygen, pH or temperature were found within the *E. densa* sites between the macrophyte-free and dense-bed vertical profiles (transect mean of vertical profiles A and D; Wilcoxon signed-rank test, $P = 0.38$, $P = 0.17$, $P = 0.83$; Table 4-1). Oxygen was the most variable physicochemical parameter in *E. densa* sites (temperature and pH $\leq$ 6 %

CV; Table 4-1). The CV values for dissolved oxygen were more variable than *C. demersum* sites in macrophyte-free profiles (6 % and 12 % CV, respectively), but similar in profiles containing macrophytes (32 – 39 % CV; Table 4-1). On the *E. densa* sampling occasion, oxygen within 20 % of the lake bottom was significantly lower on average (by 100 % oxygen) in macrophyte profiles than macrophyte-free profiles (t-test on transect mean, $P = 0.01$).

Comparison of upper-riverine and lower-lacustrine sections in January sampling occasions for macrophyte-free profiles found oxygen and pH were not significantly different on average (i.e., t-test of transect means, $P = 0.13$ and $P = 0.053$, respectively), although temperature was 1.0 ^{o}C cooler in *E. densa* sites (both macrophyte-free and macrophyte profiles) which received upstream water inflows (t-test, $P < 0.001$; Table 4-1). In vertical profiles with macrophytes (B-D; see Figure 4-2), average oxygen (range 112-113 %), pH (range 6.8-7.2) and temperature (range 22.5-22.8 ^{o}C) were not significantly different between *C. demersum* and *E. densa* in January (t-test, $P = 0.8$, $P = 0.15$, $P = 0.12$, respectively; Table 4-1).

4.4.3 **Boundary effects of macrophytes**

Detrended physicochemical variables, scaled from 0 to 100, showed clear separation between November and January related to temperature, and between the lake bottom and water surface associated with oxygen and pH (Figure 4-5). Opposing oxygen (increase) and pH (decrease) gradients in relation were more pronounced in January, when relatively low oxygen was more frequently measured at the lake bottom (Figure 4-5). Comparing the invasive macrophyte species, detrended physicochemical variables displayed separation in water surface and lake bottom, whereby *E. densa* had relatively higher oxygen and *C. demersum* more frequently displayed low oxygen, respectively (Figure 4-5).

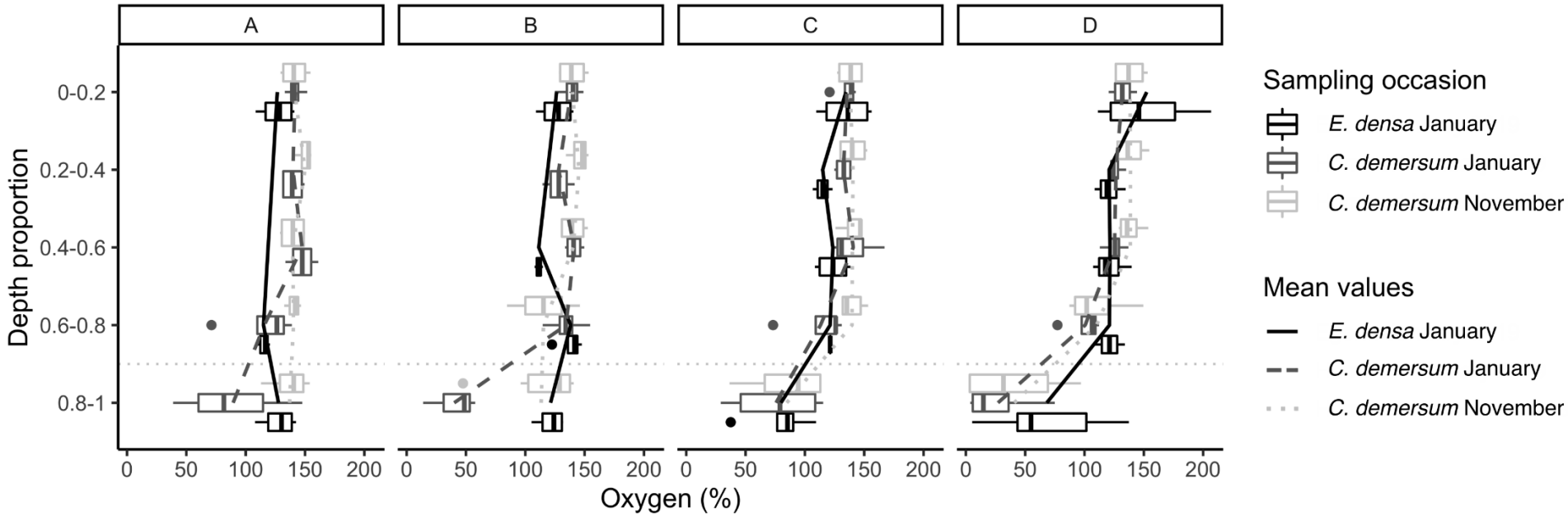

Figure 4-4: Vertical profiles of measured oxygen values across vertical profiles for *C. demersum* in November (light grey long-dash), *C. demersum* in January (dark grey short-dash), and *E. densa* in January (black solid) with coloured solid lines linking mean values. A = macrophyte-free; B = light macrophyte; C = dense-edge and; D = dense-bed (see Figure 4-2). Depth proportion was split into five groups representing 20 % intervals. Boxplots show median [black line inside boxplot]; interquartile range [box]; min/max [whiskers]; and outliers [> 1.5 x interquartile range, black dots]. Dotted grey line indicates boundary where oxygen depletion occurred.

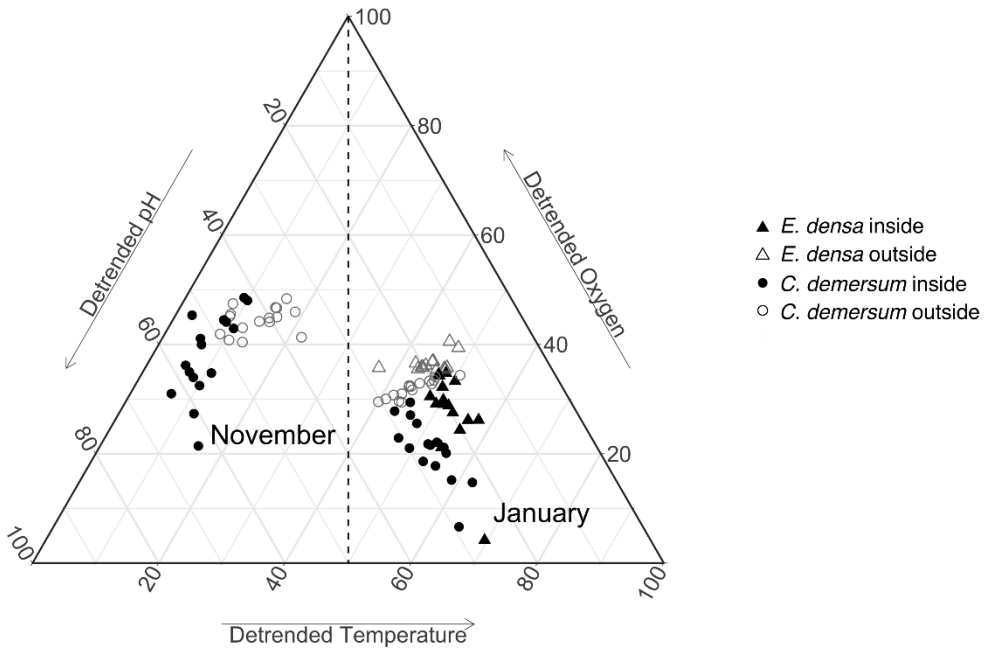

Figure 4-5: Ternary diagram showing relationships between detrended environmental variables in the water column of dissolved oxygen, pH, and temperature scaled from 0-100 (transect mean of vertical profiles). Circular points = *C. demersum*; triangular points = *E. densa*; hollow grey points = measurement collected outside the macrophyte bed; solid black points = measurement collected inside the macrophyte bed (see Figure 4-2). Vertical dotted black line separates the November (left) and January (right) sampling occasions.

For *C. demersum* sampling occasions across a gradient of proportional macrophyte height, detrended oxygen at the water surface significantly increased in January at the 90th percentile while median oxygen declined at the lake bottom on both sampling occasions (quantile regressions; Figure 4-6; 7.3.1 Table 7-6 in Appendix 7.3.5). Lake surface detrended temperature only significantly increased at the 10th percentile in January, when declines in detrended lake bottom pH and temperature were found across nearly all percentiles with increased *C. demersum* coverage. In November, a decline was only found in lake bottom detrended temperature at the 10th percentile in relation to proportion of *C. demersum* in the water column (Figure 4-6).

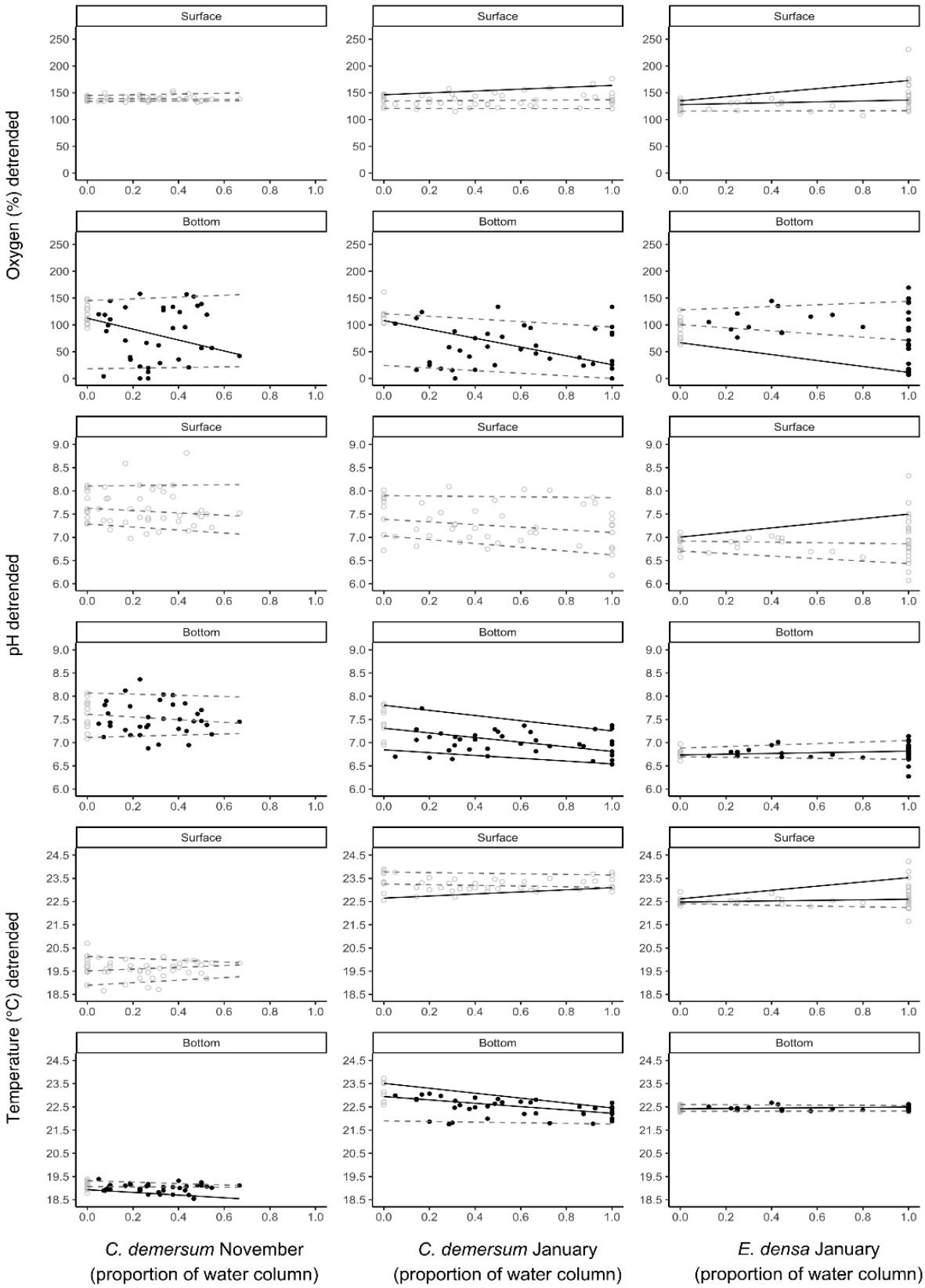

Figure 4-6: Relationship between detrended environmental variables of oxygen (%), pH and temperature (°C) with the proportion of macrophyte in the water column for November 2018 *(C. demersum*) and January 2019 (*C. demersum and E. densa*). Hollow grey points = measurement collected outside the macrophyte bed; solid black points = measurement collected inside the macrophyte bed (see Figure 4-2). Quantile regression model fit displayed for the 10th, 50th (median) and 90th percentiles, with solid lines indicating statistical significance at P = 0.05; dotted lines are not statistically significant (see Table 7-6 in Appendix 7.3.5 for model coefficients).

In January, both *E. densa* and *C. demersum* had at least a single percentile that represented: (i) increased detrended oxygen values at the water surface (median, and 90th percentile and median, respectively), and (ii) decreased values at the lake bottom (median and 10th percentile, respectively) related to the proportion of the water column occupied by macrophytes (Figure 4-5; Table 7-6 in Appendix 7.3.5). Detrended pH only increased at the water surface for *E. densa* (90th percentile), with decreased *C. demersum* and increased *E. densa* found at the lake bottom for the median (Figure 4-6). Detrended surface temperature showed a similar pattern, whereby increased proportion of *E. densa* was associated with warmer temperatures. At the lake bottom, median temperature decreased at *C. demersum* sites and increased for *E. densa* sites (Figure 4-6; Table 7-6 in Appendix 7.3.5).

4.4.4 **Herbicide-induced macrophyte decomposition**

Comparison of specific conductivity, oxygen, and pH two days before, two days after, and ten days after herbicide application indicated significant changes in physicochemical median values and variability through time (Table 4-2). Pre-herbicide median oxygen saturation declined from 19.2 % to <1 % post-herbicide application, whereas median pH and specific conductivity increased from 6.9 to 7.2-7.3 and from 221 to 230-342 µS cm^{-1} at 25 °C, respectively (Table 4-2). Specific conductivity and oxygen became more variable post-herbicide application (CVs from 4.9 % to 15.2 %, and from 100.7 % to 439.5 %, respectively), in contrast to pH which decreased in variability (CV from 3.7 % to 1.2 %; Table 4-2). The modelled oxygen control (i.e., no herbicide impact) exhibited similar diurnal changes and tracked observed oxygen before herbicide application (Figure 4-7). Comparing observed data after herbicide application with the modelled control indicated a significant increase in specific conductivity ten-days post-impact (47 %) and significant decreases in oxygen at the bottom two-days (74 %) and ten-days (91 %) post-impact (Table 4-2; Figure 4-7).

Table 4-2: Summary statistics of selected 2-day periods before, immediately after, and 10-days after herbicide application. Bold statistical tests indicate significance at $P < 0.05$.

		Specific Conductivity (µS/m at 25 °C)				Oxygen (%)				pH			
2-day period		CV	10th	50th	90th	CV	10th	50th	90th	CV	10th	50th	90th
Pre-herbicide 13-15 Feb		4.86	212	221	241.2	100.69	1.2	19.2	74.28	3.71	6.73	6.89	7.44
Post-herbicide 17-19 Feb		7.74	214	230	260	177.74	0.6	0.9	10.74	1.54	7.13	7.32	7.39
Post-herbicide (10-days) 27 Feb – 1 Mar		15.23	273.8	342.0	419	439.47	0	0	0	1.15	7.09	7.22	7.30
Comparison		Pre-Post		Pre-Post10		Pre-Post		Pre-Post10		Pre-Post		Pre-Post10	
Wilcoxon Signed-rank Test	W	**27878**		**35**		**72992**		**80836**		**17625**		**20239**	
	P	**< 0.001**		**< 0.001**		**< 0.001**		**< 0.001**		**< 0.001**		**< 0.001**	
Levene's Test	DF	**576**		**576**		**576**		**576**		**576**		**576**	
	F	**61.42**		**437.89**		**226.74**		**252.04**		**91.56**		**157.52**	
	P	**< 0.001**		**< 0.001**		**< 0.001**		**< 0.001**		**< 0.001**		**< 0.001**	
Causal Impact	O $\bar{x}$	233		**343**		**1 .72**		**0.23**		7.28		7.20	
	P $\bar{x}$	236		**232**		**46.94**		**10.94**		7.42		7.40	
	P	0.469		**0.026**		**0.009**		**0.001**		0.416		0.373	

CV = Coefficient of variation; 10th, 50th, and 90th percentiles; Feb = February and Mar = March; DF = degrees of freedom; W and F are test-statistics; O $\bar{x}$ = observed mean value; P $\bar{x}$ = predicted mean value

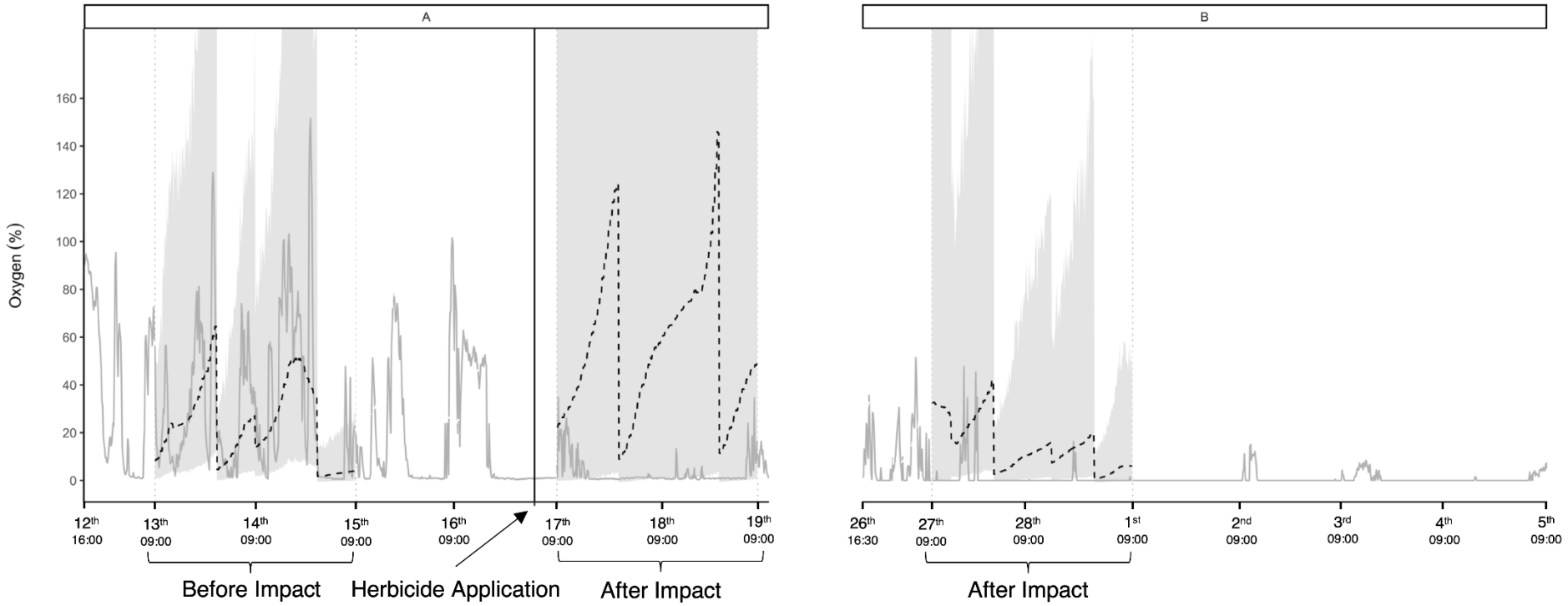

Figure 4-7: Response of observed dissolved oxygen (%) (grey solid line) to *C. demersum* decomposition induced by a single herbicide application (arrow and black vertical line). Oxygen was measured for two days before, and two and ten days after, herbicide application (see Table 4-2). A = first sonde deployment and B = second sonde deployment. Graph includes modelled Causal-Impact control values (black dashed line) and 95% confidence interval (grey smooth).

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4.5 Discussion

We were able to disentangle the complex effects of macrophytes and reservoir management on physicochemical parameters by progressive detrending to isolate the effects of covarying factors, namely variations in sampling time spanning 7.5-hours; sampling depth due to a macrophyte-free varial zone induced by hydropeaking; and daily water flow and level changes caused by hydropeaking operations. Additionally, we used causal-impact analysis to interpret the diurnal effects of an unanticipated macrophyte-spraying event on physicochemical parameters at the sediment-water interface. This combination of approaches enabled us to disentangle the relationships between physicochemical parameters and the proportional macrophyte height in the water column for two species of invasive macrophyte from management factors. Quantile regression analysis of upper and lower limits highlighted the adverse conditions that benthic biota may encounter within dense invasive macrophyte beds, which are of particular importance to species such as unionid mussels that contribute to ecosystem function and services (Vaughn, 2018; Moore, Collier& Duggan, 2019) that are abundant in Karāpiro (see Chapter 5).

Ecologically detrimental physicochemical conditions in the water column produced inside invasive macrophyte beds during their peak biomass accumulation period within this hydropeaking reservoir were primarily low dissolved oxygen events, including anoxia, but were not evident for water pH or water temperature. Hypoxic events were more pronounced at the end of summer, and notably for *C. demersum* within the bottom 20% of the water column in the lower-lacustrine section of the reservoir where proportional macrophyte height was greatest, supporting Hypothesis 1. After accounting for hydropeaking management covariates (i.e., short-term changes in water flow or level), *C. demersum* produced hypoxic conditions across a wider range of macrophyte cover than *E. densa*, likely resulting from contrasting site hydrology in the lower-lacustrine and upper-riverine sections, respectively (Hypothesis 2). The unexpected application of the herbicide diquat led to prolonged and sustained hypoxic/anoxic conditions near the bottom of the water column, highlighting the interaction of hydropeaking and macrophyte management on reservoir benthic physicochemical conditions.

These results underscore the adverse effects of invasive macrophytes on physicochemical attributes that support aquatic biota, and highlight the context-dependent nature of these effects moderated by reservoir management for hydropeaking and macrophyte control.

4.5.1 Spatial scales of invasive macrophyte effects

As well as being evident at a large spatial scale between upper and lower sections of the reservoir (discussed below), the context-dependent impacts of dense *C. demersum* and *E. densa* beds on physicochemical parameters were detectable at smaller scales, both inside and outside of macrophyte beds and within the water column. Our finding that adverse physicochemical conditions were restricted to the inside of dense invasive macrophyte beds parallels studies in a shallow lake (Vilas et al. 2017) and large river (Caraco et al. 2002), which have suggested high macrophyte cover reduces horizontal water exchange from the edge to center of the bed. Similarly, dense growths of five emergent macrophyte species in a shallow North American lake increased the probability of occurrence of hypoxia events with increased macrophyte cover (25 % and 65 % probability of < 2 mg/l dissolved oxygen at 50-64 % and 80-95 % cover, respectively), although areas with lower percentage cover were not examined (Bunch, Allen and Gwinn 2010).

Our measurement of low oxygen conditions near the bottom-water interface at low proportional macrophyte height (i.e., from 10 % of the water column) contrasts with findings of Vilas et al. (2017), who found oxygen effects at 50 % *P. crispus* cover in a shallow Australian lake following temperature stratification (not observed in the unstratified hydropeaking reservoir, but see also Andersen et al. 2017; Ribaudo et al. 2018; Torma and Wu, 2019). The main mechanisms involved in these small-scale differences likely involve reduced wind-induced hydrological exchange (i.e., water flow) as macrophyte cover and bed size increased, leading to the higher influence of solar radiation on photosynthesis rates (Torma and Wu, 2019), although we did not detect an increase in temperature associated with this inferred reduced mixing.

Benthic hypoxia and anoxia have important ecological consequences associated with the release of phosphorus, dissolved inorganic carbon and nitrogen, and toxic ions such as ammonia, sulfide, and ferrous iron from bottom sediments (Andersen et al. 2017; James, Dechamps, Turyk, & McGinley, 2007; Ribaudo et al. 2018). These impacts can be particularly pronounced during macrophyte decomposition (Godshalk & Wetzel, 1978), and were detected in this study as increased and highly variable specific conductivity measurements post-herbicide application. Furthermore, the toxic metalloids/metals arsenic and mercury, which can be high in systems with geothermal inputs such as the upper Waikato River, may be released and accumulate in freshwater fish (mercury only; Robinson, Brooks, Outred, & Kirkman, 1994) and unionid mussels (both arsenic and mercury; Hickey, Roper, & Buckland, 1995) at concentrations unsafe for human consumption. Finally, the larvae (glochidia) of unionid mussels present in Karāpiro (*Echyridella menziesii*) are highly sensitive to relatively low concentrations of copper and ammonia (Clearwater et al., 2014); therefore, benthic release of toxic compounds could be a mechanism to explain the adult-skewed size structures of mussel populations present in this system (Roper & Hickey, 1994; Chapter 5).

4.5.2 **Context-specific effects of management**

The relationship between dense invasive macrophyte beds and physicochemical conditions in shallow lakes was expected to differ in hydrolakes where differences in hydrology between sites could exacerbate or mitigate their effects. In our study, contrasting hydrological characteristics between upper and lower reservoir sections led to extensive shoreline varial zones in which macrophytes could not establish in the lower section, and were associated with the dominance of different macrophyte species contributing to context-specific effects on physicochemical conditions. Lacustrine sections in the lower reservoir have lower hydrological exchange and more adverse physicochemical parameters inside dense invasive macrophyte beds during periods of water retention compared to the upper-riverine section, associated with a higher water-level and higher flows. These findings suggest that physicochemical conditions inside dense invasive macrophyte beds in more riverine reservoir sections could be

deliberately influenced by flow management, with higher water inflows leading to increased hydrological exchange and improved physicochemical conditions inside beds.

Although physicochemical parameter measurements were taken during the daytime, continuous measurements at one site indicated a wide range of physicochemical conditions were encountered during the sampling period in the lacustrine section. Furthermore, these measurements showed that diurnal processes were disrupted by herbicide spraying due to invasive macrophyte decomposition causing prolonged benthic anoxia. Although rapid decomposition effects on oxygen conditions are considered for herbicide application in terms of frequency and area of application (Hussner et al., 2017), post-herbicide monitoring across a vertical water profile would be useful to detect the onset of hypoxic events and initiate management intervention (Parsons, Hamel, & Wierenga, 2007; Waltham & Fixler, 2017). At these times, higher water inflows from hydropeaking management may reduce the frequency of prolonged hypoxic/anoxic events near the lake bottom.

4.5.3 **Conclusions**

We have shown that dense invasive macrophyte beds produce detrimental physicochemical conditions in a hydropeaking reservoir during summer, and that site hydrology (water level and inflows) can be important covarying factors influencing the prevalence of low oxygen events. Spatial variations in the hydroreservoir due to operational effects on hydrology, and vertically and laterally within and around macrophyte beds, lead to context–specific effects on physicochemical conditions. Implementation of adjusted ecological operating guidelines has the potential to reduce the impacts of high invasive macrophyte biomass in hydropeaking reservoirs at key times. These steps may help reduce the prolonged adverse impacts of low dissolved oxygen over summer, especially for biota that reside close to, or in, the lake bed (Andersen et al. 2017). Future research is required to investigate interactions between impacts of adverse benthic physicochemical conditions on freshwater species and alternative hydropeaking management regimes.

4.6 References

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Chapter 5

Hydrology-mediated impacts of invasive macrophytes on freshwater mussels (*Echyridella menziesii*: Unionida) in a New Zealand hydropeaking reservoir

5.1 Abstract

Globally-threatened freshwater mussels belonging to the order Unionida (Bivalvia) may be adversely affected by dense beds of non-native macrophytes which modify habitat at the sediment-water interface. Such effects can be particularly pronounced in modified lentic ecosystems such as reservoirs that are subject to variable hydrology (e.g., due to hydropeaking) which exacerbate the mechanistic pathways of macrophyte-mediated impacts, including anoxic or hypoxic conditions, the related release of toxic ions (e.g., ammonia), and silt accumulation that inhibits filter-feeding. Accordingly, we investigated how population size-structure and biomass of the New Zealand mussel *Echyridella menziesii* varied inside and outside of dense beds of invasive macrophytes at two northern New Zealand hydroreservoir locations with contrasting hydrologies (lower-lacustrine location dominated by *Ceratophyllum demersum* and upper-riverine location dominated by *Egeria densa*). We found adverse sediment-water interface conditions (high sediment organic matter content and silt) were not associated with dense macrophyte beds in littoral zones, but these conditions were associated with reduced mussel density and adult skewed size-structure, inferring reduced recruitment. Structural equation modeling indicated pore-water ammonia was not related to freshwater mussel density. Prevailing hydrology appeared to moderate these relationships, such that impacts from sediment organic matter, silt, and previously recorded hypoxia and anoxia events were exacerbated in the lower-lacustrine section where variable flows promoting water mixing were not present to reduce their effects. High densities of mussels less than 40 mm in length in the upper-

riverine lake section were not associated with adverse sediment-water interface conditions, suggesting that enhanced water exchange in and around macrophyte beds may increase mussel survival in littoral zones. Our findings support the role of hydropeaking management in mitigating the development of adverse physicochemical conditions within some macrophyte beds, and underscore the context-specific effects that dense non-native macrophyte beds can have on mussel populations.

5.2 Introduction

The most speciose freshwater mussel order (Unionida, Class Bivalvia) has declined in diversity markedly over the last century, as evidenced by the International Union for Conservation of Nature classifying 40 % of mussel species as Near Threatened, Threatened, or Extinct (Lopes-Lima et al. 2018). Associated with this decline has been the loss of ecosystem services and functions that dense aggregations of mussels provide, leading them to be referred to as ‘umbrella’, ‘flagship’ or ‘keystone’ species (Geist 2011). Mussel beds can represent biogeochemical hotspots of nutrient and resource cycling that couple pelagic and benthic ecosystem compartments, potentially increasing food-web productivity and regulating water quality through biofiltration of phytoplankton (Atkinson and Vaughn 2015).

The unique life-cycle of unionid mussels, requiring larvae (glochidia) to undergo metamorphosis on a suitable host-fish, is particularly sensitive to disruption from anthropogenic activities (e.g., that impact physicochemical stream bed characteristics; Geist and Auerswald, 2007), and may lead to recruitment failure, as potentialy indicated by adult-skewed mussel population size-structures (Modesto et al. 2017). As with all sessile benthic organisms, mussels are threatened by processes that promote adverse environmental conditions near the sediment-water interface (Andersen et al. 2017). However, interactions between recognized large-scale impacts (e.g., pollution and natural system modification; Lopes-Lima et al. 2018) and poorly documented local-scale effects of invasive species are not well known (Moore et al. 2019). Understanding such interactions is important for targeting mitigation measures for mussel conservation, in particular when

accounting for context-specific effects on the ecosystem services mussels provide.

Invasive macrophytes can be considered ‘ecosystem engineers’ and ‘foundation species’ (Ramus et al. 2017, Wood and Freeman 2017), as they frequently dominate the photic zones of lentic ecosystems where they out-compete native vegetation (Yarrow et al. 2009, Thomaz et al. 2014). Dense macrophyte beds can induce adverse environmental conditions at the sediment-water interface by altering hydrology causing hypoxia or anoxia and the associated release of toxic ions (e.g., ammonia, sulfide, and ferrous iron; Andersen et al. 2017; Ribaudo et al. 2018), and by leading to the accumulation of fine sediment (Laughton et al. 2008). Benthic oxygen consumption within macrophyte beds may also be increased by the decomposition of accumulated sediment organic matter which can provide an indicator of prolonged anoxic and hypoxic events (Nogueira et al. 2011). In temperate regions, such impacts tend to be most extreme after summer following peak macrophyte biomass accumulation (Madsen et al. 2001, Zohary and Ostrovsky 2011), which reduces exchange of water between the inside and outside of dense macrophyte beds (Vilas et al. 2017, Torma and Wu 2019), and later during macrophyte senescence that results in mass decomposition of organic matter (Godshalk and Wetzel 1978).

Despite clear mechanistic pathways, field studies of invasive macrophyte interactions with mussel density, abundance, biomass, or mortality have provided inconsistent results on the direction and magnitude of such relationships depending on the species involved (for a review see Moore et al. 2019). For example, a study by Burlakova & Karatayev (2007) in Texas, USA, found density of adult unionids (both *Pyganodon grandis* and *Utterbackia imbecillis*) in two lake impoundments was negatively correlated with percentage cover of *Myriophyllum spicatum* (50 % cover) and *Nelumbo lutea* (60 % cover), but not in a third lake with 10 % cover of mainly non-native *Chara* spp. In contrast, New Zealand studies have pointed to positive relationships between density of *Echyridella menziesii* (Unionida: Hyriidae) and macrophyte biomass in some lake (Weatherhead and James 2001) and river (Nobes 1980) ecosystems, but negative relationships in other lakes (James 1985, Sorrell et al. 2007).

Human-modified environments like hydrogeneration reservoirs substantially alter hydrological regimes, with daily water level fluctuations from hydropeaking related to variable inflows and outflows leading to contrasting flow conditions within the same water body. These conditions can promote establishment and determine the distribution of invasive macrophytes (Johnson et al. 2008, Havel et al. 2015), particularly in lake littoral zones (Zhao et al. 2012, Shivers et al. 2018). Reservoir management can exacerbate or mitigate the adverse environmental conditions produced by invasive macrophytes near the lake-bed. For example, Moore et al. (2020) reported higher reservoir residence time led to reduced water mixing and promoted prolonged anoxic and hypoxic conditions within macrophyte beds in a northern New Zealand hydropeaking reservoir. Accordingly, overarching hydrology (i.e., riverine or lacustrine systems) may partly account for the context-specific nature of mussel responses to invasive macrophyte impacts at small spatial scales.

As dam construction for hydropower generation is increasing worldwide (Zarfl et al. 2014), there is a pressing need to quantify effects of the ensuing managed hydrology and environmental conditions associated with the spread of invasive macrophyte species (Johnson et al. 2008) on key biota occupying highly-affected littoral zones, such as unionid mussels (Khan et al. 2020). To address this need, a field study was conducted to compare mussel density and size-structure inside and outside dense invasive macrophyte beds across two contrasting locations (lower-lacustrine and upper-riverine) in a northern New Zealand reservoir, where the hydrology is strongly influenced by hydropeaking operations. The following hypotheses were tested: 1) macrophyte biomass will the density of freshwater mussels decline; 2) conditions within dense macrophyte beds will be associated with a reduction in small mussel density (< 40 mm) that is indicative of reduced recruitment; and 3) the magnitude of these effects will be moderated by reservoir hydropeaking activities that characterize the different hydrological regimes in the upper-riverine section (variable discharges) and lower-lacustrine section (variable water level) of the reservoir.

5.3 Materials and methods

5.3.1 Study site

The study was carried out in a dammed hydroelectric reservoir (Karāpiro: built in 1947) located on the Waikato River system, North Island, New Zealand (37° 55′ 42.82″ S, 175° 32′ 40.3″ E). This waterbody, the most downstream in a series of eight reservoirs, has a surface area of 5.4 $km^{2,}$ and mean and maximum depths of 11 m and 30.5 m, respectively (Lowe and Green 1987). The reservoir is considered eutrophic (Livingston 1986) with a residence time dependent on inflow: for example, minimum, mean and maximum annual water inflows of 208, 262 and 320 m^3 s^{-1} equate to residence times of 3.3, 2.6 and 2.2 days, respectively, given the assumptions of full water column mixing and a lake water volume of 60 x 10^6 m^3; Gibbs et al. 2015, Moore et al. 2020). Karāpiro has an upper-riverine section, where discharge from the Arapuni hydropower station produces highly variable flows (as above), and a lower-lacustrine section that has a diurnally variable water level related to hydropeaking (see Moore et al. 2020).

The two most abundant macrophyte species in Karāpiro are the invasive *Ceratophyllum demersum* and *Egeria densa* (McCarter et al. 1993, Schwarz et al. 1999, Clayton et al. 2006). *Ceratophyllum demersum* dominates the lower-lacustrine section where it forms extensive monospecific beds that occupy the majority of shallow littoral areas to c. 5 m depth (Hofstra and de Winton 2016). The upper-riverine section is dominated by large, dense and monospecific beds of *E. densa*, which are rooted to the bottom and can withstand faster flows (Clayton et al. 2009). The rapid growth of both *C. demersum* and *E. densa* in spring (e.g., 2–10 % day^{-1} and 2–8 % day^{-1} of dry biomass, respectively; Eller et al., 2015) culminates in peak biomass at the end of autumn when beds regularly reach the water surface (Hofstra and de Winton 2016). During the present study, three *C. demersum* sites were unexpectedly sprayed with herbicide immediately prior to sampling causing almost complete decomposition of macrophytes (1-KL, 2-MM and 3-BL in Table 7-7 in Appendix 7.4.3; see also Figure 5-1), similar to what might be expected following senescence. Accordingly, during sampling,

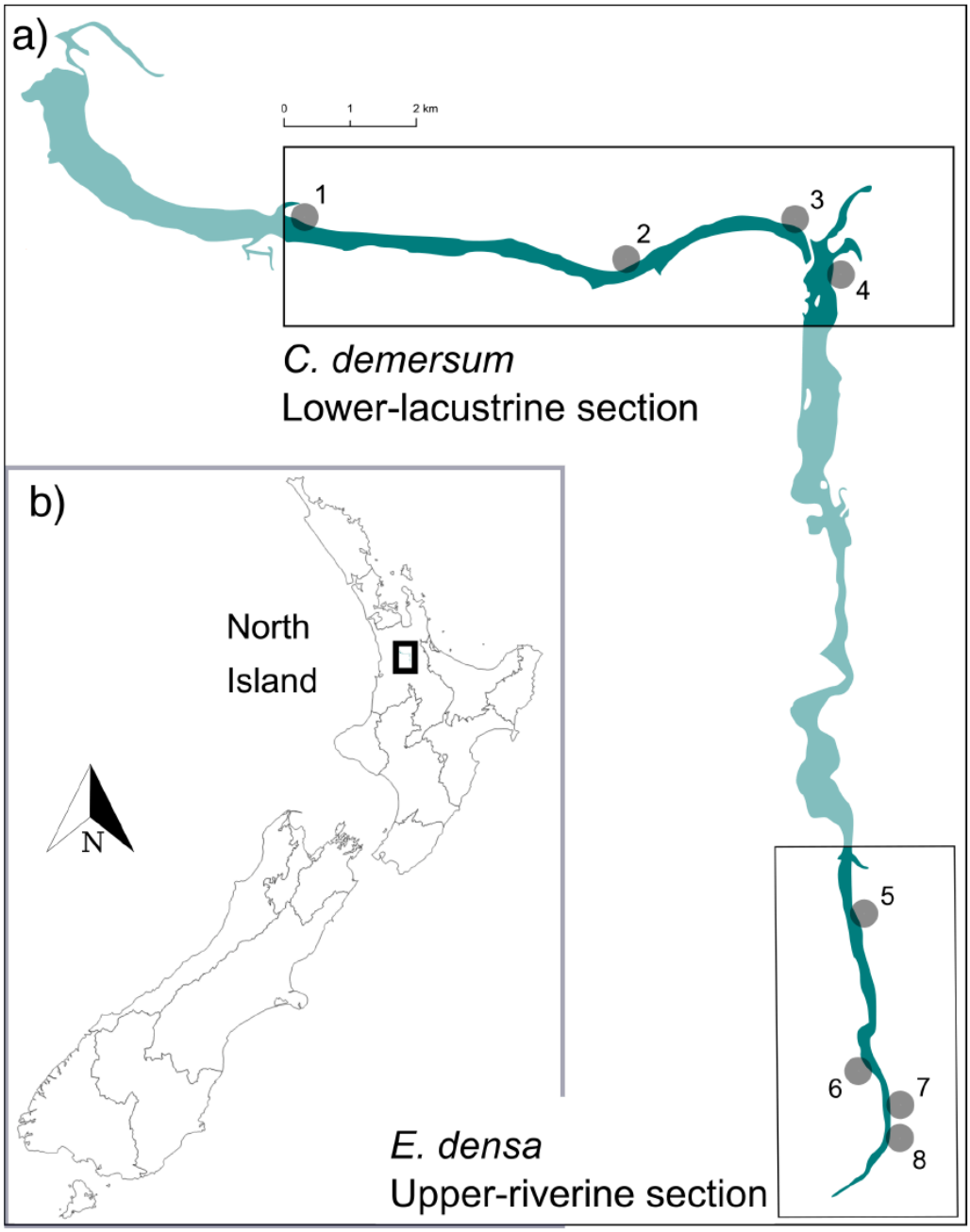

Figure 5-1: Study site locations (1-8) in *Ceratophylum demersum* and *Egeria densa* beds for the lower-lacustrine and upper-riverine sections of Karāpiro (a), North Island, New Zealand (b).

some macrophyte beds in the lower-lacustrine section were in various stages of decomposition.

5.3.2 **Mussel and macrophyte collection and processing**

A field survey of *Echyridella menziesii* density was conducted between March and April (austral summer) 2019 over 22 days at four sites in the *C.*

demersum-dominated, lower-lacustrine section and at four sites in the *E. densa*-dominated, upper-riverine section (Figure 5-1a). Within each site, divers collected measurements from 5-6 paired quadrats (1 m^2) placed on the lake bottom inside (at least 2 m from the edge) and outside (c. 1-2 m from the edge) dense macrophyte beds ($\bar{x} \pm$ SD; 4.1 $\pm$ 2.7 m distance between paired samples). Macrophyte beds comprised predominantly monospecific stands that reached the water surface and extended at least 10 m from the permanently inundated habitat near the shore into the lake and 50 m perpendicular to the shore.

Paired quadrat placements were selected to achieve similar measurement depths, and in the lower-lacustrine section at depths not exposed during low water levels. After quadrat placement, time and GPS locations (easting, northing to 3-5 m) were recorded, as well as quadrat minimum and maximum water depths. The water depth for each quadrat was calculated as the mean of minimum and maximum depths, whereas slope of the bed was calculated in degrees as $\theta = \sin^{-1}(\text{Depth}_{(max)} - \text{Depth}_{(min)})$. In total, 84 quadrats (40 lower-lacustrine and 44 upper-riverine) were sampled for (i) freshwater mussel density, biomass, and population size-structure, (ii) macrophyte fresh-weight, and (iii) water and sediment physiochemical parameters (see Section 5.3.3). Freshwater mussels and sediments were collected with gloved hands from each quadrat (excavated to c. 10 cm depth), placed into catch-bags, sieved (5000 μm mesh), and later measured for individual shell length, maximum width and wing-height (maximum height of the shell) using Vernier calipers (Jobmate model J701-2702; $\pm$ 0.5 mm). Mussel live wet-weight (including the shell) was measured on an Ohaus SP4001 Scout Pro electronic scale ($\pm$ 0.1 g).

Macrophytes were removed from entire quadrats outside of dense beds, whereas inside beds they were sampled using a Wisconsin grab sampler (500 μm mesh; 0.25 m^2). At two sites (2-MM and 3-BL; see Figure 5-1 and Table 7-7 in Appendix 7.4.3) macrophyte samples were unable to be collected since beds were almost completely decomposed. Macrophytes were transported on ice and frozen prior to biomass determination, and wet-weight was later determined based on methods by Bickel and Perrett (2016).

Ceratophyllum demersum and *E. densa* samples were completely thawed in separate clean water baths to remove non-macrophyte material and saturate with water, spun in a manual salad spinner (20 L) at a constant speed for 20 turns with no-more than half of each tier filled (< 100 g), and weighed in aluminum trays (Wedderburn W5202; ± 0.1 g). A correction was applied to account for the effect of freezing/thawing on macrophyte samples based on the wet-weight loss of six *C. demersum* and six *E. densa* samples pre- and post-freezing: *C. demersum* and *E. densa* lost $\bar{x}$ ± SD = 34.9 ± 2.3 % and 16.5 ± 3.2 % of initial fresh-weight, respectively, due to freezing and thawing. Final macrophyte biomass values were adjusted accordingly to provide fresh-weight.

5.3.3 **Water and sediment sample collection and analysis**

A water sample was collected with a Van Dorn sampler (5 L) placed horizontally 10 cm from the lake bottom (measurement range 5-15 cm from bottom) inside and outside dense macrophyte beds. From this sample, a 50 mL aliquot was filtered (Advantec glass fibre filter GC-50 (0.50 µm)) on shore into a 50 mL falcon tube for ammonia measurement in the laboratory (transported chilled in the dark). Temperature (°C), pH, dissolved oxygen saturation (%), and specific conductivity (µS/cm at 25 °C) were recorded in the remaining water sample using calibrated meters (ProSolo, YSI Incorporated, Yellow Springs, Ohio, United States; pHTestr10, Eutech, Singapore). Next, a sediment core was collected at the same location using a 50 mL syringe tube and transferred into a 50 mL falcon tube for transport to the laboratory for pore-water ammonia measurement within 12 hours of field collection.

Ammonia concentrations of water and pore-water samples were determined using the phenate method (Eaton et al. 1995). Sediment sample pore-water was separated using a benchtop centrifuge (Kubota 8420; 1800 rpm for 20 minutes) and all samples were filtered again (GC-50) prior to analysis. Sediment organic matter content was measured using the percentage weight loss on ignition method (Heiri et al. 2001), whereby pre-weighed (Sartorius BP 221S ± 0.1 mg) aluminum foil dishes with sediment samples were dried in a 60 °C oven (Contherm series 5) for at least 3 days, weighed,

and then combusted in a furnace (Nabertherm LT40/11) at 550 °C for 4 hours, followed by cooling in a desiccator and reweighing to determine ash-free dry mass.

Sediment particle size analysis followed methods by Konert & Vandenberghe (1997), whereby sample organic matter was removed using 10 % hydrogen peroxide before laser grain size analysis on a Malvern Mastersizer 3000, which quantified the percent abundance of particles between 0.01-2000 µm in diameter. Sieving separated the < 2000 µm (sand and silt) from the > 2000 µm (gravel) sediment fraction prior to Mastersizer measurement. Both sediment fractions were weighed (Denver Instrument Company TR-403 ± 0.001 g) to provide percentage weight classes according to the Wentworth (1922) scale.

5.3.4 **Data preparation and statistical analysis**

All data analyses were conducted in the R statistical software program v3.6.3 (R Core Team 2019) and presented in “ggplot2” v3.2.1 (Wickham 2016). Summary statistics of the median, mean and standard deviation were calculated for variables measured inside and outside dense macrophyte beds within each site. Detrending was performed to account for daily variability in oxygen, pH, temperature, water depth, upstream water inflow and downstream water level related to measurement time throughout the day (for detrending details see Moore et al. 2020).

Mussel population, site, physicochemical, and sediment characteristics (Table 5-1) were compared inside and outside dense macrophyte beds for the lower-lacustrine and upper-riverine sections using Generalized linear models fitted to a negative binomial distribution (i.e., for overdispersed count data; Ver Hoef & Boveng, 2007), or factorial ANOVA with transformed data as required to meet assumptions of linearity and homogeneity of variances (i.e., logit transformation for proportion data and inverse hyperbolic sine (IHS) transformation for data with extreme values that included zero; Burbidge et al. 1988).

Relationships between mussel shell length, height, width and wet weight were investigated to select mussel variables for comparison. Model

selection was used to guide if linear or polynomial models best described the relationships using the information-theoretic model-selection method (Burnham & Anderson 2002) and Akaike Information Criterion with small sample size correction (AIC_c). Since length was highly related to height, width, and weight (linear, $P < 0.001$, $R^2 = 0.79$, second-order polynomial, $P < 0.001$, $R^2 = 0.85$, and fourth-order polynomial, $P < 0.001$, $R^2 = 0.98$, respectively; Figure 7-8 in Appendix 7.4.1), only mussel lengths were analyzed subsequently to determine differences related to population size structure. Mussel length data were binned into 5 mm groups and displayed as percentage histograms, with recent recruitment at each site inferred from the density of mussels less than 40 mm in length (equivalent to 26 mm height or up to 1-2 years of age based on Herath 2018).

To explore relationships between measured and detrended environmental parameters, Principal Component Analysis (PCA) was conducted in the 'Vegan' community ecology package v2.5-4 (Oksanen 2015). Prior to the PCA, imputation of missing data (e.g., primarily macrophyte fresh-weight at two sites and pore-water ammonia measurements; Table 5-1) was performed with the iterative PCA method using 'imputePCA' in the missMDA package (Dray & Josse 2015). All data were then centered and scaled (subtracted from sample means and divided by their standard deviate, respectively) to standardize measurements to the same scale (Sergeant et al. 2016). To assess statistical significance and coefficients of determination for each environmental parameter, permutation tests (999) were performed using the "envfit" function in "Vegan" (Oksanen, 2015). Freshwater mussel density was displayed on the PCA solution as contours derived from the function 'ordisurf' in which a Generalized Additive Model (GAM with negative binomial error distribution; Ver Hoef & Boveng, 2007) fits a smoothed surface using penalized splines (Wood 2003) based on the PC1 and PC2 axes; freshwater mussel biomass contours were fitted using a GAM with Gaussian error distribution.

The form of the relationship between of freshwater mussel density with environmental parameters (i.e., parameter transformations of intercept (mean), linear (none), second-order polynomial, IHS or square-root), and how these relationships changed inside and outside dense macrophyte

beds and between the upper-riverine and lower-lacustrine sections, were explored using model selection (as above) based on AICc. Mussel density was fitted to a GLM with negative binomial distribution and a specified environmental parameter transformation (as above) with “Site” specified as a random effect. Log-likelihood ratio tests were then performed to examine if interactions should be retained in the best model; three-way interactions were retained and model assumptions of linearity and homogeneity of variances were evaluated (Supplementary material 2).

To examine direct and indirect effects of environmental parameters on freshwater mussel density (total mussels and those < 40 mm in length), piecewise structural equation modelling (SEM) was performed to construct and evaluate a network of relationships in the package ‘piecewiseSEM’ (Lefcheck 2016). Piecewise SEM evaluates if a causal network is likely to be missing relationships by comparing the hypothesized network to a network with all possible relationships using a goodness of fit test called “directed separation". This produces a Fisher’s C test statistic (Shipley 2000, 2009) and *P* value, which if greater than 0.05 indicates the hypothesized network is a good fit to the data and would likely not be improved from inclusion of unspecified relationships. AIC can be extracted from direction separation tests to compare multiple hypothesized causal networks (Shipley 2013). To test if structural equation models could be estimated based on available data, the ‘t rule’ was followed (Grace 2006).

Prior to SEM, environmental parameters were centered and scaled to allow model convergence and produce relative effect sizes with standardized estimates (Dalal and Zickar 2012). A random effect of ‘site’, allowing only the intercept to vary, and negative binomial distribution were fitted. The returned R^2 values can consider variance explained only by fixed effects (marginal) or fixed and random effects (conditional) (Lefcheck, 2016). Multicollinearity between environmental parameters was examined using variance inflation factors (‘vif’ function in the ‘car’ package; Fox et al. 2018). Where multicollinearity was detected (i.e., between silt and sediment organic matter in upper-riverine SEM), variable reduction (PCA) was conducted and the PC1 axis extracted to represent these variables. The interaction between depth and bed slope angle was specified as ‘correlated

error', which excludes it from the directed separation test. Standardized estimate values (β) from SEM were not constrained to fall between +1 and -1.

Macrophyte fresh-weight data were only available for two sites in the lower-lacustrine section due to herbicide application (1-KL and 4-HH; n = 17; Table 7-7 in Appendix 7.4.3) so we excluded this variable from models containing all sites. Instead, a factor "macrophyte (inside/outside)" was included in the SEM. In addition, depth and bed slope angle were excluded due to the influence of Site 4-HH (high densities of mussels) found in the GLM analysis (Figure 5-1; Table 7-7 in Appendix 7.4.3). Exclusion of these variables allowed the detection of broader environmental parameter relationships with freshwater mussels across all sites; these variables were not excluded for a separate SEM for the upper-riverine section where macrophyte spraying did not occur (see Appendix 7.4.5 for details).

Table 5-1: Summary statistics (mean, median (M) and standard deviation (SD)) of environmental parameters (site, physicochemical, sediment) and mussel population characteristics. Comparisons of the upper-riverine and lower-lacustrine sections of Karāpiro and between inside and outside macrophyte beds are shown with level of significance indicated following best-fit model tests indicated. Lake section coefficients are in relation to the upper-riverine section and macrophyte coefficients are in relation to outside macrophyte beds. Comparisons significant at $P < 0.05$ are shown in bold.

Lake section & dominant macrophyte	Lower-lacustrine - *C. demersum**				Upper-riverine – *E. densa*				Comparison			
	Inside		Outside		Inside		Outside			Lake section (Lower vs Upper)	Macrophyte (Inside vs Outside)	
	M	$\bar{x}$ ± SD	M	$\bar{x}$ ± SD	M	$\bar{x}$ ± SD	M	$\bar{x}$ ± SD	n	Estimate ± standard error (*p-value*)		Model: transformation
Site characteristics												
Macrophyte fresh-weight (g m^{-2})	188	725 ± 1062	12	39 ± 66	1031	1942 ± 2122	123.6	205 ± 276	62	**1.6 ± 0.5 (0.003)**	**-3.2 ± 0.5 (<0.001)**	Factorial ANOVA: IHS
Depth (m)	1.2	1.8 ± 1.1	1.2	1.5 ± 0.5	1.5	1.5 ± 0.3	0.9	1.0 ± 0.3	84	**-1.3 ± 0.08 (0.001)**	**-1.3 ± 0.08 (< 0.001)**	Factorial ANOVA
Bed slope angle (°)	0	4.8 ± 9.2	0	3.7 ± 7.1	5.7	8.5 ± 10.0	5.7	8.1 ± 9.2	84	**4.1 ± 1.9 (0.038)**	-0.7 ± 1.9 (0.69)	Factorial ANOVA
Physicochemical characteristics												
Oxygen saturation (%)	98.4	98.5 ± 4.6	96.4	98.3 ± 6.0	98.7	99.3 ± 2.8	99.6	99.5 ± 2.4	84	0.9 ± 0.7 (0.19)	0.03 ± 0.7 (0.97)	Factorial ANOVA
pH	8.3	8.1 ± 0.3	8.2	8.1 ± 0.3	8.6	8.5 ± 0.3	8.5	8.5 ± 0.3	84	**0.3 ± 0.04 (< 0.001)**	-0.03 ± 0.04 (0.48)	Factorial ANOVA
Temperature (°C)	21.7	21.7 ± 1.0	21.5	21.4 ± 1.0	21.2	21.1 ± 1.1	21.3	21.1 ± 1.0	84	**-0.5 ± 0.1 (< 0.001)**	-0.2 ± 0.1 (0.2)	Factorial ANOVA
Water ammonia (mg L^{-1})	0.1	0.8 ± 1.6	0.1	0.3 ± 0.4	0.1	0.2 ± 0.2	0.1	0.3 ± 0.8	79	-	-	Assumptions not met
Sediment characteristics												
Silt (%)	28.2	31.1 ± 19.9	23.1	30.8 ± 25.4	31.9	36.7 ± 22.1	24.3	31.8 ± 23.6	84	0.2 ± 0.2 (0.46)	-0.2 ± 0.2 (0.47)	Factorial ANOVA: logit
Sediment organic matter (%)	3.4	3.9 ± 2.8	2.8	3.9 ± 2.7	4.2	4.7 ± 2.8	3.1	4.4 ± 2.7	84	0.2 ± 0.1 (0.12)	-0.05 ± 0.1 (0.75)	Factorial ANOVA: logit
Pore-water ammonia (mg L^{-1})	2.7	2.9 ± 2.2	1.1	1.7 ± 1.6	1.0	1.2 ± 0.9	0.8	0.9 ± 0.8	61	**-1.2 ± 0.4 (0.002)**	-0.7 ± 0.4 (0.08)	Factorial ANOVA
Mussel population characteristics												
Total density (# m^{-2})	17.5	27.2 ± 24.6	20.5	44.0 ± 65.1	43.0	48 ± 36.4	17.5	25.6 ± 24.2	84	0.01 ± 0.2 (0.93)	-0.05 ± 0.2 (0.85)	glm negative binomial
Density < 40 mm (# m^{-2})	0	0.5 ± 1.1	0	0.3 ± 0.8	3.5	7.1 ± 9.6	1.0	4.4 ± 8.9	84	**2.6 ± 0.4 (<0.001)**	-0.4 ± 0.4 (0.28)	glm negative binomial
Biomass (g m^{-2})	536	803 ± 741	654	1364 ± 2003	789	901 ± 754	362	467 ± 409	84	-0.5 ± 0.4 (0.19)	-0.7 ± 0.4 (0.07)	Factorial ANOVA: IHS

$\bar{x}$ is the mean; M is the median; SD is standard deviation; n = number; *W* = Wilcoxon test-statistic; *P* = *P*-value; **C. demersum* sites 1-3 were sprayed with herbicide before sampling; model intercept is lower-lacustrine section in macrophyte.

5.4 Results

5.4.1 Site, physicochemical and sediment characteristics

A comparison of environmental parameters found lower average macrophyte fresh-weight (686-1737 g m^{-2}; $P < 0.001$), depth (0.3-0.5 m; $P < 0.001$) and pore-water ammonia (0.3-1.6 mg L^{-1}; non-significant at $P = 0.08$) outside than inside dense macrophyte beds (Table 5-1). Major differences were found between lake sections, where the upper-riverine section had higher average macrophyte fresh-weight (1383 g m^{-2}; $P <0.01$), bed slope angle (8.3 °; $P < 0.05$) and pH (0.4; $P < 0.001$) and lower average depth (0.8 m; $P = 0.001$) temperature (0.5 °C; $P < 0.001$) and pore-water ammonia (1.3 mg L^{-1}; $P <0.01$) (Table 5-1).

The PCA explained 27 % and 15 % of the variation in environmental parameters across the first and second principal components, respectively, which were associated with distinctly different environmental gradients (all vectors shown in Figure 5-2 have $P < 0.001$). PC1 was positively associated with silt, sediment organic matter, and pore-water ammonia, and negatively with slope and depth, whereas PC2 was positively associated with macrophyte fresh-weight, oxygen, and temperature, and negatively with water ammonia. Macrophyte and macrophyte-free quadrats within the lower-lacustrine section with *C. demersum* and the upper-riverine section with *E. densa* had similar environmental characteristics, although they were distinctly different between sections, separating in relation to PC2 axis (Figure 5-2).

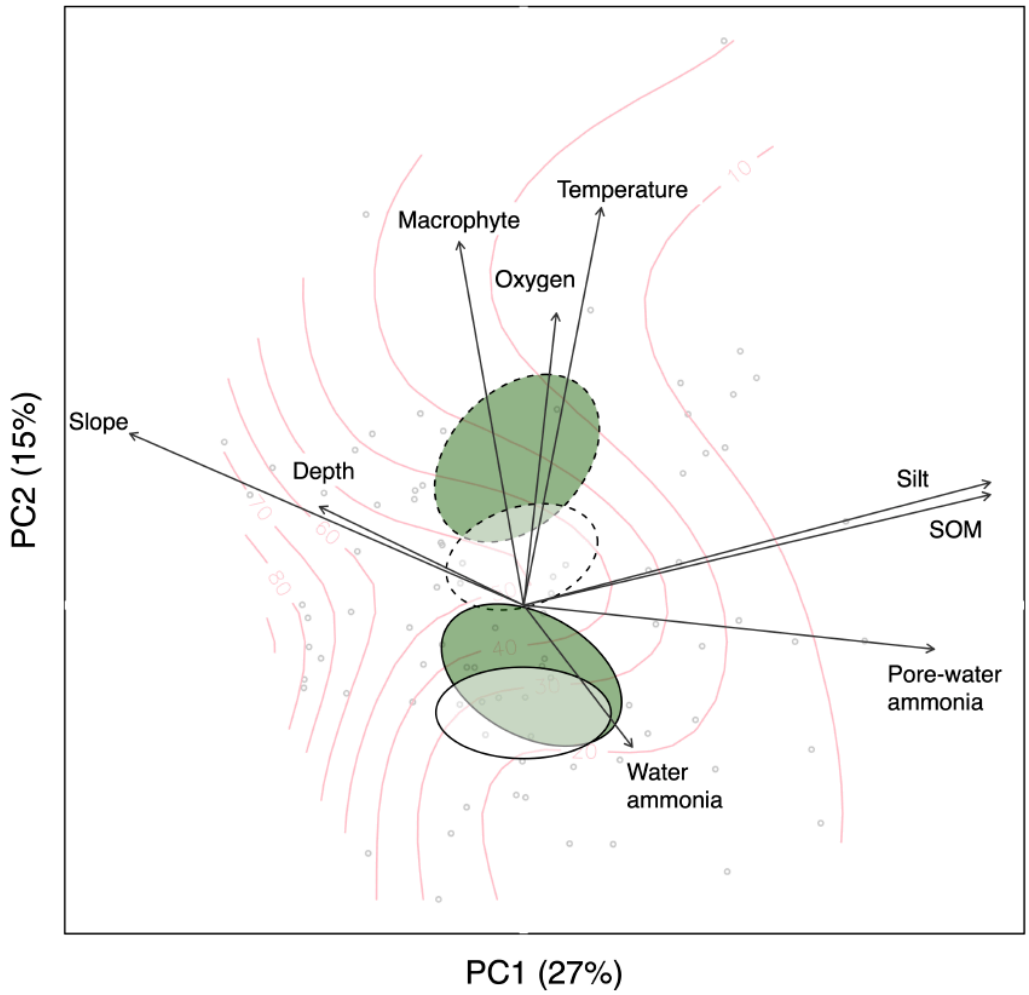

Figure 5-2: Principal component plot of axes 1 and 2 in relation to measured environmental variables with vectors significant at $P < 0.001$. Ellipses envelope sampling quadrats for *Ceratophylum demersum* lower-lacustrine (solid outline) and *Egeria densa* upper-riverine (dashed outline) sections inside (dark green) and outside (clear) dense macrophyte beds. Contours show 10 m^{-2} increments for mussel density fitted with a generalized additive model (Deviance explained = 36 %). SOM = sediment organic matter. Open circles indicate values for individual quadrats.

5.4.2 **Freshwater mussel population structure**

Neither total mussel density nor biomass were statistically different inside than outside dense macrophyte beds, although mean biomass was lower outside macrophyte beds (434-561 g m^{-2}; $P = 0.07$). Similarly, there were no differences in mussel density or biomass between the lower-lacustrine and upper-riverine sections (Table 5-1). Density of mussels < 40 mm in length was significantly higher on average in the upper-riverine section compared to the lower-lacustrine section (by 10.7 m^2; Table 5-1).

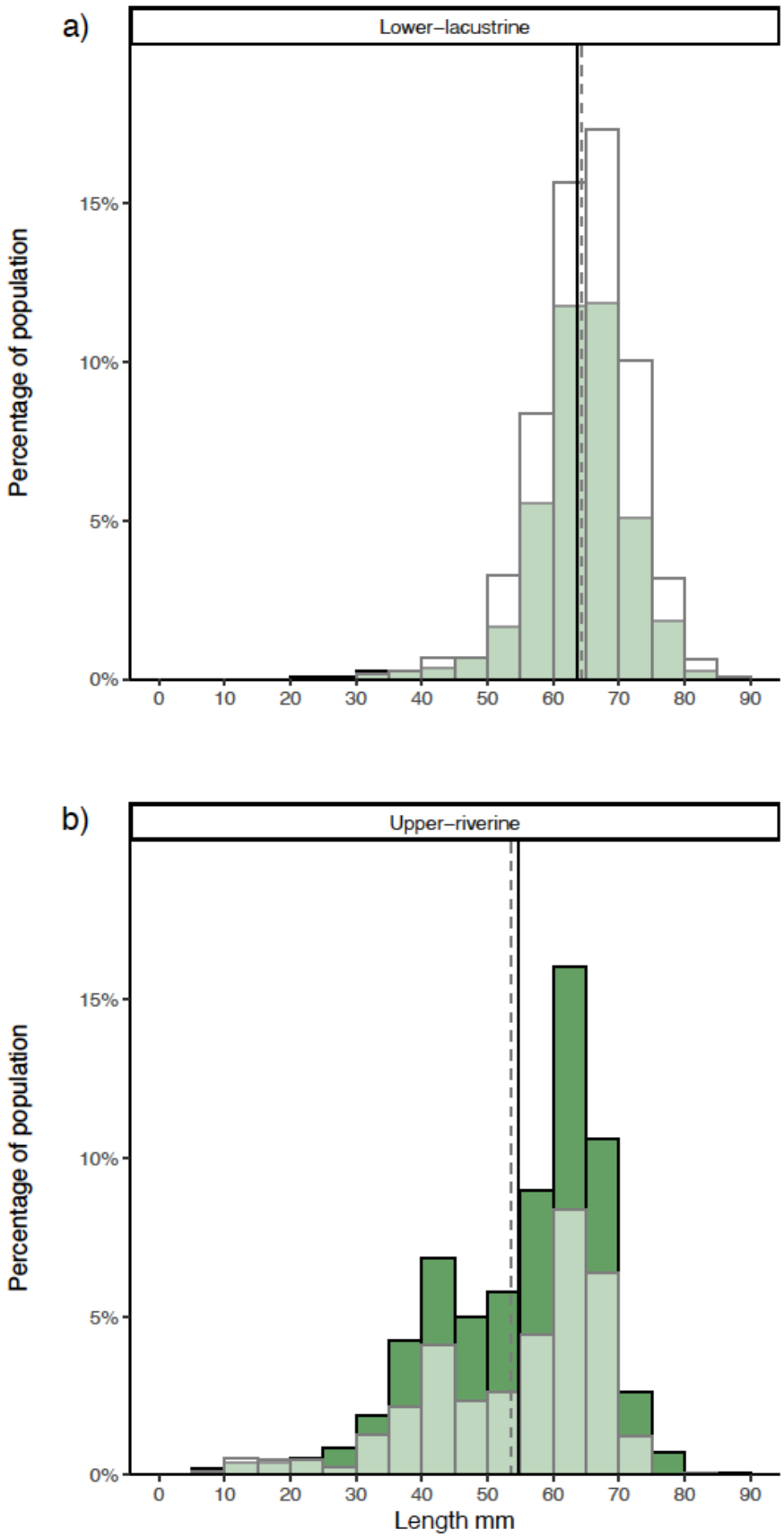

Figure 5-3: Mussel length distributions in 5 mm bins inside (dark green) and outside (white) dense macrophyte beds of (a) *Ceratophylum demersum* (lower-lacustrine) and (b) *Egeria densa* (upper-riverine). Mean lengths are shown for mussels collected inside (solid black line) and outside (dotted light-grey line) dense macrophyte beds. Transparent white bars overlaid on dark green bars are shown as light green.

Population size-structure in the lower-lacustrine section was adult-skewed based on the low percentage of mussels found under 40 mm in length (< 3 %) and mean length inside and outside macrophyte beds of 63–67 mm, respectively (Figure 5-3). In contrast, the size-structure of freshwater mussels in the upper-riverine section supporting *E. densa* beds suggested recruitment had occurred in recent years, with mussels less than 40 mm in length accounting for between 7–26 % of those collected, contributing to an overall lower mean length of 49–59 mm, respectively (Figure 5-3).

5.4.3 Relationships between mussels and environmental parameters

Freshwater mussel density displayed a non-linear relationship across environmental parameters and the two PCA axes examined (GAM; $P < 0.001$, $R^2 = 0.22$, deviance explained 36 %). Higher mussel density was associated with higher slope and depth (within the measured ranges of 0.5 – 4.5 m depth and 0 – 37 ° slope), and lower mussel density was associated with higher silt, sediment organic matter, and pore-water and water ammonia (Figure 5-2). Macrophyte fresh-weight, dissolved oxygen and water temperature were associated with higher mussel density in the middle-range of their gradients (Figure 5-2). Mussel biomass (g m^{-2}) displayed a similar pattern which appeared more linear amongst variables, but explained less deviance than mussel density across the two PCA axes (GAM; $P < 0.001$, $R^2 = 0.24$, deviance explained 29 %; Figure 7.4.2 in Appendix 7.4.2).

The relationships between freshwater mussel density and environmental parameters compared inside and outside dense macrophyte beds and between the upper-riverine and lower-lacustrine sections were best described by models with different environmental parameter transformations (Figure 5-4; see Appendix 7.4.4 for full details on model selection and coefficient tables). Freshwater mussel density was best described by a unimodal relationship with a non-significant three-way interaction with depth (standardised coefficient -0.76; $P = 0.057$), reflecting the less pronounced response of density with depth in the upper-riverine section (-0.45; $P = 0.057$) that was marginally higher inside than outside *E. densa* macrophyte beds (1.27; $P = 0.01$). The unimodal relationship did not significantly differ between lake sections (0.22; $P = 0.19$) and mussel density

was higher inside than outside *C. demersum* beds (-0.92; $P = 0.03$) driven by the 4-HH site (Figure 5-4a). Similarly, freshwater mussel density was best explained by a model with three-way interactions (-0.78; $P < 0.001$) for slope angle (IHS transformation); freshwater mussel density did not significantly vary with slope angle between lake sections (0.26; $P = 0.31$) or inside and outside *E. densa* macrophyte beds (0.23; $P = 0.64$), but it was significantly higher outside *C. demersum* beds in the lower-lacustrine section with increased slope angle (0.68; $P < 0.001$), also driven by site 4-HH.

As macrophyte fresh-weight data were influenced by or not available due to herbicide application in the lower-lacustrine section, the relationship between freshwater mussel density was only examined in the upper-riverine section, where a square-root transformation without interactions best explained the positive relationship (0.61; $P = 0.031$; Figure 5-4c). Accordingly, freshwater mussel density was negatively related with silt (linear; no interactions; -0.21; $P < 0.001$) that was marginally significantly lower outside than inside macrophyte beds across both lake sections (-0.38; $P = 0.06$; Figure 5-4d). Sediment organic matter best explained freshwater mussel density by a model with interactions (IHS transformation), where differences were not found between lake sections (0.44; $P = 0.67$) but were significant outside than inside *C. demersum* beds in the lower-lacustrine section (-1.23; $P = 0.001$; Figure 5-4e). Pore-water ammonia was best explained freshwater mussel density by a model (IHS) with three way interactions (1.72; $P = 0.021$); this relationship significantly differed between lake sections and inside and outside macrophyte beds (-2.36; $P = 0.01$). A pronounced negative relationship was indicated outside but not inside *C. demersum* beds in the lower-lacustrine section in contrast to the upper-riverine section that predicted a more pronounced relationship inside than outside *E. densa* beds (Figure 5-4f).

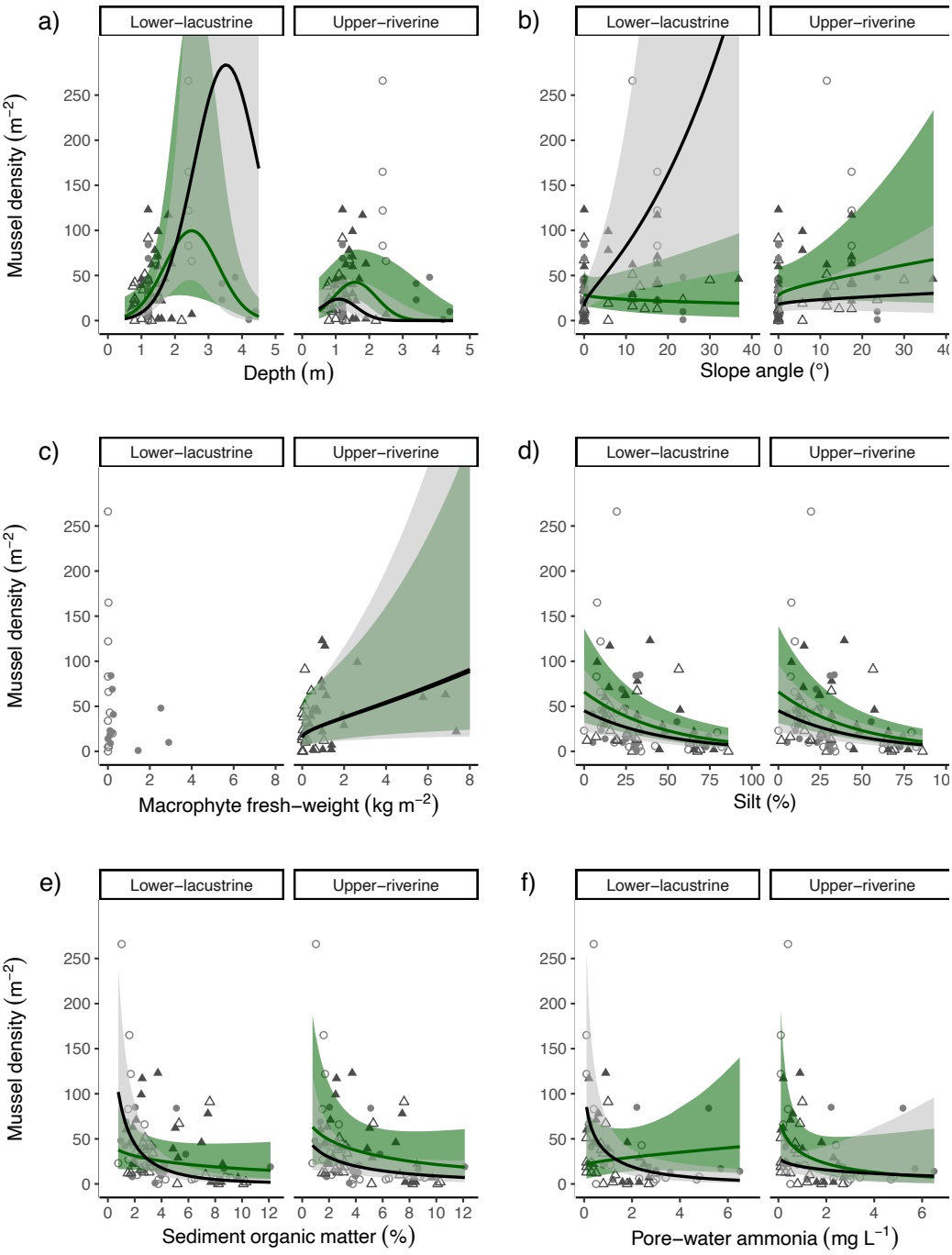

Figure 5-4: Relationships of mussel density with (a) depth, (b) bed slope angle, (c) macrophyte fresh-weight, (d) silt, (e), sediment organic matter, and (f) pore-water ammonia for lower-lacustrine (circles) and upper-riverine (triangles) sections inside (solid) and outside (hollow) dense macrophytes beds. Solid lines represent statistically significant fit of the best GLM model inside (dark green) and outside (black) macrophyte beds, and dotted lines indicate non-significant GLM model fits ($P < 0.1$). Grey smooth shows 95% confidence interval.

5.4.4 **Direct and indirect effects**

To determine direct and indirect effects of environmental parameters on freshwater mussel density (and density of mussels < 40 mm in length) between lake sections with contrasting hydrology and macrophyte species, SEM was performed across all sites (with slope, depth, and macrophyte fresh-weight excluded; see Methods), as well as for the upper-riverine section that included all variables from GLM's (Figure 5-5a-c). Variance explained by environmental parameters was influenced by between-site variability for both total mussel density ($R^2_{marginal}$ = 0.20, $R^2_{conditional}$ = 0.41) and density of mussels < 40 mm ($R^2_{marginal}$ = 0.37, $R^2_{conditional}$ = 0.62) across all sites, as well as in the upper-riverine SEM ($R^2_{marginal}$ = 0.23, $R^2_{conditional}$ = 0.64).

Across all sites, freshwater mussels had a marginaly significant higher density inside macrophyte beds (β = 0.37, P = 0.07) that was unrelated (independence claim; P = 0.18; Appendix 7.4.5) to the marginally significant negative direct effect of silt (β = -0.27, P = 0.1) and indirectly via silt on sediment organic matter (β = 0.73, P < 0.001) on mussel density (β = -0.41, P = 0.01; Figure 5-5a). Density of mussels < 40 mm was significantly higher inside macrophyte beds (β = 0.64, P < 0.001) and in the upper-riverine section (β = 2.78, P < 0.001; Figure 5-5b).

In the upper-riverine SEM, slope angle and depth were negatively (β = -0.48, P < 0.01) and positively (β = 0.31, P = 0.04) related to the silt and sediment organic matter PC1 axis (explaining 93 %), which was marginally negatively related to freshwater mussel density (β = -0.35, P = 0.07). In contrast, depth had a positive indirect effect via macrophyte fresh-weight (β = 0.40, P = 0.01) on mussel density (β = 0.47, P < 0.01; Figure 5-5c). In all SEM models, silt and sediment organic matter were positively related to pore-water ammonia, which in no cases was related to mussel density (Figure 5-5).

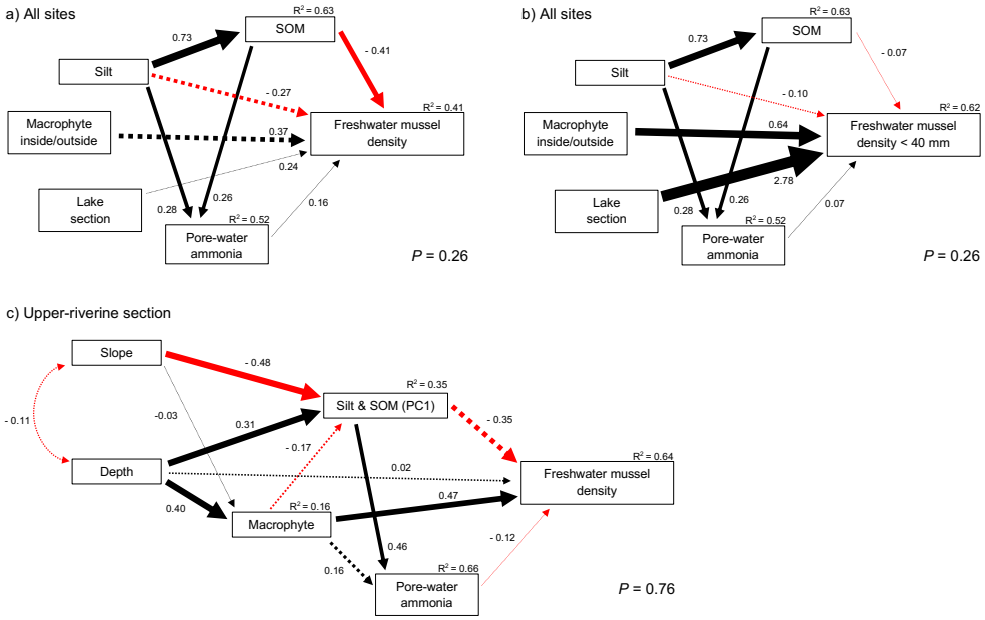

Figure 5-5: Structural equation model depicting the direct and indirect effects of environmental parameters on a) mussel density and b) mussel density less than 40 mm across all sites, and c) mussel density in the upper-riverine lake section. Black arrows indicate positive linear relationships and red arrows negative linear relationships with standardized coefficients displayed. Solid and dashed arrows indicate statistically significant ($P < 0.05$) and non-significant relationships, respectively. R2 indicates the conditional goodness-of-fit accounting for site variability. The "Macrophyte inside/outside" factor is in relation to outside the bed (i.e., mussel density is higher inside than outside dense macrophyte beds) and "Lake section" is in relation to the lower-lacustrine section (i.e., higher mussel densities less than 40 mm in the upper-riverine section) (see Appendix 7.4.5 for statistical details).

5.5 Discussion

We show that relationships of macrophyte biomass with mussel density and population size-structure in this hydropeaking reservoir differed between sites with contrasting invasive macrophyte species and hydrology (Figure 5-5). Across all sites, higher freshwater mussel density (and density of mussels < 40 mm) was found inside macrophyte beds, largely due to smaller mussels occurring in the upper-riverine section where *E. densa* dominated. The relationships and patterns detected need to be interpreted in the specific context of this study, which (i) reflected daytime conditions prevailing up to 2 m inside macrophyte beds acknowledging that conditions

further inside extensive beds may be more severe, particularly at night when respiration can lead to hypoxia or anoxia at the sediment-water interface (Moore et al. 2020), and (ii) was confined to littoral zones (i.e., < 4.5 m water depth and not deeper parts of the reservoir outside the area of dense macrophyte colonization) subject to daily variations in depth and extent due to hydropeaking. Serendipitously, macrophyte spraying unexpectedly affected three sites in the lower-lacustrine section, creating conditions potentially similar to macrophyte collapse following senescence and providing an indication of the acute concentrations of dissolved oxygen to which mussels could potentially be exposed following autumn die-off.

Somewhat unexpectedly, structural equation modelling indicated that macrophyte biomass was not related to silt, sediment organic matter, or pore-water ammonia, even though silt and sediment organic matter were implicated as the primary drivers of reduced total mussel density (but not those < 40 mm) across all sites. We were unable to determine whether this difference was driven by *C. demersum* biomass in the lower-lacustrine section (see below) or a related mechanism (e.g., prolonged anoxic and hypoxic events). However, based on findings elsewhere, we expect that where dense invasive macrophyte beds occur in sites with low hydrological exchange, such as the lower lacustrine section of Karāpiro, adverse environmental conditions occur near the sediment-water interface (see Burlakova & Karatayev, 2007; Moore et al. 2020). Notwithstanding the finding that such adverse conditions were not always associated with high macrophyte biomass, where they coincided mussel density was reduced (hypothesis 1) and population size structure was adult-skewed (hypothesis 2). Furthermore, prevailing hydrology moderated these relationships such that in the lower-lacustrine section impacts from silt and sediment organic matter were more pronounced with reduced water mixing, whereas in the upper-riverine section rooted macrophytes able to withstand highly variable flows likely experienced water exchange within their beds to create conditions apparently suitable of juvenile mussel survival (hypothesis 3).

5.5.1 **Hydrology-mediated effects on mussels**

Adult-skewed mussel population structure inside and outside dense *C. demersum* beds of the lower-lacustrine section, indicative of low recruitment,

could reflect prevailing physicochemical conditions creating adverse conditions for fish hosts and/or juvenile mussel survival. The non-native fish species that are abundant in Karāpiro littoral zones are known to be glochidial sinks (i.e., glochidia are able to attach but not develop in high numbers; Tremblay et al. 2016, Moore & Clearwater 2019), although a suitable native host (*Gobiomorphus cotidianus*) is also abundant in the lower-lacustrine section. Thus, absence of a suitable host fish can be discounted as a reason for apparently reduced recruitment in the lower lacustrine section. Furthermore, evidence from a hydrogeneration lake in the South Island of New Zealand (Lake Dunstan) found *G. cotidianus* actively inhabit dense invasive macrophyte beds of *Lagarosiphon major* (Bickel & Closs, 2008), suggesting this species of host may not be limited by dense macrophyte beds in Karāpiro.

Rather, reduced survival of transformed juvenile mussels appears to be a more likely explanation for low recruitment in the lower-lacustrine section. This is consistent with the cause of recruitment failure for populations of the European freshwater pearl mussel (*Margaritifera margaritifera*), which had limited juvenile mussel survival attributed to high levels of fine sediments, low redox potential related to low oxygen levels (at the sediment-water interface, 5 and 10 cm into the bed), and high bed compaction (not encountered in this study) (Geist & Auerswald 2007). Elsewhere, fine sediments accumulating within the roots of a recently introduced *Ranunculus* species in the River Spey (northern Scotland) have been associated with numerous dead juvenile *M. margaritifera* found during physical removal of macrophytes (Laughton et al. 2008).

In the present study, physicochemical measurements in the lower-lacustrine section were influenced by the combined effects of water level variation and herbicide application at some sites. Decomposition of macrophytes post-herbicide application resulted in prolonged anoxia and hypoxia of water near the bed (Moore et al. 2020), which can lead to the release of toxic ions such as ammonia, sulfide, and ferrous iron, further exacerbating adverse conditions found at the sediment-water interface (Andersen et al. 2017; Ribaudo et al. 2018). This is particularly relevant for water-pore ammonia release resulting from macrophyte decomposition (Godshalk & Wetzel,

1978), since unionid mussels, and in particular juveniles, are among the freshwater species most sensitive to ammonia exposure (Clearwater et al. 2013; USEPA 2013). As we did not measure pore-water pH or temperature in-situ, we were unable to assess if our measured pore-water ammonia concentrations (NH_3^- mg L^{-1}) in the lower-lacustrine exceeded the United States Environmental Protection Agency chronic criterion continuous concentration of 1.0 mg TAN L^{-1} (pH 7.8, 20 °C). However, ammonia concentrations were notably higher at sprayed sites on average (2.3-6.7 mg L^{-1}; Table 7-5 in Appendix 7.3.1). Data from most unsprayed sites indicate mussels were likely present prior to spraying, and the absence of empty shells indicates on-site mortality was not widespread, so movement away from sprayed sites seems the most likely mechanism explaining the lower numbers of larger mussels among decomposing macrophytes.

Although herbicide treatment of *C. demersum* beds in the lower-lacustrine section limited inferences that could be made about the relationship between the macrophyte biomass and the mussel population, hydrology-mediated (i.e., water level) anoxia and hypoxia events were recorded within the water column close to the bed inside dense *C. demersum* beds three months prior to the present study (see Moore et al. 2020), producing conditions likely to be lethal to juvenile mussels (Dimock & Wright 1993, Sparks & Strayer 1998). Low oxygen conditions can be inferred by high sediment organic matter content, which generates high oxygen demand for decomposition, paralleling the findings of Santos et al. (2020) and supporting the likely role of hypoxic and anoxic events in limiting mussel recruitment.

In contrast, mussel populations within dense *E. densa* beds in the upper-riverine section were clearly recruiting and had higher density of mussels less than 40 mm at sites with greater macrophyte biomass, most likely due to the variable flow hydrology enabling greater water exchange and the rooted macrophytes stabilizing mobile pumice sediments. The structural equation model suggested that *E. densa* establishes denser macrophyte beds at greater depths, within the range sampled, where shear-stress disturbance during hydropeaking is likely insufficient to dislodge mussels but sufficient to limit fine sediment accumulation and promote sufficient

water-mixing to prevent adverse physicochemical conditions from developing within macrophyte beds. This ‘shear stress water-exchange’ hypothesis postulates that a ‘goldilocks’ zone of moderate shear stress enables some macrophytes to serve as flow-refugia protecting juvenile mussels from hydropeaking effects while allowing water exchange within beds to reduce physicochemical stress (Figure 5-6). At shallower depths (< 1 m) in the upper riverine section, macrophytes and mussels occurred at lower abundances, potentially due to hydraulic limitations from the variable-flow regime (e.g., highly variable depths, periodically high velocities). These conditions contrast to the lower-lacustrine section where recruitment was not apparent and low water exchange in dense macrophyte beds was considered the key mechanism creating adverse physicochemical conditions.

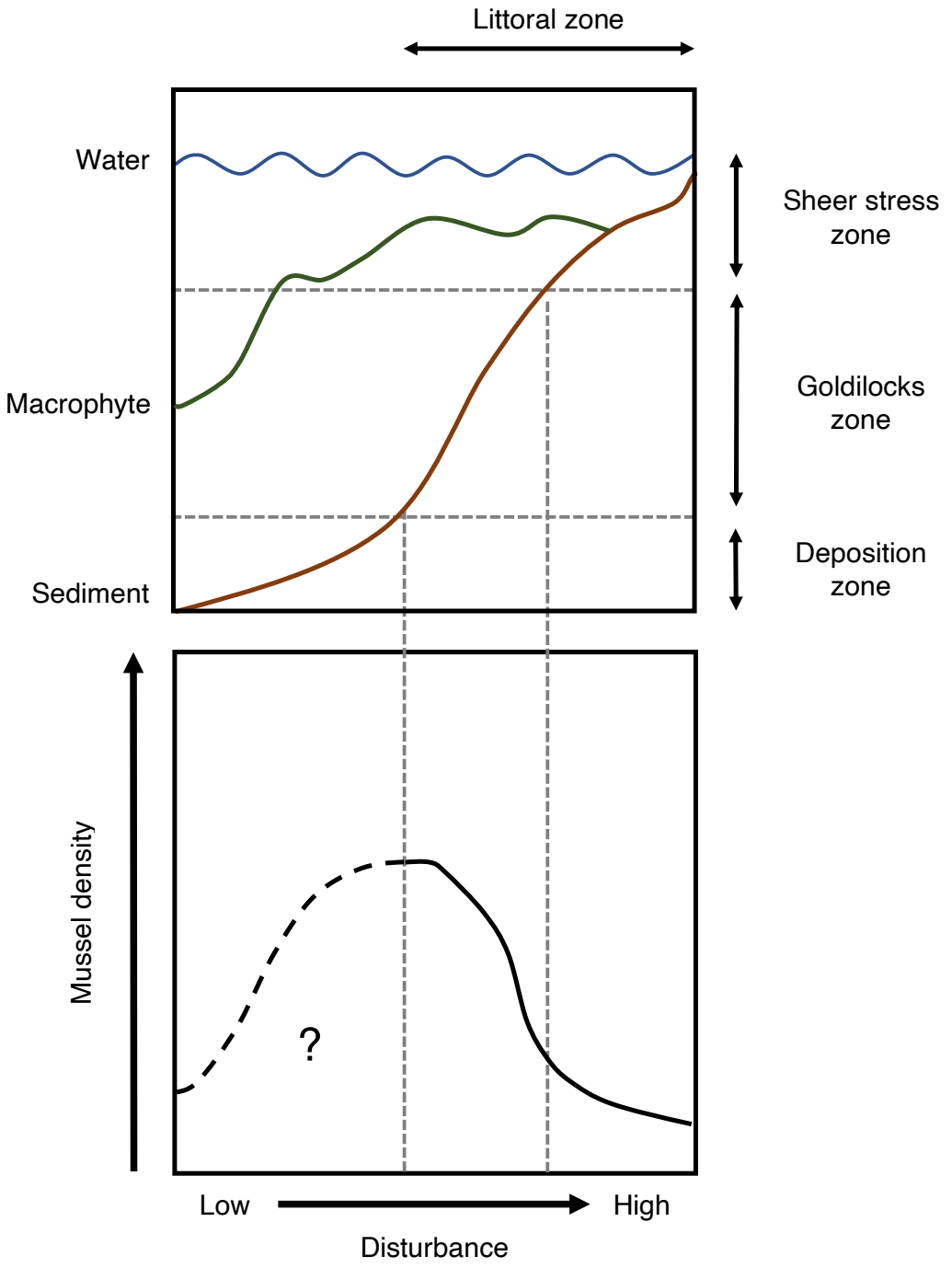

Figure 5-6: Conceptual diagram of the SEM results from the Karāpiro upper-riverine section inside the littoral zone. The dashed black line indicates the relationship between mussel density inside the low-disturbance deposition zone is unknown.

5.6 Conclusions

We show freshwater mussel density and size-structure were related to prevailing environmental conditions, but these patterns were not associated with invasive macrophyte beds in littoral zones of the hydropeaking reservoir, although site-specific hydrology and macrophyte species'

dominance may play a role in the distribution of mussels < 40 mm in length. Sediment organic matter, silt, and previously recorded hypoxia and anoxia were likely the primary factors that decreased mussel density and produced adult-skewed population size structure in lower parts of the reservoir. Since evidence of reproduction was found in littoral zones with suitable prevailing hydrology, improving conditions at the sediment-water interface through enhanced water exchange in and around macrophyte beds may increase mussel survival. Coupling flow management with macrophyte control appears particularly important where herbicide spraying is likely to exacerbate adverse benthic conditions. These findings support the role of appropriate hydropeaking management in mitigating the development of adverse physicochemical conditions that can limit mussel population density and recruitment in and around dense invasive macrophyte beds in large hydroreservoirs.

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Chapter 6

Modelling impacts of invasion intensity on mussels and implications for management

6.1 Introduction

Invasive species interact with freshwater mussels in multiple ways, ranging from disruption of critical life-cycle processes to direct predation (Moore et al. 2019). As illustrated in Figure 2-3, non-native fish are able to disrupt the critical obligate larval life-stage of freshwater mussels by serving as unsuitable hosts, and non-native macrophytes can impact mussels by producing adverse habitat conditions near the sediment-water interface. Therefore, fish and macrophyte invasions may generate sinks of mussel population reproductive output, reducing the ability of mussels to recruit, although it is also possible that suitable non-native fish-hosts and/or macrophytes as flow-refugia could improve mussel recruitment. Reduced recruitment leading to adult-skewed population size structures (e.g., Roper and Hickey 1994) may cause eventual local population extinction. This concept of a reproductive sink was explored by Tremblay et al. (2016), who examined the suitability of the non-native fish *Neogobius melanostomus* as a potential host for glochidia of North American unionid mussel species. Their findings indicated that *N. melanostomus* was likely to inhibit unionid mussel recruitment, and they therefore defined this invasive fish as an 'ecological sink'. To my knowledge, this concept has not been extended to macrophyte invasions, and the combined effects of both non-native fish and non-native macrophytes on mussel recruitment remain unstudied.

The overall aim of my thesis was to contribute knowledge of unionid mussel and non-native species interactions in modified freshwater environments to assist with species management and conservation. This thesis contributes to the field of invasion biology and mussel conservation by clarifying some of the mechanistic pathways of non-native fish and macrophyte impacts on different stages of the freshwater mussel life cycle. I used a combination of controlled laboratory experiments and field surveys to demonstrate: 1) that

certain non-native fish disrupt the obligate ectoparasitic life-stage of *Echyridella menziesii*, and 2) that, under certain conditions, non-native macrophytes produce adverse environmental conditions considered detrimental to *E. menziesii* survival. The Karāpiro hydroreservoir provided a model study system to disentangle aspects of these relationships relevant to other modified waterbodies supporting native mussel populations subject to water regime management and accelerating rates of biological invasion.

I found that three non-native fish species were unsuitable hosts of *E. menziesii*, compared to a common native fish host (Chapter 3; Moore & Clearwater 2019). This experiment provided a range of attachment and metamorphosis rates in laboratory conditions for fish species from Karāpiro. In Chapter 4 (Moore et al. 2020) and Chapter 5 field studies, I demonstrated that non-native macrophytes produced adverse physiochemical conditions at or near the surface-water interface (e.g., silt accumulation, sediment pore-water ammonia (Chapter 5) and anoxia and hypoxia (Chapter 4)) along littoral margins of the lower-lacustrine section of Karāpiro. The lower-lacustrine section did not support mussels less than 40 mm in length, suggesting recruitment failure may have been occurring along littoral margins inside and outside dense macrophyte beds, whereas juvenile mussels were collected in the upper-riverine section of the reservoir where a different species of invasive macrophyte dominated.

Combined, these studies highlight the importance of suitable native fish hosts for mussel recruitment and the requirement of suitable life-supporting conditions at the sediment-water interface for mussels following excystment from host fish. Additionally, these studies demonstrate that both requirements can be compromised by invasive species in some contexts, at least in lacustrine sections of hydroreservoirs such as Karāpiro. Here, juvenile mussels were absent in or around macrophyte beds, likely due to the prevailing sediment conditions across littoral zones in and around macrophyte beds. Such conditions were exacerbated by hydropeaking operations in the lower-lacustrine section that caused daily water-level variations of up to 1.2 m. Exposure of littoral sediments results in macrophyte bed collapse when exposed and compression when the water-level drops, effects that decrease the area of habitat suitable for mussels.

However, since size-frequency of mussels was not determined in open areas without sediments, or in other parts of the reservoir deeper than 3.8 m, recruitment in the lower-lacustrine cannot be discounted. Nevertheless, anecdotal observations during dive surveys and other sampling suggest limited recruitment in the lower lacustrine section more broadly (S. Clearwater, Department of Conservation, pers. comm.).

To synthesise the key findings of this thesis, I conducted a hypothetical modelling exercise to predict how *E. menziesii* recruitment (juvenile excystment success and survival) could be affected by different levels of fish and macrophyte species' invasions. The key focus of this model was to determine how the variability in mussel recruitment changed over an invasion gradient, and was addressed in two parts. The first part consists of an invasion model to determine the rate of juvenile excystment from host-fish across a gradient of non-native fish dominance, using the brown bullhead catfish (*Ameiurus nebulosus*) as the focal non-native species (see below). In the second part, based on the survival probability of juvenile mussels dispersed into habitats in and around dense beds of the non-native macrophytes, I examine the combined effects of non-native fish and macrophyte invasion scenarios on juvenile mussels at these locations.

This modelling exercise was based on previously collected data in the lower-lacustrine section of Karāpiro, where mussel recruitment in the littoral zone appeared to be limited, as discussed above. I hypothesised that mussel recruitment will substantially decrease across a gradient of invasion intensity, and that the combined effects of non-native fish and non-native macrophytes will exacerbate the likelihood of recruitment failure in a hypothetical mussel population.This model only considered one recruitment cycle of a mussel population and not aggregated effects of these factors over multiple-generations

Three possible response trajectories (antagonistic, synergistic, or additive) were considered to describe potential effects of fish and macrophyte invasions on mussel recruitment. An antagonistic response was not selected because mechanisms between fish and macrophyte invasions that limit each other's impact on mussel recruitment appeared unlikely; e.g., the

consumption of invasive macrophytes by invasive fish to an extent where macrophyte-mediated adverse physicochemical conditions were not produced. A synergistic response was also considered unlikely, as it may only occur in a situation where invasive macrophytes inhibit the glochidia encounter rate of invasive fish, which seemed improbable based on field observations of catfish catch locations from electrofishing boat surveys. Therefore, I postulated an additive response as the most likely response trajectory, as the strongest mechanisms of invasive fish and invasive macrophyte operate on different stages of the mussel life-cycle (Moore et al. 2019).

Information on interacting effects of different groups of invasive species is important to support their management, particularly in the context of modified flow regimes as encountered in this hydropeaking reservoir. Such knowledge will help ensure the ecosystem services provided by dense, recruiting mussel beds persist in the face of future environmental changes. I conclude this final chapter with a discussion of general implications for invasion ecology and reservoir management, and highlight future research directions.

6.2 Methods

6.2.1 **Fish invasion model**

Brown bullhead catfish was selected as the focal non-native fish species to generate a gradient of invasion intensity relative to the native common bully (*Gobiomorphus cotidianus*). These fish species were chosen since both: 1) are abundant in Karāpiro; 2) have similar habitat requirements (benthic) that increases the likelihood of interacting with *E. menziesii* glochidia and thus influencing mussel recruitment; and 3) have input data available for the majority of the required model parameters.

6.2.2 **Model specification**

The invasion model was based on Tremblay et al. (2016) who examined whether, on balance, *N. melanostomus* had a role as a host fish or a glochidial sink for unionid mussels (*Epioblasma torulosa rangiana*, *Epioblasma triquetra, Lampsilis fasciola*, *Villosa iris*, and *Actinonaias*

ligamentina) in the Laurentian Great Lakes region. I used a similar approach to examine the potential for reduced *E. menziesii* recruitment across a hypothetical gradient of invasion intensity (ratio of non-native catfish to native common bully). The model end-points were: 1) total excysted juveniles; and 2) juvenile excystment from host fish as a proportion of the glochidia attached. Since field data on the density of mussels were available for 1 m^2 patches, this was selected as the model scale. Furthermore, I assumed model processes would be for a single exposure, not across the entire reproductive period of *E. menziesii* (October – March).

The gradient of invasion intensity (*GI*) expressed as a ratio (0-1) was given by:

$$GI = \frac{N_{cf}}{N_{cb} + N_{cf}} \quad (1)$$

where: N_{cf} is the number of catfish and N_{cb} the number of common bullies.

The reproductive output (*O*) from mussels in a patch was given by:

$$O = U \times F \quad (2)$$

where: *U* is the density of female *E. menziesii* and *F* is fecundity (total glochidia produced by a single female) (Figure 6-1).

The infestation rate for common bully (IR_{cb}) or catfish (IR_{cf}) was given by:

$$IR_{cb} = O \times ER_{cb} \times I_{cb} \times N_{cb} \quad (3)$$

$$IR_{cf} = O \times ER_{cf} \times I_{cf} \times N_{cf} \quad (4)$$

where: *ER* is the encounter rate and *I* is the infestation rate (initial attachment of glochidia) specific to each species, with *N* the hypothetical number of fish in a patch.

Finally, juveniles excysted in total (J_t) and as a proportion (J_p) for a patch were given by:

$$Jt = (IR_{cb} \times MR_{cb}) + (IR_{cf} \times MR_{cf}) \quad (5)$$

$$Jp = \frac{Jt}{IR_{cb} + IR_{cf}} \quad (6)$$

where: *MR* is the metamorphosis rate specific to each species.

The model to predict invasion intensity effects (*GI*) on juvenile excystment (J_t and J_p) was run 10,000 times with model parameters specified as distributions (see below) to introduce model variability when determining effects on juvenile excystment. For repeatability, a random seed (13579) was selected for model parameter draws. All data analyses were conducted in the R statistical software program v4.0.1 (R Core Team 2019) and plotted in "ggplot2" v3.2.1 (Wickham 2016). Additive quantile regression smoothing ('rqss' function in the 'quantreg' package) at the 5th, 50th, and 95th percentiles was used to show the upper and lower limits of the relationship between juveniles excysted and invasion intensity (Koenker et al. 2019).

6.2.3 Data and model parameterisation

The density of female *E. menziesii* was calculated by multiplying the sex ratio of mussels recorded in Karāpiro brood pouch assessments (50:50; from Chapter 3) and mussel density (m^2) determined from the Karāpiro field survey (Chapter 5). It was assumed that all females reached gravidity and expelled glochidia. The estimated female *E. menziesii* density (mean 18 m^2; range 0-133 m^2) was represented in the invasion model as a gamma distribution Γ (1,0.05) rounded to integer values (Bolstad, 2007; Figure 6-1a). Fecundity (total glochidia in a brood pouch) for *E. menziesii* was estimated by Melchoir et al. (2019) (mean 44,016; range 28,840-72,000; n = 6) and was represented by a Gaussian distribution *N* (45000,10000) rounded to integer values (Figure 6-1b).

The encounter rate of host fish with glochidia was unknown but, in line with Tremblay et al. (2016), was given the value of 0.001 for common bully and 0.01 for catfish (10x higher) to reflect differences in length, and therefore surface area, between species (16-140 mm for common bully and 42-420 mm for catfish; Jellyman et al. 2013). Furthermore, the difference in encounter rate between species is consistent with initial field observation of

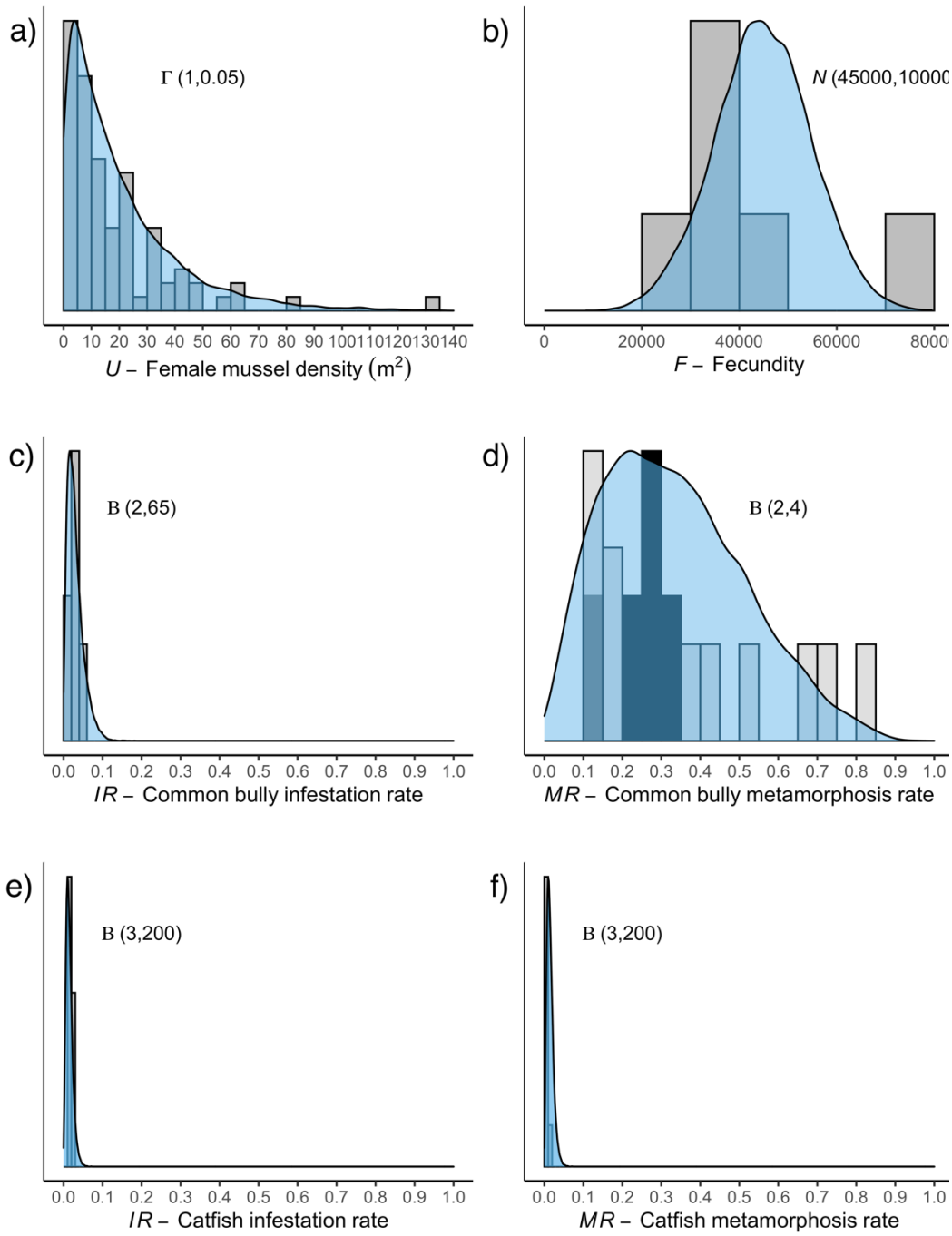


Figure 6-1: Data as density histograms overlaid with invasion model parameter value distributions (blue) for (a) female mussel density, (b) fecundity (total glochidia produced by mussels), (c) common bully infestation rate, (d) common bully metamorphosis rate with data from Hanrahan (2019) indicated in black, (e) catfish infestation rate, and (f) catfish metamorphosis rate. Y-axes are not presented with a scale as there are no units: all data presented are on the same scale. Text on plot shows the distribution used: Γ is gamma distribution, B is beta distribution, and N is normal distribution. Numbers in parentheses are parameters used to define the distributions.

an approximately 10-fold difference in glochidia load between the two species (T. Moore, unpubl. data). A second model with encounter rate set to 0.001 for both fish species was also run to test the sensitivity of the model

outputs to different encounter rates. The number of fish to be used in the invasion model was randomly generated (i.e., range 0-19 m^2) for each species from a Poisson distribution ($\lambda = 5$) (Bolstad, 2007).

Infestation rates (*I*) for common bully and catfish were sourced from Moore & Clearwater's (2019) (Chapter 3) laboratory trials, and calculated as the proportion of glochidia attached to the fish (G_a) from the total number of glochidia available to infest the fish (i.e., glochidia total (G_t) = infestation bath volume (3-L) multiplied by infestation bath concentration (~ 2000 viable glochidia L^{-1}) minus glochidia attached to other fish (G_o) in the infestation bath as given by:

$$I = \frac{G_a}{G_t + G_o} \quad (7)$$

The metamorphosis rate for each fish (*MR*) was calculated as the proportion of glochidia that excysted as juvenile mussels (G_M) relative to the proportion attached as given by:

$$MR = G_m/G_A \quad (8)$$

To inform distribution selection of the common bully metamorphosis rates, additional data were used from Hanrahan (2019). Beta distributions (values drawn were bound between 0-1) represented the infestation and metamorphosis rates of common bully ((B(2,65) and (B(2,4), respectively) and catfish (both B(3,200)) in the invasion model (Figure 6-1c,d,e,f) (Bolstad 2007).

6.2.4 **Combined fish and macrophyte invasion scenarios**

To model survival of juvenile mussels associated with macrophyte beds, three different scenarios, represented as three different distributions, were compared. Scenario 1 assumed adverse physicochemical conditions at the sediment-water interface inside dense macrophyte beds, with an associated higher juvenile mortality specified using a positively skewed B(4,1) distribution (red in Figure 6-2). This represents the situation in the lower-lacustrine section of Karāpiro, where juvenile mussels were almost entirely absent, and therefore findings may be extended to juveniles deposited inside and outside dense macrophyte beds in littoral zones. Scenario 2

assumed that random physicochemical conditions would occur inside dense macrophyte beds to represent an intermediate situation between scenario 1 and scenario 3, and therefore a uniform beta distribution was specified B(1,1)) with a neutral effect (blue in Figure 6-2). For scenario 3, favourable physicochemical conditions were selected at the sediment-water interface that promoted juvenile survival, which was specified using a negatively-skewed B(1,8) beta distribution (green Figure 6-2). Scenario 3 represents the upper-riverine section of Karāpiro inside dense macrophyte beds, where juveniles were abundant and associated with favourable conditions inside dense macrophyte beds that provided hydraulic refugia from the prevailing variable flow-regime.

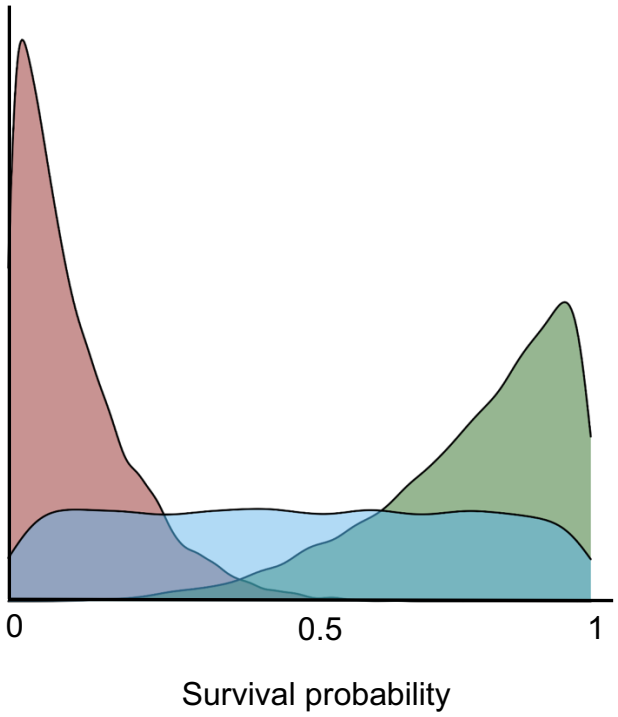


Figure 6-2: Probability distributions for macrophyte invasion scenarios: red is low survival; blue is random survival; and green is high survival of juveniles. Y-axes are not presented with a scale as there are no units.

To combine fish and macrophyte invasion models and predict the probability of mussel survival across different gradients of fish and macrophyte invasion, the probability of juveniles excysted (J_p: equation 6) was multiplied by the probability of survival inside dense macrophyte beds, as given by:

$$S = Jp \times Mp \quad (9)$$

where: S is survival (%) and Mp is the proportion of juveniles that survive across a gradient of macrophyte cover.

6.3 Results

6.3.1 **Non-native fish**

The modelled total number of juveniles excysted across a gradient of invasion intensity was relatively consistent across all quantiles (5th, median, and 95th), although the number of juveniles excysted declined more steeply at the median and 95th percentile with higher non-native fish abundances (Figure 6-3a). The median number of juveniles excysted was predicted to remain above 10 in total across most of the invasion intensity gradient (0-0.9), whereas the potential for no juvenile excystment was always possible (Figure 6-3a). Juveniles excysted as a proportion of total glochidia attached was predicted to decline steeply across the invasion intensity gradient at the median and 95th percentile (Figure 6-3b).

The reproductive output parameter (O) was not a major determinant of the total number of juveniles excysted in total or as a proportion, since glochidial production was not a limiting factor in the invasion model. However, the assumed encounter rate (R_e) parameter was a major determinant. Adjusting the encounter rate parameter to an equivalent value (0.001) for both species showed a steeper decline in the total number of juveniles excysted, but the effect on the proportion of juveniles excysted was weaker (Figure 7-10 in Appendix 7.5.1), highlighting the need for field validation of this parameter.

6.3.2 **Combined invasion scenarios**

Across gradients of fish invasion (catfish:bully ratio) and macrophyte invasion (the percentage of dense invasive beds covering the littoral zone), the survivability of juvenile mussels was examined for three scenarios (Figure 6-2). Across all scenarios, survival increased with decreasing non-native fish and macrophyte invasion (Figure 6-3). However, where adverse conditions inside and outside dense macrophyte beds were specified (scenario 1), macrophyte invasion strongly influenced juvenile mussel survivability (indicated by the density of points between 10 and 40% survival

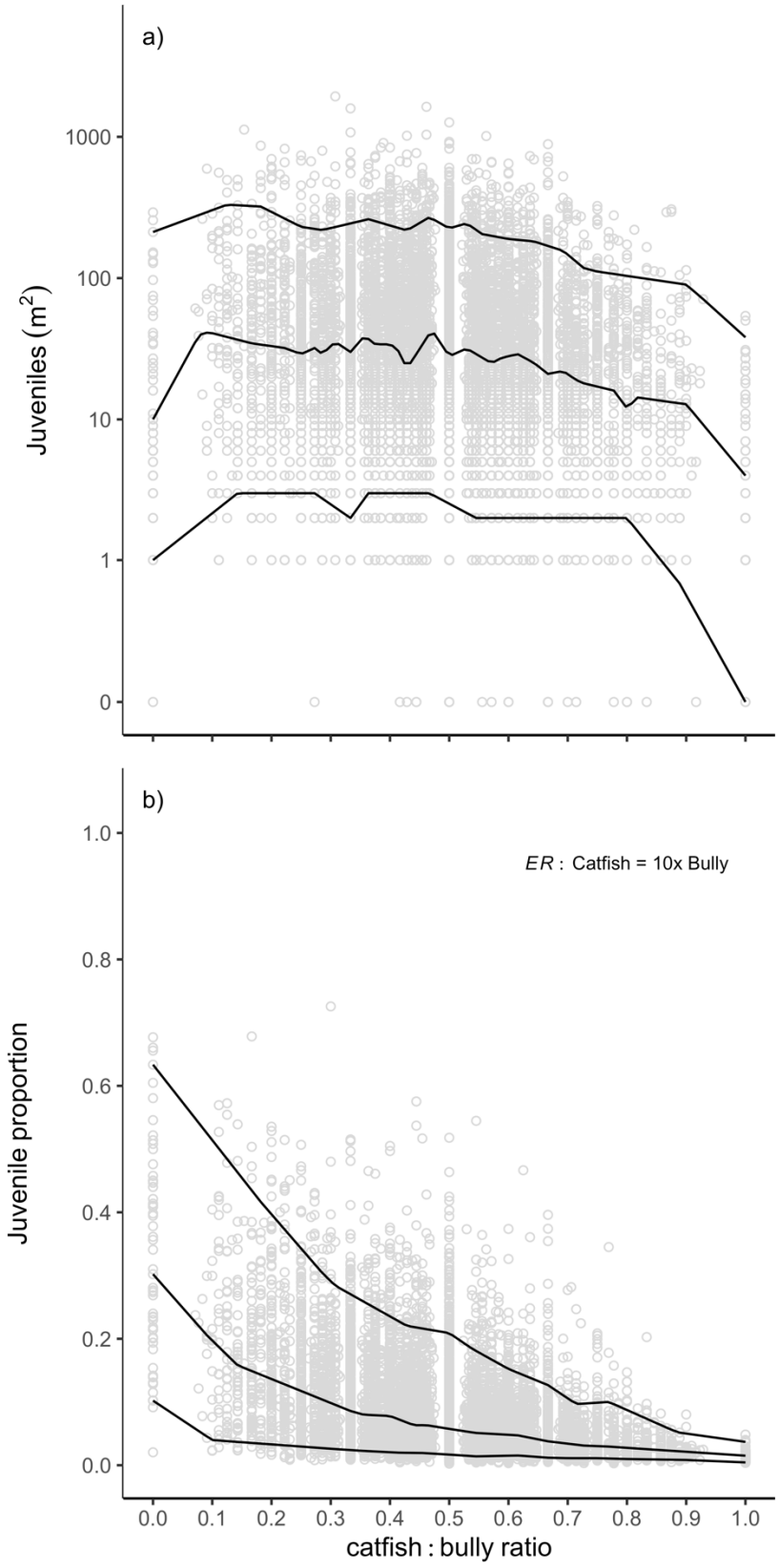
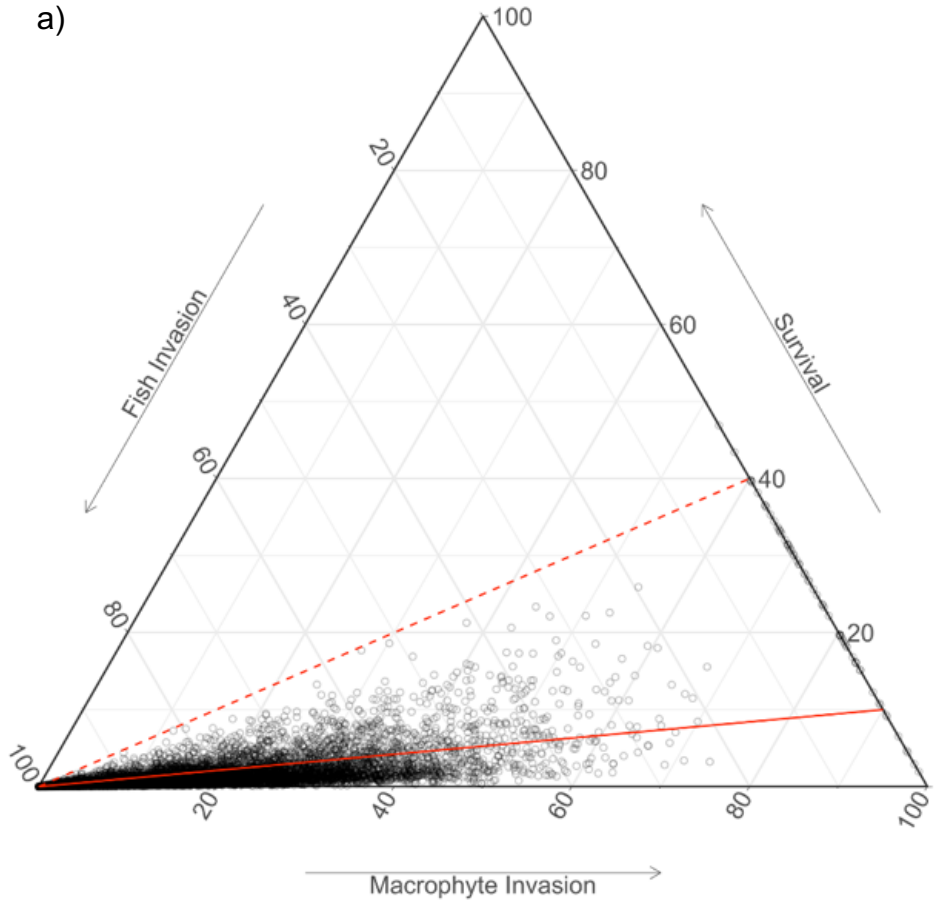


Figure 6-3: Modelled juveniles excystment in total (a) and as a proportion of total glochidia attached (b) across a gradient of invasion intensity expressed as the ratio of catfish to common bully. Encounter rate (*ER*) was specified as 10x higher for catfish than common bully (see text; equivalent encounter rates shown in Figure 7-10 in Appendix 7.5.1). Black lines display the 5th , 50th, and 95th quantiles fitted using additive quantile regression smoothing.

a)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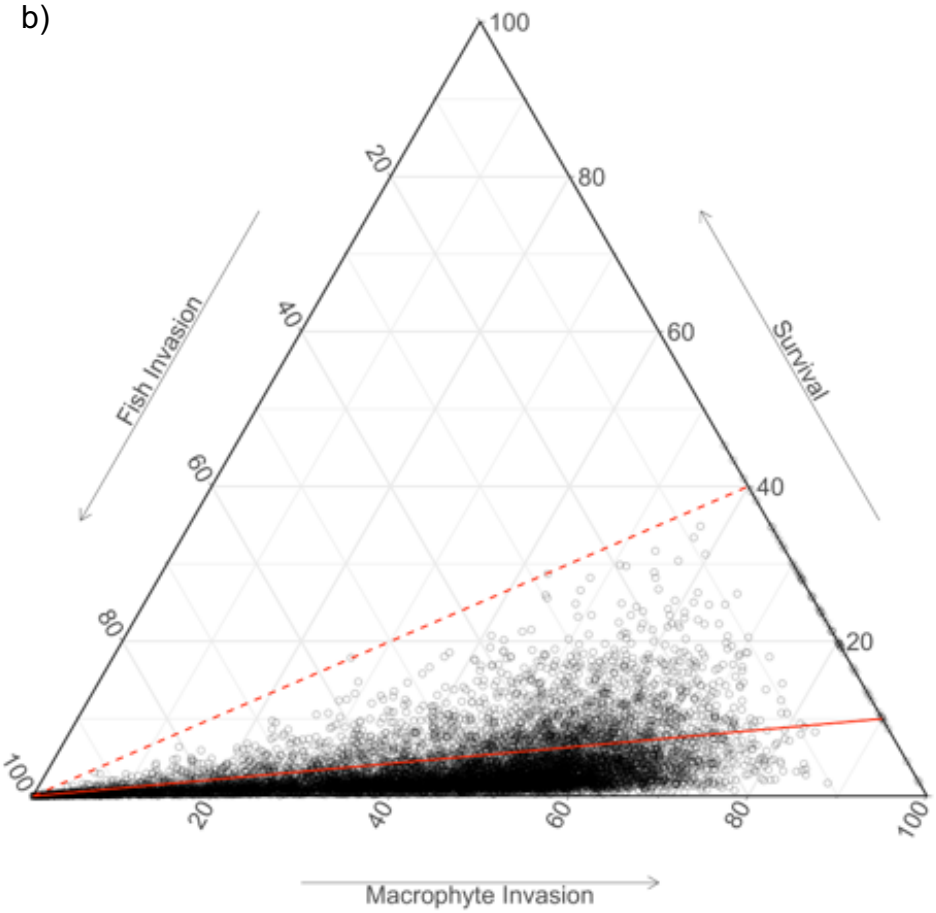
100
Fish Invasion
Survival
Macrophyte Invasion

b)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Macrophyte Invasion

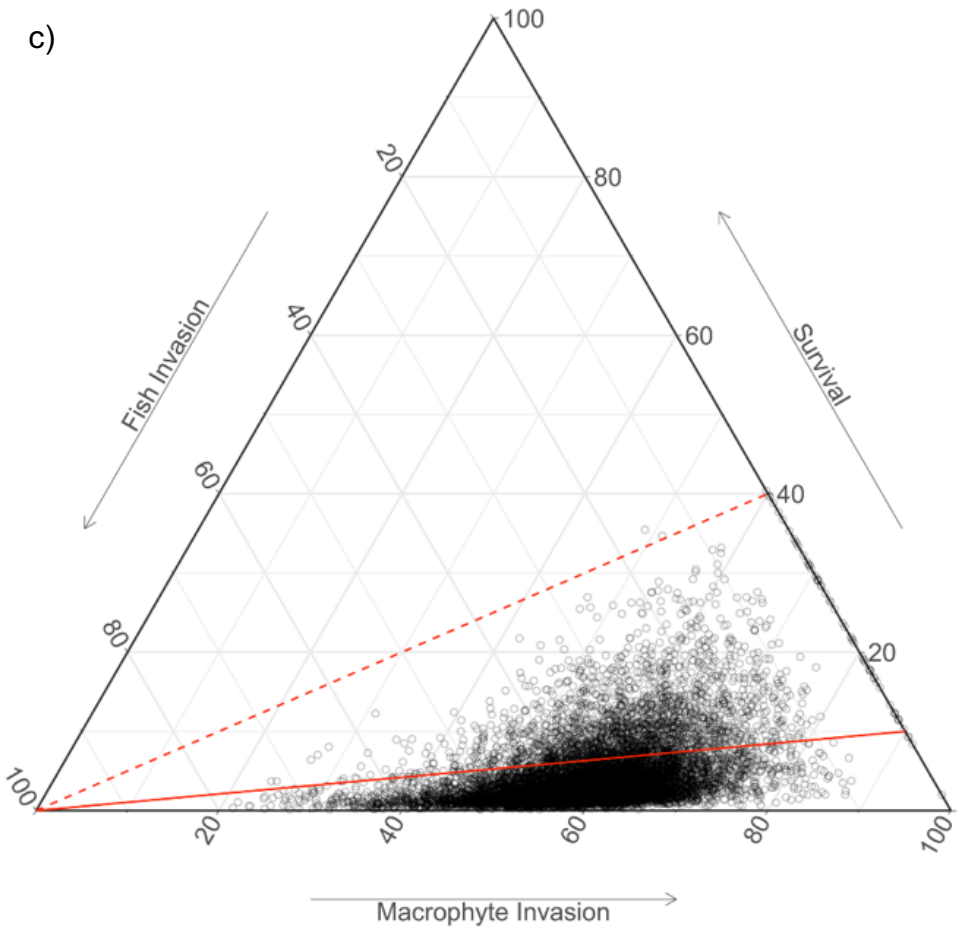

Figure 6-4: Ternary plot displaying the hypothetical relationship between juvenile mussel survival across gradients of fish invasion (catfish: bully ratio) and macrophyte invasion (percentage littoral zone cover). Red solid line indicates 10 % juvenile mussel survival and red dotted line indicates 40 % survival. Scenarios presented are where juvenile survival inside dense macrophyte beds are a) –low (scenario 1); b) –random (scenario 2); or c) -high (scenario 3; see text and Figure 6-2 for details).

in Figure 6-3). The influence of macrophyte invasion on juvenile survival decreased when random conditions were specified inside dense macrophyte beds (scenario 2), and even more so when conditions were favourable for mussel survival (scenario 3).

6.4 Discussion

6.4.1 Predictions of juvenile excystment

The invasion model showed that, while juvenile recruitment was possible across the entire gradient of catfish invasion intensity, increasing dominance of non-native fish substantially decreased the likelihood of high numbers of juveniles being excysted and recruited to the benthos.

Furthermore, juvenile recruitment became less 'efficient', since the proportion of those excysted relative to the number that attached sharply declined with increasing fish invasion. This decline occurred despite the high amount of variability specified in the invasion model, which represented a broad range of values to encompass the potential situations that may occur in Karāpiro and similar managed ecosystems.

My findings confirm the important role of native common bullies as host fish for *E. menziesii* at the population level, although the metamorphosis rate specified for common bullies was based on laboratory conditions and potentially may be different in the field (Chapter 3). However, the total predicted number of juveniles attached per fish using laboratory data appeared to be within the range observed on fish in the field (i.e., 95% had 0-20 glochidia per common bully; range 0-226; T. Moore, unpubl. data), although this needs to be confirmed for catfish. These predicted metamorphosis rates may still be relevant since common bullies are repeatedly exposed through the glochidial release period (October – March), with preliminary evidence suggesting no resistance to multiple exposures (Hanrahan 2019). However, based on the invasion model and various assumptions (notably the glochidia encounter rate), it appears unlikely that non-native fish could impact *E. menziesii* recruitment as unsuitable hosts when suitable native hosts are present and relatively abundant, as is currently the case in the lower-lacustrine section of Karāpiro (Pepper 2015).

The glochidia encounter rate is an important factor when considering the potential of host fish to successfully excyst juveniles, but less influential when determining the role of host-fish as glochidia sinks. This is because the vast majority of glochidia are lost to potential recruitment before host attachment, and thus as a proportion would only be a fraction of those that did not encounter fish hosts initially. Nevertheless, field data on infection rates for both common bully and catfish would provide added confidence to model predictions.

The possibility of multiple unsuitable non-native hosts was not included in the model, even though data were available for non-native rudd and goldfish from Karāpiro (Chapter 3). These fish species were not selected because

of their relatively low probability of encountering glochidia in the field given they are more pelagic species. A situation where multiple non-native benthic species were likely to encounter glochidia would be an added complexity worth examining in other ecosystems, especially if the non-native fish encountering glochidia represented a spectrum of host suitability. However, in Karāpiro attachment or non-attachment of glochidia to non-native fish is equivalent to lost recruitment as excystment rates appear to be low, and therefore indirect mechanisms that impact the native common bully's ability to act as a mussel-host may be more important to consider than invasive fish control for mussel conservation. However, if competition with other fish species reduces common bully abundance or confines them to habitats where deposited juveniles are unlikely to survive (e.g., non-native macrophyte beds), then management of competing species may be important.

6.4.2 **Combined fish and macrophyte invasion**

Juvenile mussels are extremely sensitive to adverse environmental conditions present at the surface-water interface, especially from anoxia, hypoxia, and ammonia toxicity (Clearwater et al. 2014, Černá et al. 2018). Therefore, it is likely that these adverse conditions will be a strong driver of reduced juvenile mussel recruitment after excystment, as highlighted by macrophyte scenario 1 that specified poor juvenile survival inside dense macrophyte beds. Across gradients of fish and macrophyte invasion, mussel recruitment declined but appeared to be more strongly related to unsuitable macrophyte-mediated habitat conditions than disruption of the obligate host stage by non-native fish. Although this difference is dependent on juvenile mussel survival inside dense macrophyte beds, as specified in the model but for which there are no measured data, the findings of scenario 1 appear to be consistent with observations in Karāpiro where: 1) adult skewed-size population structure is present in the lower-lacustrine section; and 2) there is clear evidence for glochidial attachment on native host fish (i.e., all common bullies collected during the release season had glochidia attached; T. Moore, pers. obs.). Scenario 3 was more indicative of conditions in the upper-riverine section where *E. densa* beds support

juvenile mussels, and increasing dominance of non-native fish is likely to be a more important issue for juvenile recruitment.

The apparently stronger impact of macrophyte invasion may be amplified by the comparatively longer time mussels spend in the juvenile mussel stage (and thus a longer time exposed to adverse environmental conditions) relative to attachment on a host fish (9–21 days; Chapter 3). Any hypoxia or anoxia event or toxic sediment conditions (e.g., pore-water ammonia) that occur when juvenile mussels are present is almost certain to be fatal, whereas mussel survival through the obligate larval life-stage is more dependent on the host-fish immune system (Sparks & Strayer 1998, O'Connell & Neves 1999). Regardless, fish and macrophyte invasions have potential to impact mussel recruitment through different mechanisms at different stages of the mussel life-cycle, and both likely contribute to the observed adult-skewed mussel population size-structures in Karāpiro, similar to many other invaded aquatic ecosystems (Bailey & Green 1989, Hastie & Toy 2008, Moore et al. 2019). However, to assess the degree to which non-natives species contribute to mussel decline, a population viability analysis would be useful to estimate how many individuals and habitat could be required for long-term surivial of mussel populations(Reed et al. 2002). Furthermore, models that account for effecs over multiple generations would provide insights into mussel population extinction rates over the long-term.

6.4.3 **Implications for reservoir management**

My findings for Karāpiro reinforce general observations around the world that reservoirs are hotspots of biological invasions (Johnson et al. 2008; Havel et al. 2015). In terms of macrophyte invasion and subsequent proliferation, my work shows that adverse conditions are most pronounced when the peak biomass period coincides with high water temperatures in summer and autumn, as well as during senescence induced either by natural phenology or by herbicide application (Godshalk & Wetzel 1978; Moore et al. 2020). Although these findings were spatially confounded (i.e., flow and macrophyte species effects could not be teased apart), multiple lines of evidence suggest both hydrology and macrophytes likely interact to influence mussel density, for example, by provisioning of flow-refugia or

higher flows limiting adverse physicochemical conditions at the sediment-water interface.

Research is required to determine whether reduction of macrophyte-induced impacts at these times could be achieved by increasing flows to promote water circulation and re-oxygenation at the sediment-water interface in an attempt to limit the development of hypoxic and anoxic conditions in the littoral zone (Chapter 4). Furthermore, variable flows may help reduce silt accumulation and associated adverse physicochemical conditions, which was highlighted in the structural equation model by a weak influence of silt on mussel density in the upper-riverine section (Chapter 5). However, information on the distribution and habitat associations of juvenile mussels needs to be expanded to target management actions for sustaining recruitment, particularly within the substrate where juveniles are thought to live (Ferreira-Rodríguez et al. 2019).

Reservoir management can also limit impacts of fish invasions, firstly by preventing further establishment of non-native fish, particularly from downstream environments where controls can be effectively implemented, and secondly by reducing the abundance of previously established non-native fish if they interact with native hosts, for example by generating hydrological regimes unfavourable to them at critical times. Related to this, regulation of hydropeaking operation regimes so that common bully eggs survive to spawning could improve the *E. menziesii* population recruitment pool. Ensuring suitable fish-hosts are sufficiently abundant at key times for mussel recruitment is essential, as it was highlighted in the model as the most important factor determining juvenile excystment. Accordingly, management should also focus on maintaining fish host populations at densities where glochidia encounter rates are sufficient to produce ecologically relevant numbers of juvenile mussels (see discussion above). Such actions could be supported by development of mussel rearing programs or translocations from source populations to re-populate areas where local die-offs have occurred, for example as a result of wide-scale herbicide applications or extreme natural events (Strayer et al. 2019).

6.4.4 **Implications for mussel conservation**

Non-native species are potentially under-recognized globally as a threat to unionid mussels and this thesis contributes to the expanding literature clarifying the mechanistic pathways of their interactions (IUCN 2018) (Chapter 2). Although non-native species are one amongst a multitude of threats to mussels, their mode of action operates on the life-stages critical for mussel recruitment, and therefore they may have disproportionately high effects on population density (Moore et al. 2019). Since their impacts are often recognisable through adult-skewed population size structures, it may be possible to identify locations of potentialy reduced recruitment and take remedial actions to counteract invasive species impacts. As such, conservation management plans that identify the status of native fish hosts and the role of non-native species in disrupting mussel recruitment can be initiated when recruitment failure is indicated by adult-skewed population size structures. This is particularly relevant for situations where non-native hosts are abundant and act as glochidial sinks (Tremblay et al. 2016). Accordingly, re-population via enhancing native fish host populations may not be required if barriers to mussel recruitment are addressed when recruiting adults producing viable glochidia are still present.

6.4.5 **Theoretical implications and future research directions**

Affiliate relationships, such as those involving host fish and mussel glochidia, are vulnerable to disruption since they are based on ecologically-balanced associations that have developed over evolutionary timescales (Douda et al. 2013). Some non-native fish are suitable affiliate partners to mussels, but often these species are similar to the native host fish (e.g., in terms of lineage (Watters and O'Dee 1998) or morphology (Huber and Geist 2017); Chapter 3). Based on the meta-analysis (Moore et al. 2019) I found the replacement of mussel-fish host associations with invasive species may be unlikely, as confirmed when low rates of transformation success were found for the *E. menziesii* on non-native fish, albeit under laboratory conditions (Chapter 3). Regardless, within the context of the multiple interacting stressors prevalent in hydropeaking reservoirs, the ability to transform glochidia on invasive fish is unlikely to significantly boost mussel recruitment due to the time required to develop co-evolutionary relationships.

Future research investigating non-native species impacts on *E. menziesii*, and unionid mussels in general, should examine the general ecology of the focal mussel species with a view to quantifing conditions that are required to complete their life-cycle, in particular for juvenile mussels, over multiple generations. This could be achieved by a field manipulation experiment that relocates juvenile mussels into habitats across multiple sites that represent gradients of sediment and macrophyte biomass, with growth, survival and recruitment as end-points. Additionally, host-fish compatibility should be determined in the field across a range of environmental conditions suitable for juvenile mussel survival, so the transferability of laboratory results can be determined. Validation of model parameters for the fish invasion model of glochidia encounter rate could be achieved by dissecting fish captured during the mussel spawning season. Furthermore, the tolerances of juvenile mussels to multiple interacting stressors operating within natural and managed waterbodies could be addressed by determining the key times reservoir management should enable flushing/water movement along littoral zones. Understanding of the longer-term impacts of herbicide application on macrophyte recovery and the build-up of habitat with high organic matter content, should be sought to explain potential mechanisms leading to reduced mussel densities in littoral zones.

In addition to their inherent conservation value, the functions that mussels provide underpin ecosystem services important for maintaining water quality and aquatic ecosystem health (Vaughn 2018). Management of non-native fish and macrophyte invasions will play an important role in informing future management decisions aimed at conserving mussels and sustaining these values, particualry in Karāpiro over the summer season. Mitigating pressures on freshwater mussel populations will become more important in a changing future environment, where globalisation and the demand for energy production will facilitate continued biotic homogenisation and associated loss of ecosystem services in modified freshwater systems. Due to their long life-spans, mussels may be slow to replace if populations become locally extinct. However, this longevity also provides an opportunity to restore disrupted mechanisms that support their recruitment before adult mussel populations die out.

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Appendices

7.1 Interactions between Unionida and non-native species: a global meta-analysis (Chapter 2)

7.1.1 **Bibliometrix package output**

Table 7-1: Comparison of literature searches on interactions between freshwater mussels with all non-native species, non-unionid species, and all non-native species excluding non-unionid mussels. Search date 20.10.17.

	Literature search output of freshwater mussel interactions with					
	All non-native species		Non-unionid species		All non-native species excluding non-unionid mussels	
Articles	1422		1141		315	
Authors	3240		2502		1002	
Annual growth rate	13.5 %		15.7 %		10.4 %	
Most relevant keywords	Keyword	Articles	Keyword	Articles	Keyword	Articles
	Invasive species	178	Invasive species	163	Invasive species	22
	Zebra mussel	87	Zebra mussel	87	Unionidae	17
	Dreissena polymorpha	85	*Dreissena polymorpha*	85	Freshwater mussels	16
	Dreissena	79	*Dreissena*	79	Distribution	9
	Zebra mussels	64	Zebra mussels	64	Fish	8
	Great Lakes	46	Lake Erie	42	Glochidia	8
	Lake Erie	43	Great Lakes	40	Unionid	8
	Exotic species	41	*Corbicula fluminea*	38	Alien species	7
	Corbicula fluminea	39	Exotic species	38	Great lakes	7
	Unionidae	38	Phytoplankton	32	Species	7

7.1.2 **Literature review summary tables**

Table 7-2: Summary of articles that examine the interaction between freshwater mussels (Unionida) and non-native fish.

Native Freshwater mussels (Unionidae)				Non-native fish		Study Design/Analysis						
Species	Ecosystem	Life-stage	Reproductive Strategy	Species	Ecological niche	Study type	Response variable	Statistical method	Effect direction	Significance of effects	Country	Reference
Anodonta anatina	N/A	Larvae & Juveniles	Host Generalist	*Cyprinus carpio, Gobio lozanoi, Lepomis gibbosus, Micropterus salmoides,* & *Oncorhynchus mykiss, Carassius auratus, Carassius gibelio, Psudorasbora parva,* & *Rhodeus amarus*	Mixed: benthic and pelagic	Laboratory experiment	Transformation rate	Fisher's exact test & Generalised linear models	-	*S – P* < 0.05; higher proportion of native fish considered to be suitable hosts (94%) over non-indigenous (20%). GLM *P* < 0.01; Mean transformation rate higher on native (33.6 ± 20.3%) than non-indigenous (6.0± 15.4%) species	Portugal & Czech Republic	(Karel Douda et al., 2013)
Anodonta anatina, Unio pictorum, Unio tumidus, & *Pseudoanodonta complanata*	Lake	Larvae	Host Generalists (Haag, 2012)	*Neogobius fluviatilis, Babka gymnotrachelus,* & *Proterorhinus semilunaris*	*N. fluviatilis* prefers sandy bottoms; *B. gymnotrachelus* & *P. semilunaris* prefer muddy and overgrowth habitats	Field survey	Prevalence & mean intensity	None	+ & -	*N. fluviatilis* (p=1% MI = 5) was a poor host. *B. gymnotrachelus* (p=21.7%; MI = 10.2) & *P. semilunaris* (p=24.6%; MI = 8.3) better hosts; probably due to ecological niche differences.	Poland	(Mierzejewska et al., 2014)
Anodonta cygnea	N/A	Larvae & Juveniles	Host Generalist	*Pseudorasbora parva* & *Ctenopharyngodon idella*	Benthic: feed on aquatic weeds	Laboratory experiment	Excysted juvenile mussels per fish	Post-hoc pairwise Wilcoxon rank sum test with Bonferroni correction	+ & -	*S – P* < 0.05; *C. idella* had higher numbers of dropped of living mussels (9.1) than some of the native host species. *P. parva* was a highly unsuitable host (0.5 excysted mussels per fish)	Germany	(Huber & Geist, 2017)
Anodonta sp.	River	Larvae	Host Generalist (Haag, 2012)	*Neogobius melanostomus*	Benthic: guarding cavity spawners	Field survey	Prevalence % & mean intensity		(-)	*N. melanostomus* (p = 23.3 %; MI = 1.4 ± 0.8 (intensity range; 1-3)). Low MI compared with (Mierzejewska et al., 2014)	Czech Republic	(Kvach, Ondračková, Janáč, & Jurajda, 2017)

Lampsilis cardium	N/A	Larvae & Juveniles	*Host* Specialist: Black Basses (Haag, 2012)	42 non-indigenous fish		Laboratory experiment	Mean percent of all glochidia that metamorphosed		-	*L. cardium* 6 species successful however, lower mean percent of all glochidial that metamorphosed; Native hosts mean 62% non-indigenous host mean 14%	U.S.A	(Watters & O'Dee, 1998)
Margaritifera auricularia	N/A	Larvae & Juveniles	Salmon/Trout (Haag, 2012)	*Accipenser baeri*	Benthic	Laboratory experiment	Metamorphosis		?	Suitable host: Not Quantitative. One month after infestation, 15 live juveniles and many empty juvenile valves were found	Spain	(Araujo & Ramos, 2000)
Margaritifera margaritifera	River	Larvae & Juveniles	Salmon/Trout (Haag, 2012)	*Salvelinus fontinalis*	Pelagic	Laboratory experiment & Field survey	Number & size of larvae & encystment in field	Chi-squares & Mann-Whitney *U* tests	-	Generally, brook trout were less suitable hosts than native hosts with respect to numbers and size of *M. margaritifera* larvae. Only in one river were a few larvae encysted on brook trout for at least 9 months.	Finland	(Salonen, Marjomäki, & Taskinen, 2016)
Popenaias popeii	River	Larvae & Juveniles	Host Generalist; Lab trails) **Host specalist;** Field survey	17 non-indigenous species		Field survey & Laboratory data	Relative host suitability		-	Relative host suitability (when able to be calculated) very low; *Micropterus punctulatus* (<0.010); *Gambusia affinis* <0.001). Best native host *Moxostoma congestum*; 0.122	U.S.A	(Levine, Lang, & Berg, 2012)
Psilunio littoralis, Anodonta cygnea, Unio elongatulus, & *Margaritifera auricularia*	River	Larvae & Juveniles	Mostly Host Generalists; *M auricularia* Salmon/Trout (Haag, 2012)	*Cyprinus carpio, Gobio gobio* & *Alburnus alburnus*		Field survey	Glochidia attachment		-	No non-indigenous fish were found with glochidia	Spain	(Araujo, Bragado, & Ramos, 2000)

Strophitus undulatus	N/A	Larvae & Juveniles	Host Generalist	*Oncorhynchus mykiss, Ambloplites rupestris, Micropterus salmoides,* & *Etheostoma zonale*	Benthic (*Etheostoma zonale*) & Pelagic	Laboratory experiment	Transformation rate		-	All (except *Etheostoma zonale*) produced juveniles, although at much low rates than suitable natives (2-12% non-indigenous; 3-52% native)	U.S.A	(van Snik Gray, Lellis, Cole, & Johnson, 2002)
Unio & *Anodonta*	River	Larvae	Host Generalists	*Pseudorasbora para, Gobio albipinnatus,* & *Carassius auratus*	Benthic	Field Survey	Prevalence		-	*Anodonta* glochidia found on *Gobio albipinnatus* (p = 11.8%, n=34); *Unio* glochidia found on *Pseudorasbora para* (p = 20%, n=5) & *Carassius auratus* (p= 8.3%, n=12). Low prevalence compared to native fish hosts	Czech Republic	(Gelnar, 2006)
Unio crassus	N/A	Larvae & Juveniles	Host Generalist	*Oncorhynchus mykiss* & *Neogobius melanostomus*	*O. mykiss;* Pelagic. *N. melansotmus*; benthic guarding cavity spawners	Laboratory experiment	Infestation rate	Kruskal-Wallis sum of ranks test	-	*O. mykiss;* glochidial per fish weight 13 - 0.1 (2-16 days): Not hosts - Glochidial Sink *N. melansotmus*; glochidia per fish weight 29.4 - 0 (2-16 days): Not Hosts - Glochidial Sink	Germany	(Taeubert, Gum, & Geist, 2012)
Unio crassus	N/A	Larvae & Juveniles	Host Generalist	*Pseudorasbora parva, Salmo trutta, Cyprinus carpio, Rhodeus amarus, Ctenopharyngodon idella, Oncorhynchus mykiss& Salvelinus fontinalis*		Laboratory experiment	Transformation rate		-	2 Suitable hosts with low transformation rate (*Pseudorasbora parva*, 13% & *Salmo trutta* 0.02%); Unsuitable hosts - *Cyprinus carpio, Rhodeus amarus, Ctenopharyngodon idella, Oncorhynchus mykiss& Salvelinus fontinalis*	Czech Republic	(K Douda, Horký, & Bílý, 2012)

Unionidae gen. sp.	Lake	Larvae	Not specified	*Proterorhinus semilunaris*	Prefer muddy and overgrown habitats	Field survey	Prevalence % & mean intensity	?	Present at 2/4 sites; Channel p=68.6 MI=1.69; Shumarovka River p=42.1, m=0.68	Russia	(Zhokhov, Pugacheva, & Molodozhnikova, 2017)
Utterbackia imbecillis	N/A	Larvae & Juveniles	Host Generalist	42 non-indigenous fish		Laboratory experiment	Mean percent of all glochidial that metamorphosed	+	*U. imbeciles* 30 species successful, with some equal or higher rates of mean percent of all glochidial metamorphosed than native hosts; native mean 43% (20 -65%); non-indigenous host mean 35% (0-82.6%).	U.S.A	(Watters & O'Dee, 1998)

Native freshwater mussel (Unionidae) response; prevalence % (P); & mean intensity (MI).

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Table 7-3: Summary of articles that examine the interaction between freshwater mussels (Unionida) and non-native macrophytes.

Native Freshwater mussels (Unionidae)				Non-native macrophytes			Study Design/Analysis					
Species (**dominant**)	Ecosystem	Life-stage	Response	Species **(dominant)**	Structure	Cover	Study type	Statistical method	Effect direction	Significance of effects	Country	Reference
Anodonta cygnea	Lake	Adult	Mortality	*Eichhornia crassipes*	Floating	High	Field Survey		-	*NR* - Reports unpublished data. Mass mortality of seasonal macrophtyes resulted in high freshwater mussel mortality.	Portugal	(Lopes-Lima et al., 2016)
Anodontoides ferussacianus, *Stophitus undulatus, Lasmigona compressa,* & *Pyganodon grandis*	River	Adult	Density & Presence	Not specified	Submerged	Mussels present, mean 6%. Mussels absent, mean 41%	Field Survey	Step-wise regression & T-test	-	*S – P* = 0.002; Negative relationship with percent vegetation cover (coefficient -4.11). *NS* – percent vegetation and mussel presence/absence.	U.S.A	(Harriger, Moerke, & Badra, 2009)
Echyridella menziesii	Lake	Adult	Density & Biomass	***Elodea* spp.**, *Typha orientalis*, Potamogeton perfoliatus,* Turf, & Charophyte	Submerged	Mean 44%; range 0-100%	Field Survey	Correlation	0	*NS* – Reports positive association with freshwater mussel density (+0.26) and biomass (+0.40).	New Zealand	(Butterworth, 2008)
Echyridella menziesii	Lake	Adult	Density	*Lagarosiphon major*	Submerged	Mean of 3%; range 0-10%; 100% at 2-5 m for a separate site.	Field Survey		-	One site had very low density of mussels in a large macrophyte bed (1.2 m^2); and another site with 100% weed cover from 2-5 m excluded mussels completely.	New Zealand	(James, 1985)
Echyridella menziesii	Lake	Adult	Density	***Lagarosiphon major***, *Egeria densa, Elodea canadensis,* & *Ceratophyllum demersum*	Submerged	*Lagarosiphon major,* ~ 50%; range 5-80%	Field Survey	T-test	0	*NS* – Reports mean of 244 m^2 live mussels amongst and 182 m^2 outside *Lagarosiphon major* beds, respectively.	New Zealand	(Lodge, 2012)
Echyridella menziesii	River	Adult	Density	*Elodea canadensis*	Submerged		Field Survey		+	"Mussels were found within the weed bed where the substrate is stabilized by macrophyte roots"; 4.6 m^2 live mussels amongst and 0 m^2 outside macrophtye beds.	New Zealand	(Nobes, 1980)
Echyridella menziesii	Lake	Adult	Density	***Elodea canadensis*** & *Ranunculus trichophyllus*	Submerged	*Elodea canadensis*, median 76-100% category. *Ranunculus trichophyllus,* median 96-100%	Field Survey		-	"In general, mussel density decreased considerably within macrophyte beds". Mussels ranged 8-59 m^2.	New Zealand	(Sorrell, Phillips, Wells, & Sykes, 2007)
Echyridella menziesii	Lake	Adult	Abundance	Not specified	Submerged	Max. macrophtye biomass range 7-2123 g/m^2	Field Survey	Multiple regression	(+)	*S – P* < 0.01; mussels had weak positive relationship with macrophyte biomass Beta coefficient (0-1): + 0.17.	New Zealand	(Weatherhead & James, 2001)
Elliptio complanata, *Alasmidonta varicosa, Alasmidonta heterodon,*	River	Adult	Presence	Not specified			Field Survey	Discriminant analyses	(-)	*S – P* = 0.007; Weak negative association between *A. varicosa* and aquatic macrophyte presence/absence (x^2 =3.23).	U.S.A	(Strayer & Ralley, 1993)

Strophitus undulatus, Anodonta implicate, & *Alasmidonta undulata*												
Margaritifera margaritifera	River	Adult & Juveniles	Density	Not specified	Submerged	0-100% cover mean 19%; median 5%	Field Survey	Chi-square	0	*NS*	Scotland	(Hastie, Boon, & Young, 2000)
Margaritifera margaritifera	River	Adult	Distribution	Reeds/sedges/herbs (e.g., *Phragmites australis*)	Emergent		Field Survey	Chi-square & logistic regression	-	*S* – *P* < 0.01; Reports negative association with emergent reeds/sedges/herbs. However, *NS* in logistic regression; possibly due to small sample size of interactions between variables (n=50).	Scotland	(Hastie et al., 2003)
Pyganodon grandis & *Utterbackia imbecilli*	Lake	Adult	Density	***Myriophyllum spicatum*** & *Neluumbo lutea**	Submerged & Emergent	> 50% cover	Field Survey	Correlation	-	*S* – *P* < 0.001; Reports mussel density significantly lower in macrophyte beds (- 0.49).	U.S.A	(Burlakova & Karatayev, 2007)
Villosa iris, *Elliptio dilatata, Stropitus undulatus, Fusconaia flava, Alasmidonta calceola, Lampsilis radiata,* & *Pleurobema cordatum coccineum.*	River	Adult	Density	Not specified	Submerged		Field Survey		+	"Ignoring species, the clams occur most frequently in the shallow, slow-current, more vegetated areas"	Canada	(Salmon & Green, 1983)

* Native macrophyte species. Effect direction: positive +; negative –; neutral 0; weak magnitude indicated by parentheses. Significance of effects were reported as statistically significant (*S*; with *P*-value if provided); not significant (*NS*); not reported (*NR*).

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Table 7-4: Summary of articles that examine the interaction between freshwater mussels (Unionida) and non-native predators.

Native Freshwater mussel				Non-native predators			Study Design/Analysis					
Species (**non-indigenous**)	Ecosystem	Life-stage	Response	Species	Common name	Predator type	Study type	Statistical method	Effect direction	Significance of effects	Country	Reference
Anodonta cygnaea & *Unio pictorum*	River & lake	Adult	Observation	*Rattus norvegicus*	Norway rat	Terrestrial	Observation		-	Unionidae remains were found only in rat feeding remain piles near flowing waters; piles on lake shores did not contain Unionidae, even though both *R. norvegicus* & Unionidae mussels were present.	Italy	(Parisi & Gandolfi, 1974)
Echyridella menziesii	River	Adult	Observation	*Rattus* spp.		Terrestrial	Observation		-	Rats observed taking freshwater mussels.	New Zealand	(O'Donnell, Weston, & Monks, 2017)
Echyridella menziesii	River	Adult	Observation	*Rattus* spp.		Terrestrial	Observation		-	Eighteen freshwater mussel shells, typical of rat predation, were found in what was likily a rat den.	New Zealand	(Theobald & Coad, 2002)
Echyridella menziesii	Lake	Adult	Observation	*Rattus norvegicus*	Norway rat	Terrestrial	Observation		-		New Zealand	(Beveridge & Daniel, 1965)
Elliptio icterina	Stream	Adult	Observation	*Sus scrofa*	Feral hog	Terrestrial	Observation		-	Describes stream banks rooted and littered with broken mussel shells.	North America	(Williams & Benson, 2004)
Margaritifera margaritifera	River	Adult	Observation	*Mustela vison*	American mink	Terrestrial	Observation		(-)	No direct evidence suggesting natural predation causes significant mortalities of *Margarifera margaritifera*: appears rare and opportunistic.	Scotland	(Cosgrove, Hastie, & Sime, 2007)
Microcondylaea compressa & *Unio mancus elongatulus*	River	Adult	Observation	*Ondatra zibethicus*	Muskrat	Terristrial	Observation		-	Suspected predation of endangered *M. compressa* mussels by muskrats	Croatia	(Reischüz & Reischüz, 2001)
Pseudanodonta complanata	Lake	Adult	Burrowing depth	*Ondatra zibethicus*	Muskrat	Terrestrial	Field Survey	One-way ANOVA	-	*P. complanata* burrowed deeper than *Anodonta piscinalis* & *Unio pictorum*. The shell of *P. complanata* was observed to be thiner than these other speices, and therefore predicted to exhibit this behaviour to avoid predation by muskrats.	Finland	(Saarinen & Taskinen, 2003)
Uniomerus carolinianus	Stream	Adult	Observation	*Sus scrofa*	Feral hog	Terrestrial	Observation		-	Predation observed in small blackwater streams	North America	(Zengel & Conner, 2008)
Unionacea Superfamily	Stream	Adult	Observation	*Sus scrofa*	Feral hog	Terrestrial	Observation		-	Hogs may consume freshwater mussels in shallow water from head water streams – personal communication.	North America	(Kaller, Hudson III, Achberger, & Kelso, 2007)
Unionidae Family	Lake		Relative frequency of occurance in stomach	*Lithobates catesbeianus*	American bullfrog	Reptile	Observation	Descriptive statistics	(-)	Unionidae found in stomach contence of male American bullforgs. Relatively minor component of bullfrog diet.	China	(Xuan et al., 2015)
Velesunio ambiguous	River	Adult	Observation	*Hydromys chrysogaster* & *Vulpes vulpes*	Water rat & red fox	Terrestrial	Observation		-	After stroms large numbers of mussels are stranded onshore. Hypotheised that these may be prey for animals frequenting the shoreline. Also, humans may use them for fishing bait.	Australia	(Walker, 1981)

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7.2 Non-native fish as glochidial sinks: elucidating disruption pathways for *Echyridella menziesii* recruitment (Chapter 3)

7.2.1 Infestation trail schematic overview

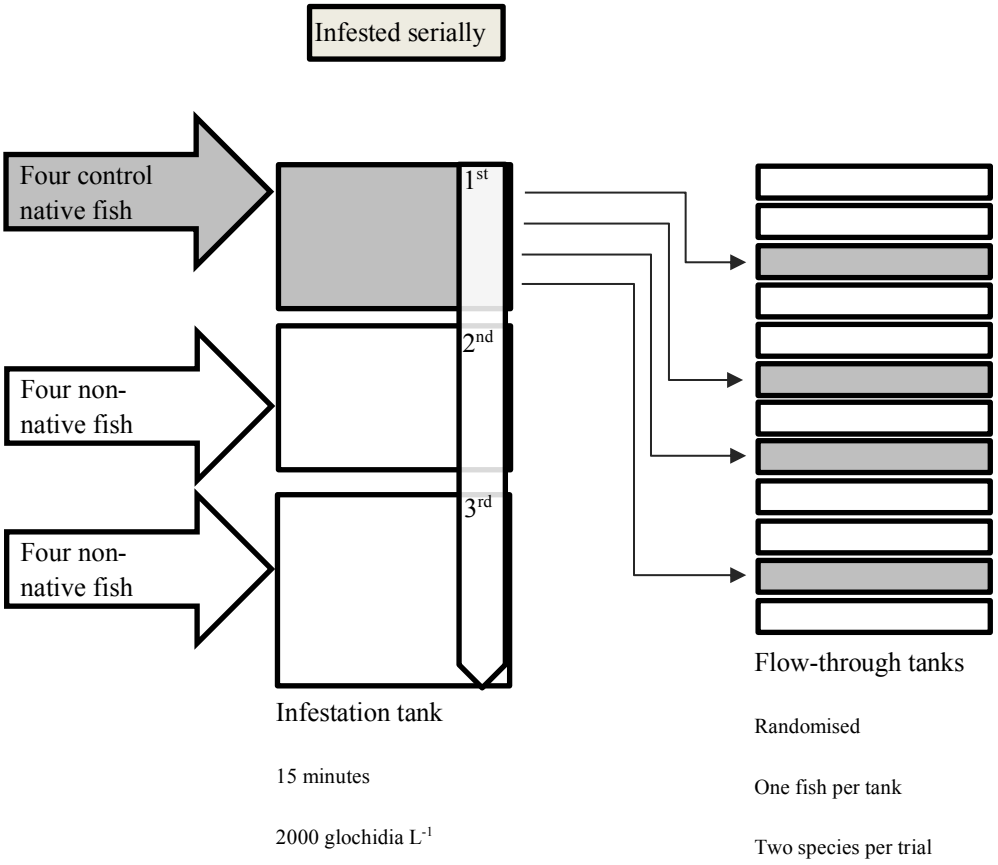


Figure 7-1: Schematic overview of methods used in fish glochidial infestation for one trial (e.g., catfish).

7.2.2 R-INLA code for recruitment models

Install packages and set working directory

```
ipak <- function(pkg){
  new.pkg <- pkg[!(pkg %in% installed.packages()[, "Package"])]
  if (length(new.pkg))
    install.packages(new.pkg, dependencies = TRUE)
  sapply(pkg, require, character.only = TRUE)}

packages <- c("ggplot2", "dplyr","egg","INLA", "lattice")
ipak(packages)

setwd("~/...")
```

Import data & define covariates

```
GEXP_df <- read.csv("GEXP_Controls.csv")
GEXP_df <- GEXP_df %>%
           mutate(Gloch_loss = (Gloch_C+Gloch_O)) %>%
           mutate(Day2 = Day) %>%
           mutate(Tank2 = as.factor(Tank))


# Factors
GEXP_df$Tank          <- as.factor(GEXP_df$Tank)
GEXP_df$Day           <- as.factor(GEXP_df$Day)

# Standardise Continuous Covariates
GEXP_df$Temp          <- scale(GEXP_df$Temp)
GEXP_df$Surface_Area  <- scale(GEXP_df$Surface_Area)
GEXP_df$Length        <- scale(GEXP_df$Length)
GEXP_df$Weight_total  <- scale(GEXP_df$Weight_total)
GEXP_df$Surface_Area_Fins <- scale(GEXP_df$Surface_Area_Fins)
GEXP_df$Fin_Edge <- scale(GEXP_df$Fin_Edge)
```

R-INLA model: Glochidial loss and Juvenile excystment

```
# Random intercept:              f(Tank,model="iid")

# Random slope:          f(Tank2,Day2,model="iid")

# Autocorrelation structure:     f(Day, model ="ar1")

# Poisson likelihood distribution   family = "poisson"
```

```
#### Glochidial loss ####


formula.GL  <- GEXP_df$Gloch_loss ~ 1 + Temp + Surface_Area_Fins +
                                       f(Tank,model="iid") +
                                       f(Tank2,Day2,model="iid") +
                                       f(Day, model ="ar1")
model.GL    <- inla(formula.GL,
                    family = "poisson",
                    data=GEXP_df,
                    control.predictor=list(compute=TRUE),
                    control.compute = list(dic = TRUE))
summary(model.GL)
## Call:
## c("inla(formula = formula.GL, family = \"poisson\", data = GEXP_df, ",  "    contro
l.compute = list(dic = TRUE), control.predictor = list(compute = TRUE))" )
##
## Time used:
##  Pre-processing    Running inla Post-processing           Total
##          2.3326          0.6940          0.3239          3.3505
##
## Fixed effects:
##                       mean     sd 0.025quant 0.5quant 0.975quant   mode kld
## (Intercept)       3.4356 0.3066     2.7890   3.4456     4.0213 3.4609   0
## Temp              0.2266 0.0500     0.1282   0.2267     0.3246 0.2268   0
## Surface_Area_Fins 0.4745 0.2354     0.0103   0.4721     0.9525 0.4675   0
##
## Random effects:
## Name   Model
##  Tank   IID model
## Tank2   IID model
## Day   AR1 model
##
## Model hyperparameters:
##                          mean      sd 0.025quant 0.5quant 0.975quant    mode
## Precision for Tank   2.1239  1.0565     0.7030   1.9205     4.7520  1.5348
## Precision for Tank2  9.1584  3.8324     3.6022   8.5398    18.4194  7.3168
## Precision for Day   36.2991 29.8995     5.9080  28.2128   114.6507 15.6192
## Rho for Day          0.5234  0.2747    -0.1163   0.5693     0.9137  0.7194
##
## Expected number of effective parameters(std dev): 27.84(1.922)
## Number of equivalent replicates : 8.083
##
## Deviance Information Criterion (DIC) ...: 910.78
## Effective number of parameters .........: 28.78
##
## Marginal log-Likelihood:  -527.22
## Posterior marginals for linear predictor and fitted values compute
```

```
#### Juvenile Excystement ####


formula.JE  <- GEXP_df$Gloch_A ~ 1 + Temp + Surface_Area +
                                    f(Tank, model="iid") +
                                    f(Tank2,Day2, model="iid") +
                                    f(Day, model ="ar1")
model.JE    <- inla(formula.JE,
                    family = "poisson",
                    data=GEXP_df,
                    control.predictor=list(compute=TRUE),
                    control.compute = list(dic = TRUE))
```

```
summary(model.JE)
## Call:
## c("inla(formula = formula.JE, family = \"poisson\", data = GEXP_df, ",  "
control.compute = list(dic = TRUE), control.predictor = list(compute = TRUE))" )
##
## Time used:
##  Pre-processing    Running inla Post-processing           Total
##          1.6951          1.4740          0.9889          4.1579
##
## Fixed effects:
##                  mean     sd 0.025quant 0.5quant 0.975quant    mode kld
## (Intercept)  -2.0289 2.0482    -6.8094  -1.8611     1.6615 -1.6551   0
## Temp          0.4723 0.1366     0.2071   0.4712     0.7433  0.4691   0
## Surface_Area  0.4361 0.2155     0.0321   0.4289     0.8798  0.4138   0
##
## Random effects:
## Name   Model
##  Tank   IID model
## Tank2   IID model
## Day   AR1 model
##
## Model hyperparameters:
##                           mean        sd 0.025quant  0.5quant 0.975quant
## Precision for Tank  1.847e+04 1.805e+04  1226.4596 1.315e+04  6.664e+04
## Precision for Tank2 2.531e+02 1.234e+02    84.9905 2.301e+02  5.590e+02
## Precision for Day   2.228e-01 1.486e-01     0.0390 1.904e-01  5.944e-01
## Rho for Day         9.132e-01 5.250e-02     0.7833 9.234e-01  9.825e-01
##                           mode
## Precision for Tank  3329.0270
## Precision for Tank2  185.4956
## Precision for Day      0.1117
## Rho for Day            0.9501
##
## Expected number of effective parameters(std dev): 25.09(0.6248)
## Number of equivalent replicates : 8.968
##
## Deviance Information Criterion (DIC) ...: 847.87
## Effective number of parameters .........: 25.24
##
## Marginal log-Likelihood:  -479.17
## Posterior marginals for linear predictor and fitted
```

7.3 Invasive macrophytes induce context-specific effects on oxygen, pH, and temperature in a hydropeaking reservoir (Chapter 4)

7.3.1 Aquatic vegetation mapping

Boat-based sounder and transducer set-up.

Sounder: HDS Carbon 9

Transducer: TotalScan Skimmer Med/High 455/800 transducer

Transducer depth: 50 cm below boat

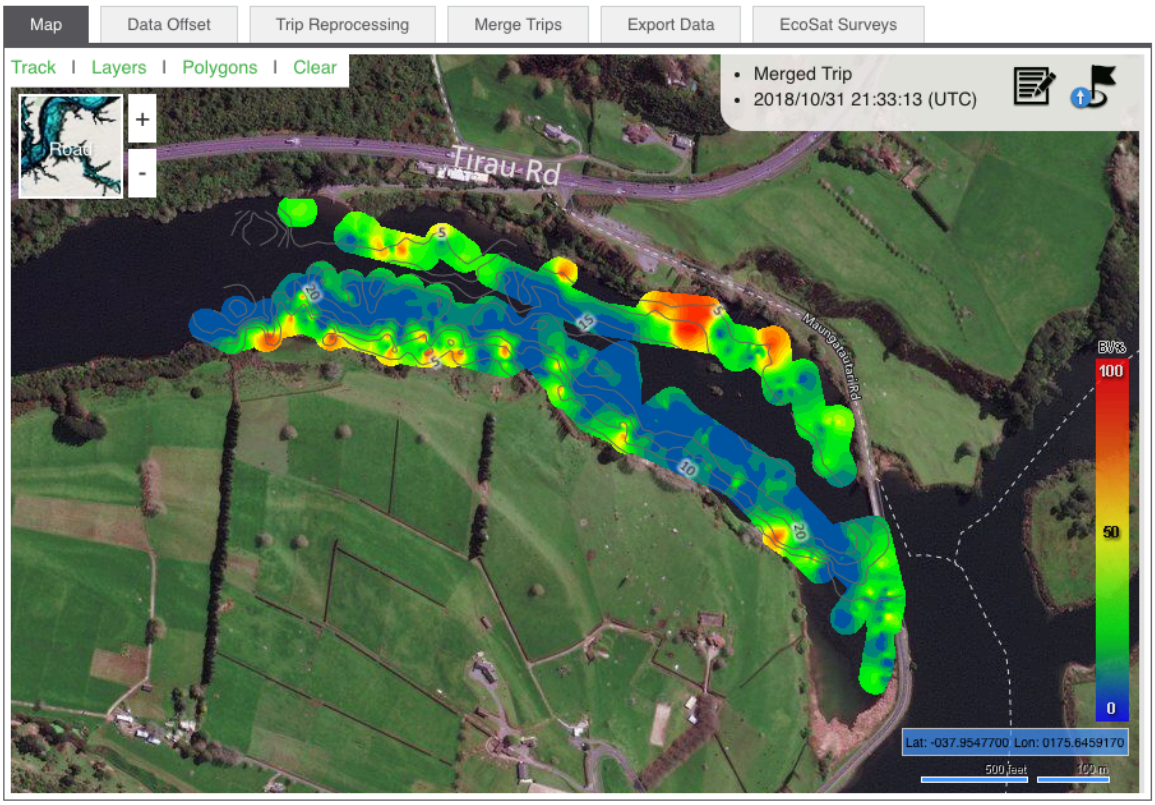
Range: Auto

Frequency: 200 kHz and 800 kHz

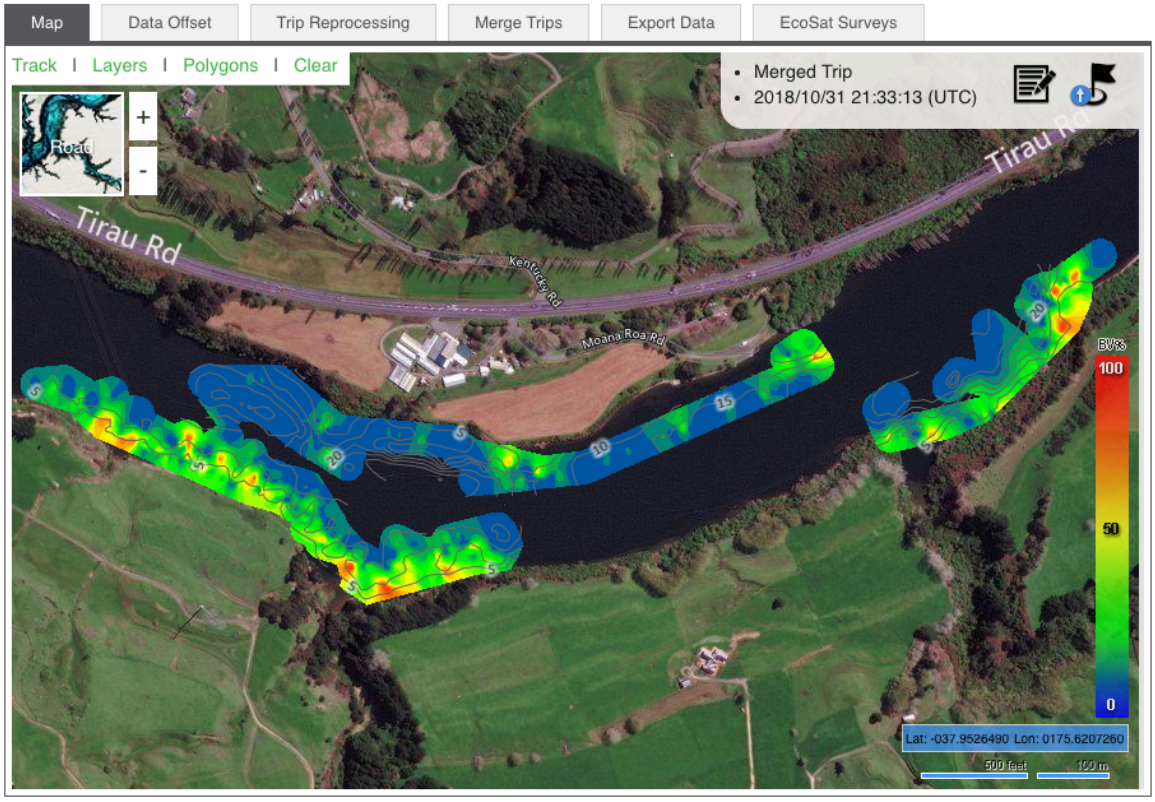
Ping: 15

Analysis: BioBase Ecosound – cloud-based automated data processing tool.

a)



b)


Map
Data Offset
Trip Reprocessing
Merge Trips
Export Data
EcoSat Surveys
Track I Layers I Polygons I Clear
Merged Trip
2018/10/31 21:33:13 (UTC)
Tirau Rd
Kentucky Rd
Moana Roa Rd
BVR
100
50
0
Lat: -037.9526490 Lon: 0175.6207260
500 feet
100 m

c)


Map
Data Offset
Trip Reprocessing
Merge Trips
Export Data
EcoSat Surveys
Track I Layers I Polygons I Clear
Merged Trip
2018/10/31 21:33:13 (UTC)
Tirau Rd
Tirau Rd
BVR
100
50
0
Lat: -037.9433970 Lon: 0175.5796760
250 feet
50 m

d)

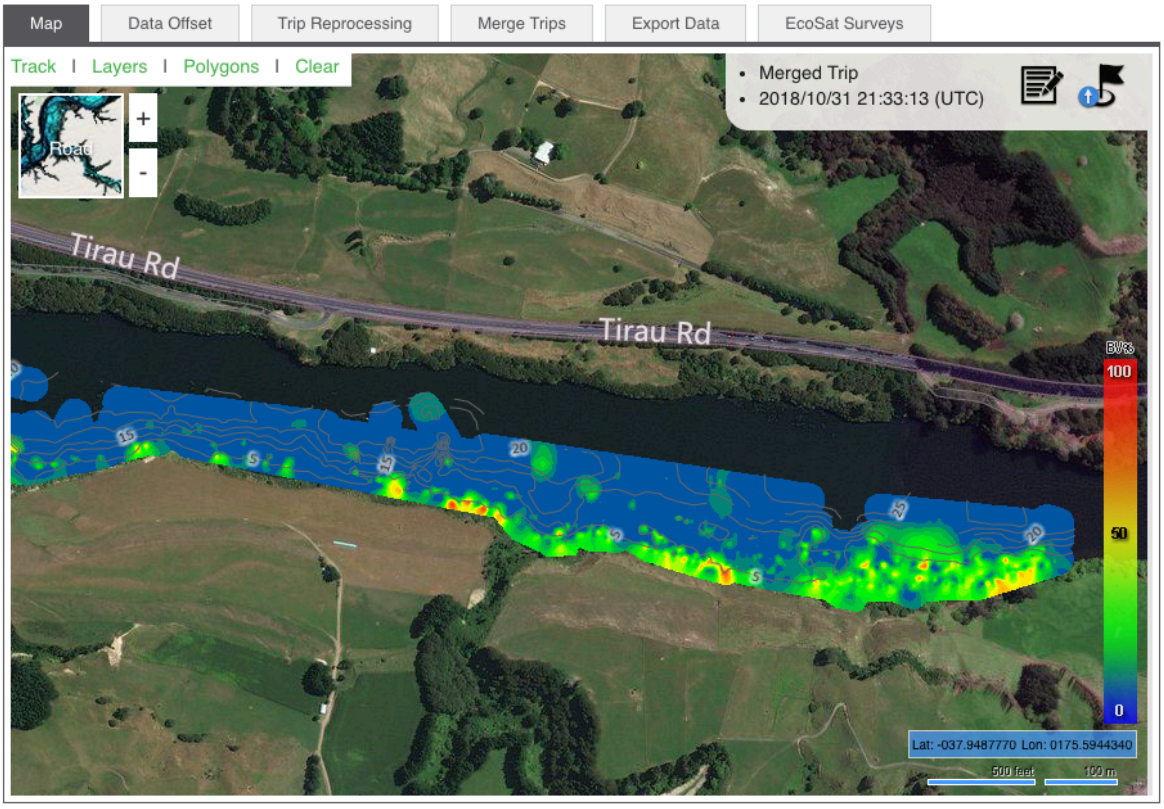
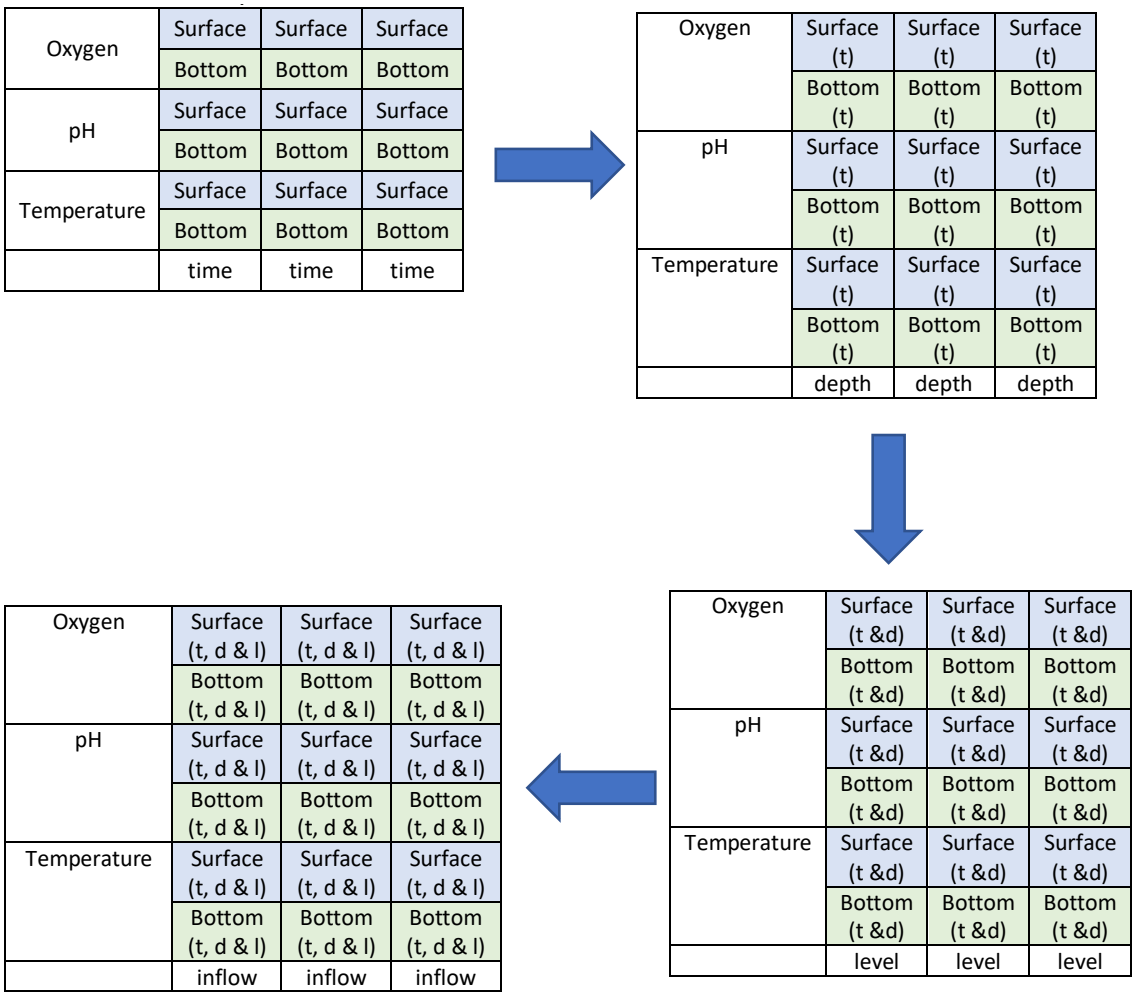


Figure 7-2: Aquatic vegetation maps in the lower-lacustrine hornwort section of Lake Karāpiro displaying percentage biomass volume: a) Bob's Landing North; b) Moana Roa Reserve; c) Keeley's Landing; and d) Keeley's Landing East. All were used to collect water quality measurements except Keeley's landing East.

7.3.2 **Detrending flow-diagram for isolating macrophyte effects**



Detrended data

Oxygen	Surface (t, d, l, & i)	Surface (t, d, l, & i)	Surface (t, d, l, & i)
	Bottom (t, d, l, & i)	Bottom (t, d, l, & i)	Bottom (t, d, l, & i)
pH	Surface (t, d, l, & i)	Surface (t, d, l, & i)	Surface (t, d, l, & i)
	Bottom (t, d, l, & i)	Bottom (t, d, l, & i)	Bottom (t, d, l, & i)
Temperature	Surface (t, d, l, & i)	Surface (t, d, l, & i)	Surface (t, d, l, & i)
	Bottom (t, d, l, & i)	Bottom (t, d, l, & i)	Bottom (t, d, l, & i)
	Hornwort November	Hornwort January	Egeria January

Figure 7-3: Detrending flow-diagram of how covaraites were progressitvely detrended by time (t), depth (d), level (l) and inflow (i) for each of the 18 plots presented in Figure 4-6.

7.3.3 **Detrending time example**

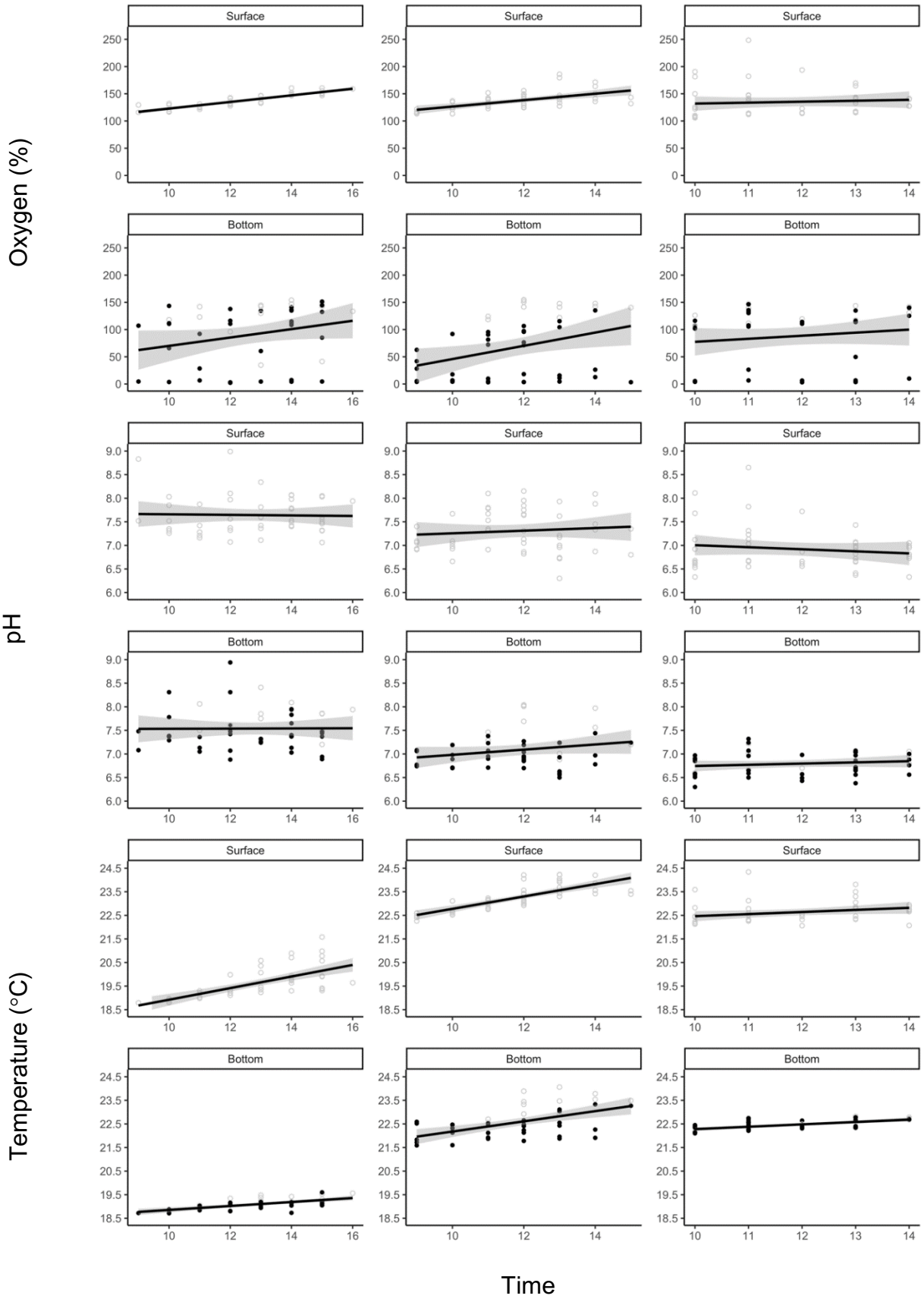


Figure 7-4: Relationships between raw oxygen, pH, and temperature and time prior to detrending at the surface and bottom of vertical profiles. Linear regression model fit indicated with 95 % confidence interval as a grey smooth.

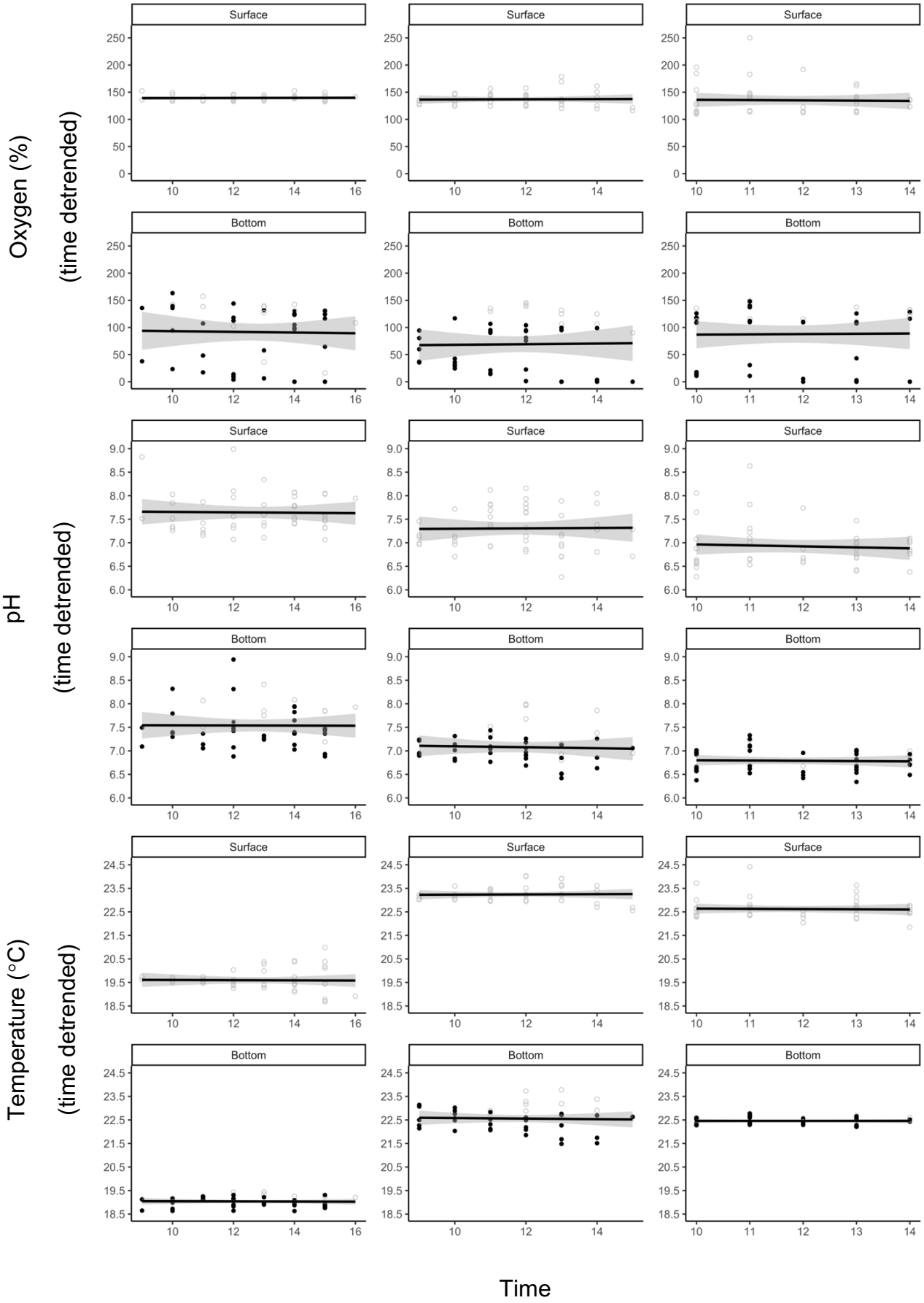


Figure 7-5: Relationships between time detrended oxygen, pH, and temperature and time at the surface and bottom of vertical profiles. Linear regression model fit indicated with 95 % confidence interval as a grey smooth.

7.3.4 **Vertical profiles of measured pH and temperature**

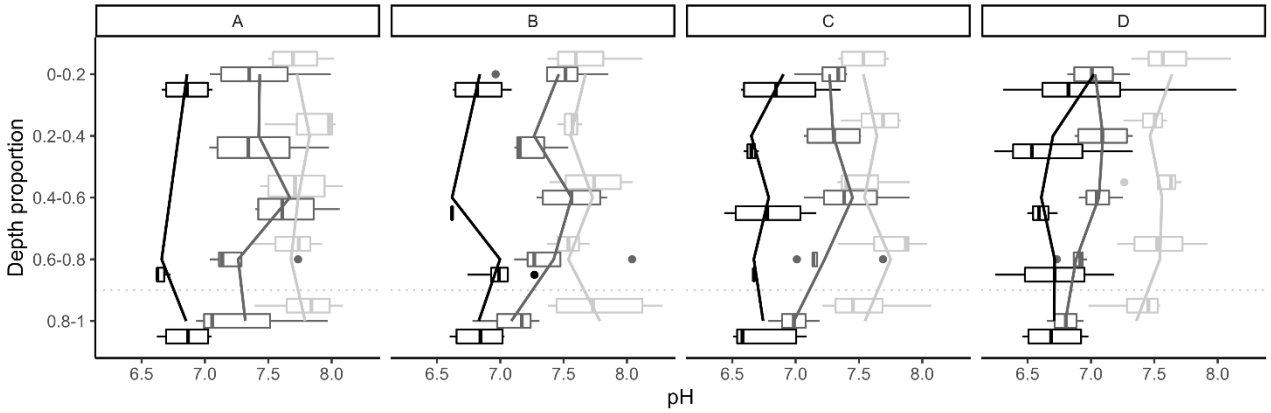


Figure 7-6: Vertical profiles of measured pH values across vertical profiles for *Ceratophyllum demersum* in November (light grey long-dash), *C. demersum* in January (dark grey short-dash), and *Egeria densa* in January (black solid) with coloured solid lines linking mean values. A = macrophyte-free; B = light macrophyte; C = dense-edge; and D = dense-bed (see Figure 4-2). Depth proportion was split into five groups representing 20% intervals. Boxplots show median (black line inside boxplot); interquartile range (box); min/max (whiskers); and outliers (>1.5 Å~interquartile range, black dots). Dotted grey line indicates boundary where oxygen depletion occurred.

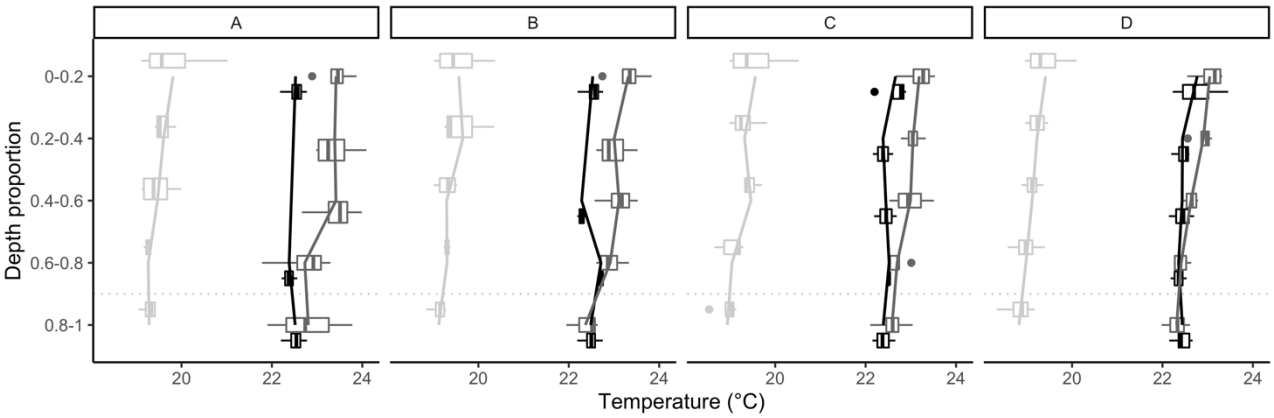


Figure 7-7: Vertical profiles of measured temperature values across vertical profiles for *Ceratophyllum demersum* in November (light grey long-dash), *C. demersum* in January (dark grey short-dash), and *Egeria densa* in January (black solid) with coloured solid lines linking mean values. A = macrophyte-free; B = light macrophyte; C = dense-edge; and D = dense-bed (see Figure 4-2). Depth proportion was split into five groups representing 20% intervals. Boxplots show median (black line inside boxplot); interquartile range (box); min/max (whiskers); and outliers (>1.5 Å~interquartile range, black dots). Dotted grey line indicates boundary where oxygen depletion occurred.

7.3.5 Regression model coefficients

Table 7-5: Linear model regression coefficients of relationships between measured oxygen, pH, and temperature with measurement time, depth, water inflow and water level for *C. demersum* (November 2018 and January 2019) and *E. densa* (January 2019) at the water surface or lake bottom.

			Oxygen (%)				pH				Temperature (°C)			
Sampling Occasion	Depth		time	depth	inflow	level	time	depth	inflow	level	time	depth	inflow	level
C. demersum November 2018	Surface	*p*	**<0.001**		0.093	**0.05**	0.965		0.071	0.706	**<0.001**		0.222	**0.004**
		a	**57.283**	NA	166.472	**-684.112**	7.662	NA	10	20.674	**16.116**	NA	21.39	**-89.249**
		b	**0.104**		-0.095	**15.654**	<0.001		-0.008	-0.248	**0.004**		-0.006	**2.069**
	Bottom	*p*	**0.005**	**0.022**	0.971	0.109	0.89	**0.0216**	**0.015**	0.178	**<0.001**	**<0.001**	0.867	0.695
		a	**-91.905**	**148.224**	106.863	-7097.729	7.7	**8.069**	**10.828**	-45.826	**17.088**	**19.287**	18.923	25.46
		b	**0.248**	**-28.141**	-0.018	136.808	<0.001	**-0.275**	**-0.011**	1.015	**0.002**	**-0.168**	<0.001	-0.123
C. demersum January 2019	Surface	*p*	**<0.001**		0.304	0.927	0.614		0.468	0.705	**<0.001**		0.074	0.166
		a	**63.767**	NA	179.222	57.307	7.033	NA	8.296	18.217	**19.936**	NA	21.391	-5.915
		b	**0.099**		-0.186	1.504	<0.001		-0.004	-0.207	**0.004**		0.008	0.553
	Bottom	*p*	**0.021**	0.509	0.187	0.622	0.218	0.093	0.795	0.598	**0.003**	0.79	0.49	0.144
		a	**-107.48**	30.671	-178.834	2007.135	6.391	7.135	6.635	21.363	**20.266**	22.336	21.124	85.497
		b	**0.204**	7.242	0.978	-37.238	<0.001	-0.125	0.001	-0.274	**0.003**	0.031	0.006	-1.196
E. densa January 2019	Surface	*p*	0.409		**<0.001**	0.939	0.627		**<0.001**	0.954	**0.032**		**0.002**	0.966
		a	105.993	NA	**12.196**	225.701	7.197	NA	**4.93**	8.124	**21.374**	NA	**21.381**	21.598
		b	0.039		**0.444**	-1.72	<0.001		**0.007**	-0.023	**0.002**		**0.004**	0.019
	Bottom	*p*	0.329	**<0.001**	**0.021**	0.994	0.187	**0.004**	**<0.001**	0.905	**<0.001**	**<0.001**	**<0.001**	0.901
		a	21.037	**171.962**	**174.313**	69.92	6.389	**7.025**	**5.768**	7.828	**21.181**	**22.613**	**21.749**	23.149
		b	0.09	**-82.861**	**-0.314**	0.331	<0.001	**-0.23**	**0.004**	-0.02	**0.002**	**-0.152**	**0.003**	-0.013

p = *p* value; *a* = intercept; *b* = slope; NA = model unable to run (i.e., no change in depth at surface); bold = statistical significance at $p < 0.05$

Table 7-6: Quantile regression coefficients of relationships between measured oxygen, pH, and temperature with macrophyte as a proportion of the water column at the 10th, 50th, and 90th percentiles for *C. demersum* (November 2018 and January 2019) and *E. densa* (January 2019) at the water surface or lake bottom.

			Oxygen (%)			pH			Temperature (°C)		
Sampling Occasion	Depth		10th	50th	90th	10th	50th	90th	10th	50th	90th
C. demersum November 2018	Surface	*p*	0.772	0.382	0.533	0.327	0.507	0.952	0.555	0.351	0.324
		a	133.96	139.32	144.83	7.29	7.63	8.11	18.89	19.51	20.14
		b	1.11	-3.06	7.03	-0.32	-0.25	0.04	0.55	0.39	-0.41
	Bottom	*p*	0.97	**0.042**	0.476	0.774	0.401	0.76	**0.044**	0.821	0.055
		a	17.97	**112.34**	145.15	7.11	7.61	8.07	**18.93**	19.07	19.32
		b	6	**-101.82**	16.79	0.13	-0.29	-0.13	**-0.58**	-0.05	-0.31
C. demersum January 2019	Surface	*p*	0.942	0.741	**0.047**	0.224	0.43	0.824	**0.026**	0.355	0.514
		a	120.75	134.52	**146.28**	7.03	7.39	7.9	**22.65**	23.26	23.77
		b	-0.39	2.29	**17.36**	-0.41	-0.29	-0.05	**0.45**	-0.15	-0.131
	Bottom	*p*	0.269	**<0.001**	0.381	**0.039**	**0.004**	**0.014**	0.77	**<0.001**	**<0.001**
		a	24.36	**107.91**	120.97	**6.85**	**7.31**	**7.81**	21.90	**22.95**	**23.51**
		b	-24.36	**-82.22**	-24.8	**-0.30**	**-0.5**	**-0.553**	-0.135	**-0.72**	**-1.06**
E. densa January 2019	Surface	*p*	0.898	**0.039**	**0.014**	0.125	0.619	**0.043**	0.385	**0.027**	**0.01**
		a	115.88	**127.86**	**134.94**	6.71	6.92	**7.01**	22.40	**22.48**	**22.12**
		b	0.8	**8.48**	**37.821**	-0.27	-0.06	**0.49**	-0.16	**0.12**	**0.91**
	Bottom	*p*	**<0.001**	0.148	0.409	0.638	**0.049**	0.108	0.747	**0.029**	0.481
		a	**66.64**	100.42	127.90	6.70	**6.74**	6.88	22.31	**22.42**	22.60
		b	**-55.30**	-29.24	15.92	-0.056	**0.08**	0.16	0.016	**0.08**	-0.04

p = *p* value; *a* = intercept; *b* = slope

7.4 Hydrology-mediated impacts of invasive macrophytes on freshwater mussels (*Echyridella menziesii*: Unionida) in a New Zealand hydropeaking reservoir (Chapter 5)

7.4.1 Mussel length, width and fresh weight relationships

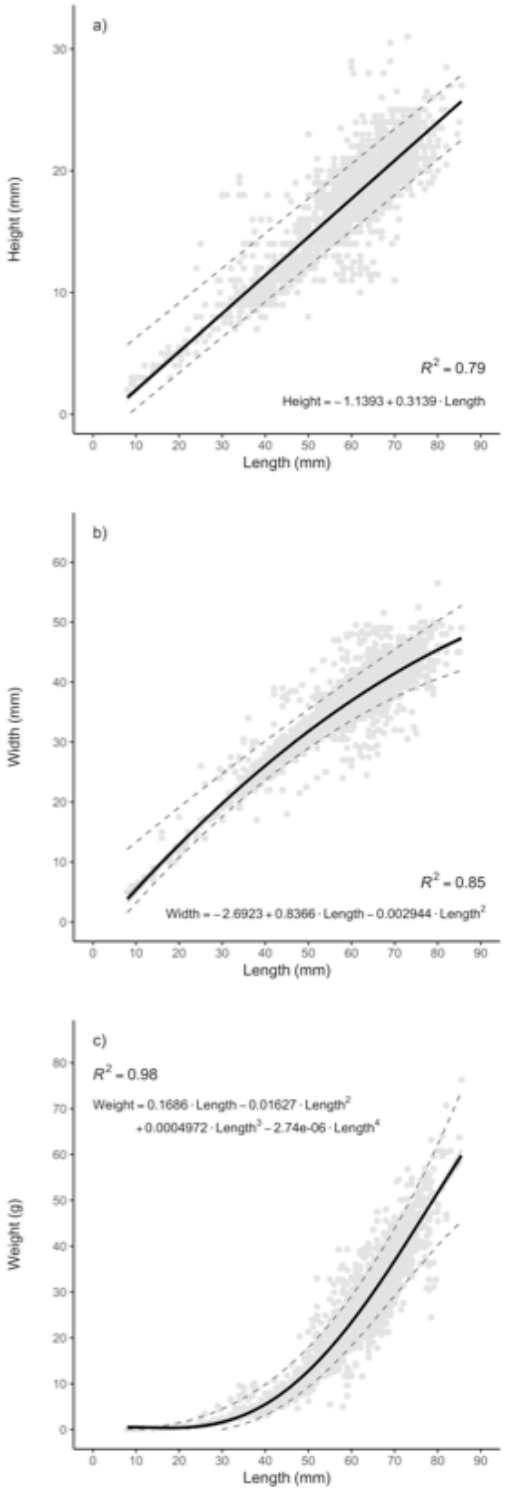


Figure 7-8: Relationships between mussel length, height, width, and fresh weight with goodness-of-fit statistics and line-fit equation displayed. Solid black line is predicted linear or polynomial model fit. Dotted grey line indicate 5th and 95th percentiles and grey smooth shows 95 % confidence interval.

7.4.2 **Mussel biomass principal component analysis**

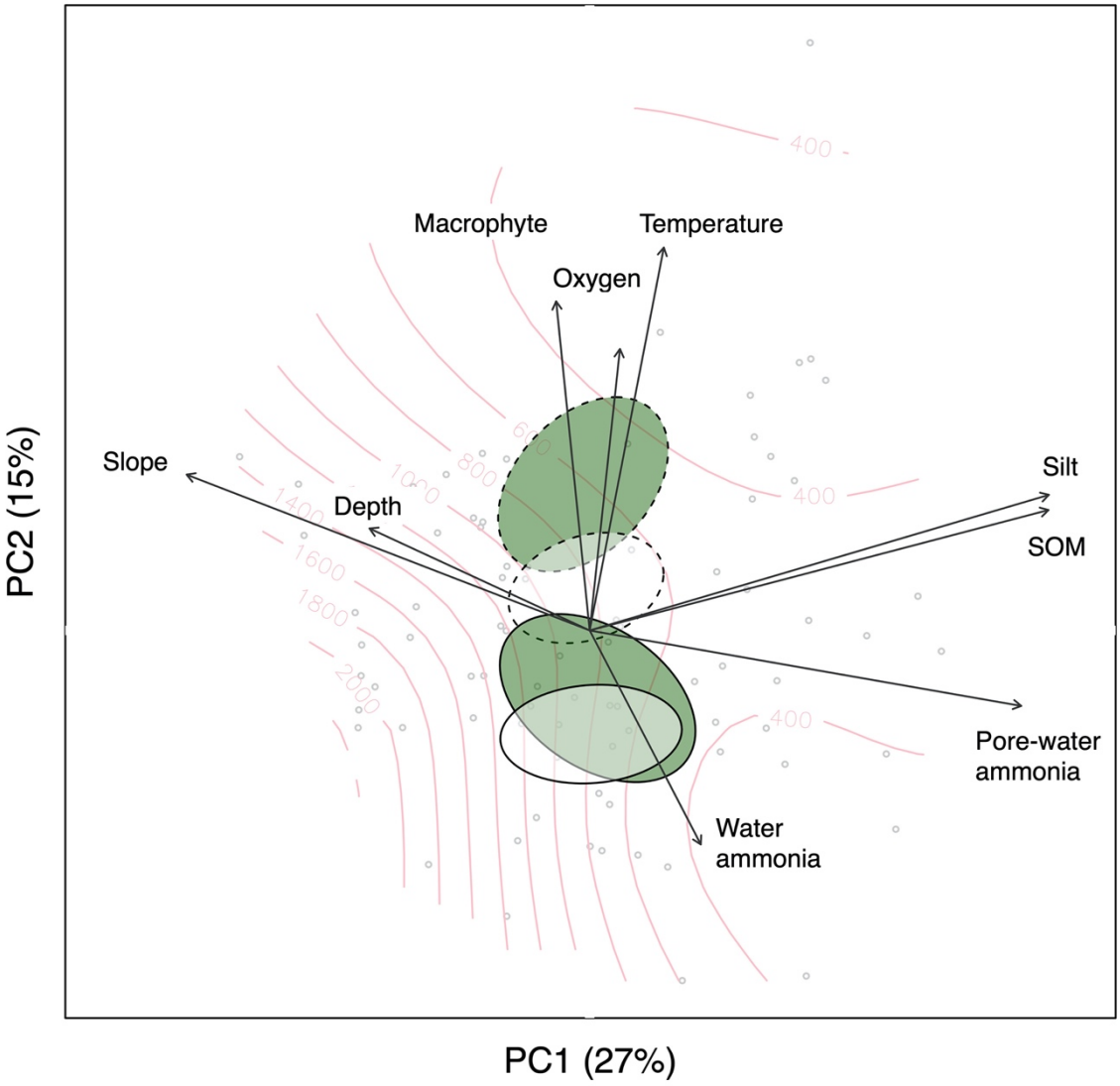


Figure 7-9: Principal component plot of axes 1 and 2 in relation to measured environmental variables with vectors significant at $P < 0.001$ and mussel biomass contours (200 g m^{-2}) fitted with a generalized additive model (Deviance explained = 29 %). Ellipses envelope sampling quadrats for *C. demersum* at the lower-lacustrine sites (solid outline) and *E. densa* at the upper-riverine sites (dashed outline) with (dark green fill) and without (white) fill macrophyte. SOM = sediment organic matter.

7.4.3 **Summary table of environmental parameters**

Table 7-7: Summary statistics of environmental parameters (site, physicochemical, sediment) and mussel population characteristics for each site, outside and inside dense macrophyte beds for each site. Values are means and standard deviations (SD) except for depth where the minimum (min) and maximum (max) are shown: a single number is presented if min and max are equivalent. The lower-lacustrine section contains *Ceratophyllum demersum* (Sites 1-4) and upper-riverine section *Egeria densa* (Sites 5-8) (see Table 1). Sites 1-KL, 2-MM, and 4-BL were sprayed with herbicide prior to sampling.

Location and dominant macrophyte		Lower- lacustrine section – *C. demersum*								Upper-riverine Egeria							
Site		1 – KL*		2 – MM*		3 – BL*		4 - HH		5 - LW		6 - OR		7 - PI		8 - CT	
Treatment		Outside	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside	Inside
Site characteristics																	
Macrophyte fresh-weight (g m^{-2})	$\bar{x}$ ± SD	61 ± 86	125 ± 75	N.D	N.D	N.D	N.D	13 ± 12	1445 ± 1275	221 ± 256	1315 ± 606	43 ± 50	2412 ± 2610	371 ± 447	2329 ± 2830	184 ± 160	1713 ± 2275
Depth (m)	min-max	1.2	1.2	1.2	1.2	1.2	1.2	2.1-2.4	3.5-3.8	0.5-0.9	1.2-1.5	0.7-0.9	1.4-1.6	1.2	1.8	1.1	1.2
Bed slope angle (°)	$\bar{x}$ ± SD	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	16 ± 2.24	21.2 ± 3.83	19.6 ± 7.23	20 ± 9.75	11.8 ± 3.90	12.8 ± 4.55	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Physicochemical characteristics																	
Oxygen saturation (%)	$\bar{x}$ ± SD	103.2 ± 1.2	100.9 ± 2.4	98 ± 4.4	98.7 ± 6.7	98.1 ± 8.9	98.4 ± 4.2	92.7 ± 2.2	95.1 ± 0.6	100.4 ± 0.4	81.3 ± 40	99.4 ± 3.1	98.8 ± 2.2	100.0 ± 4.0	101.9 ± 3.3	20.0 ± 1.6	19.8 ± 1.3
pH	$\bar{x}$ ± SD	8.3 ± 0.1	8.3 ± 0.0	8.1 ± 0.1	8.3 ± 0.1	7.5 ± 0.2	7.6 ± 0.2	8.3 ± 0.2	8.4 ± 0.2	8.7 ± 0.2	8.7 ± 0.1	8.4 ± 0.2	8.3 ± 0.1	8.7 ± 0.1	8.7 ± 0.1	8.2 ± 0.1	8.1 ± 0.2
Temperature (°C)	$\bar{x}$ ± SD	21.3 ± 0.5	21.6 ± 0.7	22.0 ± 0.5	22.3 ± 0.5	22.3 ± 0.7	22.5 ± 0.7	20.1 ± 0.3	20.2 ± 0.3	21.6 ± 0.2	21.7 ± 0.3	22.2 ± 0.3	22.6 ± 0.7	20.6 ± 0.4	20.9 ± 0.3	20.0 ± 0.4	19.8 ± 0.3
Water ammonia (mg L^{-1})	$\bar{x}$ ± SD	1.0 ± 0.5	2.8 ± 2.4	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.1	0.2 ± 0.3	0.1 ± 0.0	0.2 ± 0.3	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.8 ± 1.6	0.1 ± 0.0	0.3 ± 0.3	0.3 ± 0.5
Sediment characteristics																	
Silt (%)	$\bar{x}$ ± SD	25.1 ± 8.2	28.5 ± 4.4	60.6 ± 19.7	48.3 ± 22.1	22.0 ± 28.5	27.8 ± 24.6	10.8 ± 5.0	16.8 ± 10.8	19.3 ± 7.9	30.4 ± 17.3	19.9 ± 9.8	16.8 ± 7.5	48.9 ± 36.1	65.1 ± 12.4	39.2 ± 19.7	39.2 ± 16.1
Sediment organic matter (%)	$\bar{x}$ ± SD	1.3 ± 1.0	4.2 ± 3.0	3.1 ± 1.4	3.0 ± 1.7	1.8 ± 2.2	3.6 ± N/A	0.2 ± 0.2	0.2 ± 0.1	0.5 ± 0.2	0.9 ± 0.9	0.1 ± 0	0.2 ± 0	1.8 ± 1.0	2.2 ± 0.4	1.0 ± 0.2	0.9 ± 0.6
Pore-water ammonia (mg L^{-1})	$\bar{x}$ ± SD	3.3 ± 1.1	3.7 ± 1.3	6.7 ± 2.2	6.6 ± 3.9	3.1 ± 3.4	2.3 ± 1.1	1.7 ± 0.6	2.3 ± 1.1	2.6 ± 0.5	2.8 ± 1.4	2.9 ± 0.3	2.8 ± 1.3	6.4 ± 4.4	8.5 ± 0.8	5.7 ± 1.7	4.9 ± 2.5
Mussel population characteristics																	
Total density (# m^{-2})	$\bar{x}$ ± SD	17.8 ± 17.6	35.8 ± 32.1	7.8 ±6.7	29.0 ± 28.9	22.6 ± 15.6	17.4 ± 13.9	140.4 ± 79.9	24.6 ± 19.9	32.2 ± 13.7	41.6 ± 18.9	24.6 ± 15.3	74.2 ± 34.2	5.2 ± 6.3	7 ± 8.7	40.4 ± 38.3	69.2 ± 33.6
Density < 40 mm (# m^{-2})	$\bar{x}$ ± SD	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.4	0 ± 0	0.8 ± 1.3	1.6 ± 1.1	1.2 ± 1.9	1.2 ± 1.6	4.4 ± 2.7	2.2 ± 1.3	4.2 ± 5.1	0.6 ± 1.3	1.0 ± 1.7	13.4 ± 15.4	18.8 ± 12.9
Biomass (g m^{-2})	$\bar{x}$ ± SD	596.2 ± 568	1145.8 ± 968.5	222.5 ± 198.7	839.5 ± 881.8	663.5 ± 461.8	480.0 ± 400.7	4354 ± 2400.4	672.2 ± 495.7	763.9 ± 337.1	880.8 ± 402.8	533.7 ± 374.9	1642.7 ± 949.9	66.2 ± 71.4	112.6 ± 106.9	502.1 ± 466.4	969.6 ± 392.9

O is outside the macrophyte bed; I is inside the macrophyte bed; $\bar{x}$ is the mean; SD is standard deviation; min = minimum; max = maximum; *hornwort sites 1-3 were sprayed with herbicide before sampling; N.D is no data.

7.4.4 **Model selection for mussel relationships with physicochemical parameters**

S2 – GLM model selection and coefficient tables

GLM: Depth

```
Model selection based on AICc:
               K   AICc Delta_AICc AICcWt Cum.Wt      LL
Second-order* 11 755.03       0.00   0.41   0.41 -364.68
Second-order   7 755.12       0.09   0.39   0.80 -369.82
Linear*       10 758.85       3.83   0.06   0.86 -367.92
SQRT*         10 758.96       3.93   0.06   0.92 -367.97
IHS*          10 758.99       3.97   0.06   0.98 -367.99
Intercept*     3 762.24       7.22   0.01   0.99 -377.97
Linear         6 764.45       9.43   0.00   0.99 -375.68
Intercept      5 764.48       9.45   0.00   1.00 -376.86
SQRT           6 766.43      11.40   0.00   1.00 -376.67
IHS            6 766.76      11.74   0.00   1.00 -376.84
```

```
Models:
Second-order*: Density ~ response + I(response^2) + USDS + Treatment + (1 | Site)
 Second-order: Density ~ response + I(response^2) * USDS * Treatment + (1 | Site)

             npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
Second-order    7 753.64 770.66 -369.82   739.64
Second-order*: 11 751.36 778.10 -364.68   729.36 10.282  4    0.03594 *
---
```

```
Fixed effects: Second-order*
                                                Estimate Std. Error z value Pr(>|z|)
(Intercept)                                      -0.1863     1.5655  -0.119  0.90526
response                                          3.8363     1.5776   2.432  0.01502 *
I(response^2)                                    -0.7684     0.2966  -2.591  0.00958 **
USDSUpper-riverine                                0.9165     0.7089   1.293  0.19607
TreatmentNone                                    -0.9240     0.4370  -2.114  0.03448 *
I(response^2):USDSUpper-riverine                 -0.4491     0.2551  -1.761  0.07828 .
I(response^2):TreatmentNone                       0.2240     0.1740   1.287  0.19804
USDSUpper-riverine:TreatmentNone                  1.2670     0.7697   1.646  0.09973 .
I(response^2):USDSUpper-riverine:TreatmentNone   -0.7585     0.3979  -1.906  0.05663 .

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’
```

GLM: Slope

```
Model selection based on AICc:
              K   AICc Delta_AICc AICcWt Cum.Wt      LL
IHS*         10 758.64       0.00   0.42   0.42 -367.81
SQRT*        10 759.28       0.64   0.31   0.73 -368.13
Linear*      10 761.07       2.43   0.13   0.85 -369.03
Intercept*    3 762.24       3.60   0.07   0.92 -377.97
IHS           6 764.48       5.84   0.02   0.95 -375.69
Intercept     5 764.48       5.84   0.02   0.97 -376.86
SQRT          6 765.16       6.51   0.02   0.99 -376.03
Linear        6 766.38       7.74   0.01   0.99 -376.64
Second-order  7 767.21       8.56   0.01   1.00 -375.87
```

```
Models:
IHS*: Density ~ asinh(response) + USDS + Treatment + (1 | Site)
IHS : Density ~ asinh(response) * USDS * Treatment + (1 | Site)

            npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
IHS            6 763.39 777.97 -375.69   751.39
IHS*          10 755.63 779.94 -367.81   735.63 15.759  4    0.00336 **
```

```
Fixed effects: IHS*
                                                 Estimate Std. Error z value Pr(>|z|)
(Intercept)                                       3.35896    0.34403   9.763  < 2e-16 ***
asinh(response)                                  -0.06227    0.18765  -0.332 0.739994
USDSUpper-riverine                               -0.10157    0.54699  -0.186 0.852687
TreatmentNone                                    -0.60995    0.30122  -2.025 0.042877 *
asinh(response):USDSUpper-riverine                0.25272    0.24843   1.017 0.309027
asinh(response):TreatmentNone                     0.68338    0.17122   3.991 6.57e-05 ***
USDSUpper-riverine:TreatmentNone                  0.23370    0.49698   0.470 0.638179
asinh(response):USDSUpper-riverine:TreatmentNone -0.78168    0.23396  -3.341 0.000834 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

GLM: Macrophyte biomass (Upper-riverine section only)

```
Model selection based on AICc:
              K   AICc Delta_AICc AICcWt Cum.Wt      LL
SQRT          5 361.02       0.00   0.25   0.25 -174.63
SQRT*         6 361.41       0.38   0.20   0.45 -173.43
IHS           5 362.02       0.99   0.15   0.60 -175.13
IHS*          6 362.90       1.88   0.10   0.69 -174.18
Linear*       6 363.15       2.13   0.08   0.78 -174.30
Intercept     4 363.21       2.19   0.08   0.86 -177.03
Linear        5 363.56       2.53   0.07   0.93 -175.90
Second-order  6 365.27       4.25   0.03   0.96 -175.36
Intercept*    3 365.27       4.25   0.03   0.99 -179.30
Second-order* 7 366.85       5.82   0.01   1.00 -174.67
```

```
Models:
 SQRT: Density ~ sqrt(response) + Treatment + (1 | Site)
SQRT*: Density ~ sqrt(response) * Treatment + (1 | Site)

      npar    AIC   BIC  logLik deviance Chisq Df Pr(>Chisq)
SQRT     5 359.26 367.7 -174.63   349.26
SQRT*    6 358.86 369.0 -173.43   346.86 2.396  1     0.1216
```

```
Fixed effects:
               Estimate Std. Error z value Pr(>|z|)
(Intercept)     2.77401    0.61531   4.508 6.53e-06 ***
sqrt(response)  0.61433    0.28429   2.161   0.0307 *
TreatmentNone  -0.02595    0.33206  -0.078   0.9377

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

GLM: Silt

```
Model selection based on AICc:
            K   AICc Delta_AICc AICcWt Cum.Wt      LL
Linear      6 754.63       0.00   0.53   0.53 -370.77
SQRT        6 755.77       1.14   0.30   0.84 -371.34
IHS         6 758.82       4.19   0.07   0.90 -372.86
Linear*    10 759.37       4.74   0.05   0.95 -368.18
SQRT*      10 760.55       5.92   0.03   0.98 -368.77
Intercept*  3 762.24       7.61   0.01   0.99 -377.97
Intercept   5 764.48       9.85   0.00   1.00 -376.86
IHS*       10 764.51       9.88   0.00   1.00 -370.75
```

```
Models:
Linear:  Density ~ response + Treatment + (1 | Site)
Linear*: Density ~ response * Treatment + (1 | Site)

        npar    AIC   BIC  logLik deviance   Chisq Df Pr(>Chisq)
Linear     6 753.54 768.12 -370.77   741.54
Linear*   10 756.36 780.66 -368.18   736.36 5.1839  4     0.2689
```

```
Fixed effects:
                   Estimate Std. Error z value Pr(>|z|)
(Intercept)        4.187698   0.371445  11.274  < 2e-16 ***
response          -0.021035   0.005783  -3.638 0.000275 ***
USDSUpper-riverine 0.002285   0.434658   0.005 0.995805
TreatmentNone     -0.378637   0.203423  -1.861 0.062698 .

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

GLM: Sediment organic matter

```
Model selection based on AICc:

               K   AICc Delta_AICc AICcWt Cum.Wt      LL
IHS*          10 747.48       0.00   0.48   0.48 -362.23
SQRT*         10 749.16       1.69   0.21   0.69 -363.08
IHS            6 750.20       2.72   0.12   0.82 -368.56
SQRT           6 751.43       3.95   0.07   0.89 -369.17
Linear*       10 751.84       4.36   0.05   0.94 -364.41
Second-order   7 753.05       5.57   0.03   0.97 -368.79
Linear         6 753.44       5.96   0.02   0.99 -370.17
Second-order* 11 756.50       9.03   0.01   1.00 -365.42
Intercept*     3 762.24      14.77   0.00   1.00 -377.97
Intercept      5 764.48      17.00   0.00   1.00 -376.86
```

```
Models:
IHS : Density ~ asinh(response) + USDS + Treatment + (1 | Site)
IHS*: Density ~ asinh(response) * USDS * Treatment + (1 | Site)

     npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
IHS     6 749.11 763.70 -368.56   737.11
IHS*   10 744.46 768.77 -362.23   724.46 12.648  4    0.01313 *
```

```
Fixed effects:
                                                 Estimate Std. Error z value Pr(>|z|)
(Intercept)                                       4.00705    0.62035   6.459 1.05e-10 ***
asinh(response)                                  -0.39486    0.28440  -1.388  0.16502
USDSUpper-riverine                                0.44901    1.06188   0.423  0.67241
TreatmentNone                                     2.09603    0.75122   2.790  0.00527 **
asinh(response):USDSUpper-riverine               -0.02477    0.47110  -0.053  0.95806
asinh(response):TreatmentNone                    -1.22827    0.38390  -3.199  0.00138 **
USDSUpper-riverine:TreatmentNone                 -2.11167    1.34250  -1.573  0.11573
asinh(response):USDSUpper-riverine:TreatmentNone  0.92770    0.65859   1.409  0.15895

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

GLM: Pore-water ammonia

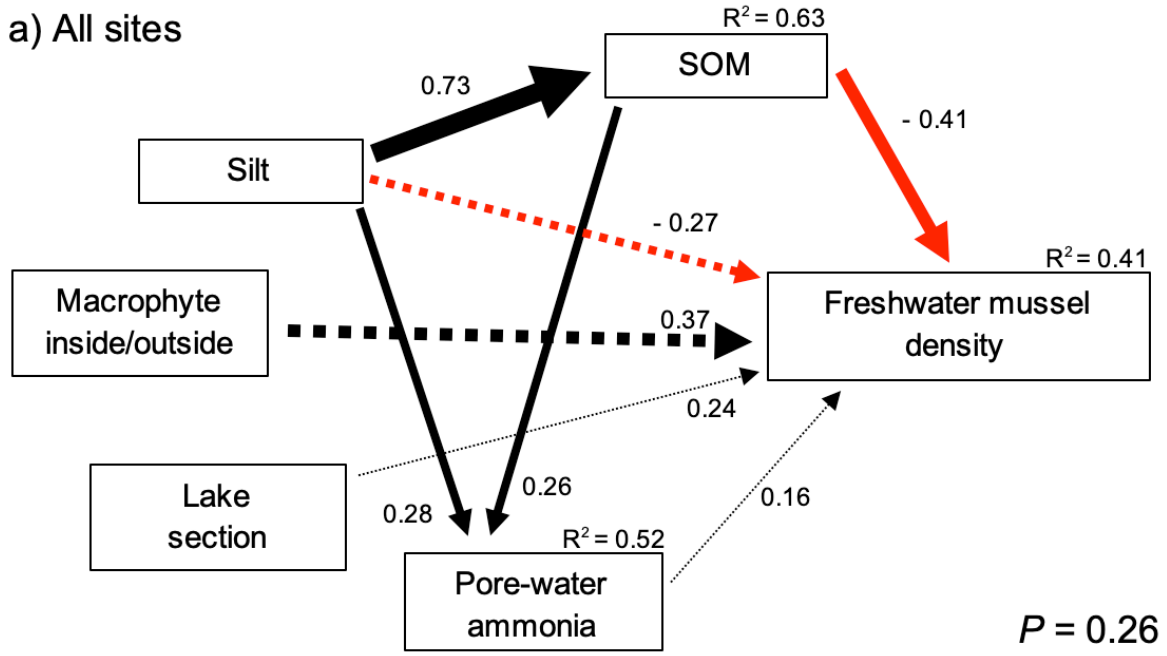
```
Model selection based on AICc:
               K   AICc Delta_AICc AICcWt Cum.Wt      LL
IHS            6 558.19       0.00   0.30   0.30 -272.32
SQRT           6 558.43       0.24   0.26   0.56 -272.44
Linear         6 558.93       0.74   0.20   0.76 -272.69
Second-order   7 560.73       2.55   0.08   0.85 -272.31
IHS*          10 561.15       2.96   0.07   0.91 -268.37
SQRT*         10 561.64       3.45   0.05   0.97 -268.62
Linear*       10 562.62       4.43   0.03   1.00 -269.11
Second-order* 11 568.66      10.48   0.00   1.00 -270.64
Intercept*     3 762.24     204.06   0.00   1.00 -377.97
Intercept      5 764.48     206.29   0.00   1.00 -376.86
```

```
Models:
IHS : Density ~ asinh(response) + USDS + Treatment + (1 | Site)
IHS*.N: Density ~ asinh(response) * USDS * Treatment + (1 | Site)
     npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
IHS     6 556.63 569.30 -272.31   544.63
IHS*   10 556.75 577.86 -268.37   536.75 7.8825  4    0.09598 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
Fixed effects:
                                                Estimate Std. Error z value Pr(>|z|)
(Intercept)                                       2.9984     0.7107   4.219 2.45e-05 ***
asinh(response)                                   0.2811     0.4118   0.683  0.49482
USDSUpper-riverine                                1.2142     0.8572   1.417  0.15662
TreatmentNone                                     1.5071     0.7163   2.104  0.03537 *
asinh(response):USDSUpper-riverine               -1.0557     0.6058  -1.743  0.08138 .
asinh(response):TreatmentNone                    -1.4030     0.4645  -3.021  0.00252 **
USDSUpper-riverine:TreatmentNone                 -2.3754     0.9035  -2.629  0.00856 **
asinh(response):USDSUpper-riverine:TreatmentNone  1.7251     0.7475   2.308  0.02101 *

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

7.4.5 **Structural equation models of freshwater mussel density**



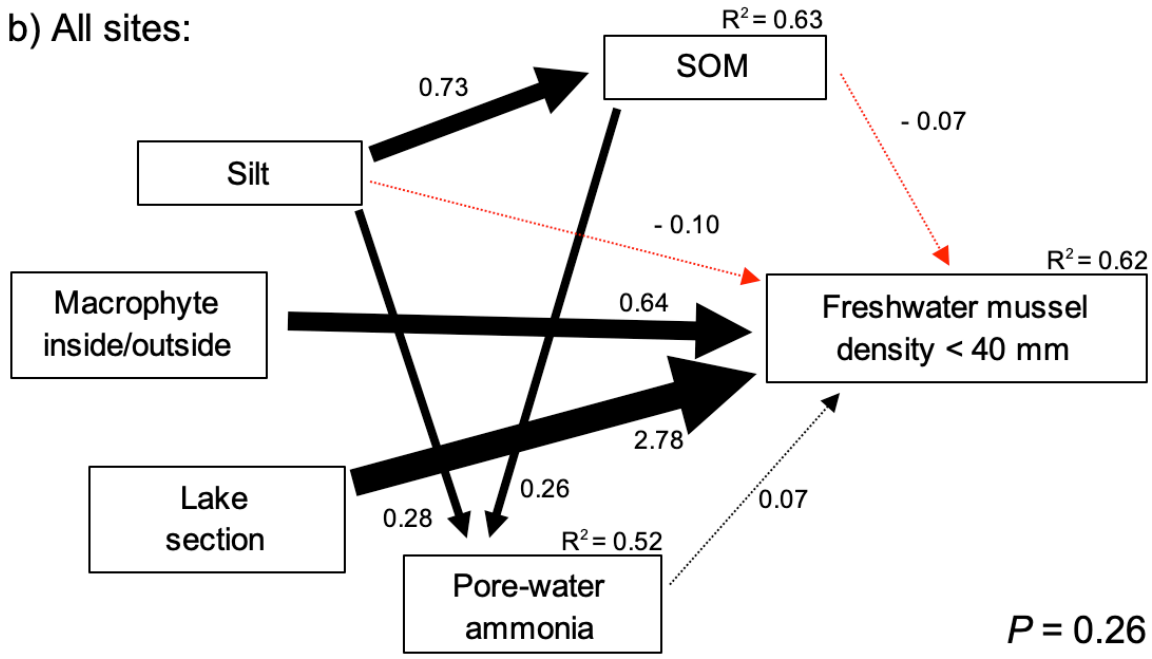
Global goodness-of-fit:

Fisher's C = 10.056 with P-value = 0.261 and on 8 degrees of freedom

Independ.Claim <chr>	Test.Type <chr>	DF <dbl>	Crit.Value <dbl>	P.Value <dbl>	<chr>
OC_Percent ~ USDS + ...	coef	6	1.0652	0.3277	
NH4_Sediment ~ USDS + ...	coef	6	-1.8014	0.1217	
OC_Percent ~ Treatment + ...	coef	74	-0.1142	0.9094	
NH4_Sediment ~ Treatment + ...	coef	73	1.3518	0.1806	

Response <chr>	Predictor <chr>	Estimate <dbl>	Std.Error <dbl>	DF <dbl>	Crit.Value <dbl>	P.Value <dbl>	Std.Estimate <chr>	<chr>
NH4_Sediment	OC_Percent	0.2630	0.1260	74	2.0873	0.0403	0.263	*
NH4_Sediment	Silt	0.2822	0.1242	74	2.2718	0.0260	0.2822	*
OC_Percent	Silt	0.7302	0.0742	75	9.8355	0.0000	0.7302	***
Density	Silt	-0.2732	0.1662	84	-1.6440	0.1002	-	
Density	NH4_Sediment	0.1583	0.1596	84	0.9915	0.3214	-	
Density	OC_Percent	-0.4134	0.1607	84	-2.5729	0.0101	-	*
Density	USDS	0.2404	0.4652	84	0.5168	0.6053	-	
Density	Treatment	0.3675	0.2002	84	1.8357	0.0664	-	

Response <chr>	method <chr>	Marginal <dbl>	Conditional <dbl>
NH4_Sediment	none	0.31	0.52
OC_Percent	none	0.58	0.63
Density	trigamma	0.20	0.41



Global goodness-of-fit:

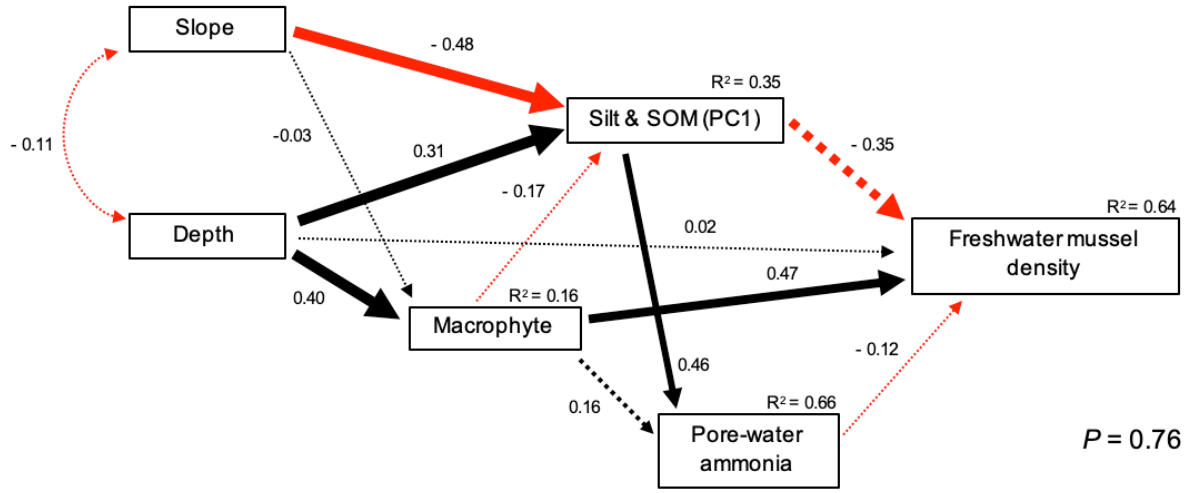
Fisher's C = 10.056 with P-value = 0.261 and on 8 degrees of freedom

Independ.Claim <chr>	Test.Type <chr>	DF <dbl>	Crit.Value <dbl>	P.Value <dbl>	<chr>
OC_Percent ~ USDS + ...	coef	6	1.0652	0.3277	
NH4_Sediment ~ USDS + ...	coef	6	-1.8014	0.1217	
OC_Percent ~ Treatment + ...	coef	74	-0.1142	0.9094	
NH4_Sediment ~ Treatment + ...	coef	73	1.3518	0.1806	

Response <chr>	Predictor <chr>	Estimate <dbl>	Std.Error <dbl>	DF <dbl>	Crit.Value <dbl>	P.Value <dbl>	Std.Estimate <chr>	<chr>
NH4_Sediment	OC_Percent	0.2630	0.1260	74	2.0873	0.0403	0.263	*
NH4_Sediment	Silt	0.2822	0.1242	74	2.2718	0.0260	0.2822	*
OC_Percent	Silt	0.7302	0.0742	75	9.8355	0.0000	0.7302	***
DU40	Silt	-0.0958	0.3502	84	-0.2736	0.7844	-	
DU40	NH4_Sediment	0.0699	0.3664	84	0.1908	0.8487	-	
DU40	OC_Percent	-0.0741	0.3072	84	-0.2411	0.8095	-	
DU40	USDS	2.7776	0.9776	84	2.8413	0.0045	-	**
DU40	Treatment	0.6445	0.3184	84	2.0242	0.0430	-	*

Response <chr>	method <chr>	Marginal <dbl>	Conditional <dbl>
NH4_Sediment	none	0.31	0.52
OC_Percent	none	0.58	0.63
DU40	trigamma	0.37	0.62

c) Upper-riverine section



Global goodness-of-fit:

Fisher's C = 3.41 with P-value = 0.756 and on 6 degrees of freedom

Independ.Claim <chr>	Test.Type <chr>	DF <dbl>	Crit.Value <dbl>	P.Value <dbl>	<chr>
NH4_Sediment ~ Depth_max + ...	coef	33	-0.0026	0.9979	
NH4_Sediment ~ Slope_angle + ...	coef	33	-1.2649	0.2148	
Density ~ Slope_angle + ...	coef	40	0.1914	0.8482	

Response <chr>	Predictor <chr>	Estimate <dbl>	Std.Error <chr>	DF <dbl>	Crit.Value <dbl>	P.Value <dbl>	Std.Estimate <chr>	<chr>
NH4_Sediment	Mac_biomass	0.1575	0.0863	34	1.8255	0.0767	0.1575	
NH4_Sediment	SS_PCA	0.4598	0.1108	34	4.1507	0.0002	0.4598	***
SS_PCA	Mac_biomass	-0.1715	0.1424	33	-1.2045	0.2370	-0.1715	
SS_PCA	Depth_max	0.3114	0.1455	33	2.1411	0.0397	0.3114	*
SS_PCA	Slope_angle	-0.4788	0.1521	33	-3.1486	0.0035	-0.4788	**
Mac_biomass	Depth_max	0.3998	0.1513	34	2.6430	0.0123	0.3998	*
Mac_biomass	Slope_angle	-0.0325	0.1513	34	-0.2147	0.8313	-0.0325	
Density	SS_PCA	-0.3478	0.1909	40	-1.8221	0.0684	-	
Density	Mac_biomass	0.4706	0.1516	40	3.1039	0.0019	-	**
Density	NH4_Sediment	-0.1183	0.249	40	-0.4752	0.6347	-	
Density	Depth_max	0.0222	0.1477	40	0.1502	0.8806	-	
~~Depth_max	~~Slope_angle	-0.1119	-	38	-0.6942	0.4918	-0.1119	

Response <chr>	method <chr>	Marginal <dbl>	Conditional <dbl>
NH4_Sediment	none	0.28	0.66
SS_PCA	none	0.33	0.35
Mac_biomass	none	0.16	0.16
Density	trigamma	0.23	0.64

7.5 Modelling impacts of invasion intensity on mussels and implications for management (Chapter 6).

7.5.1 Modelled mussel recruitment

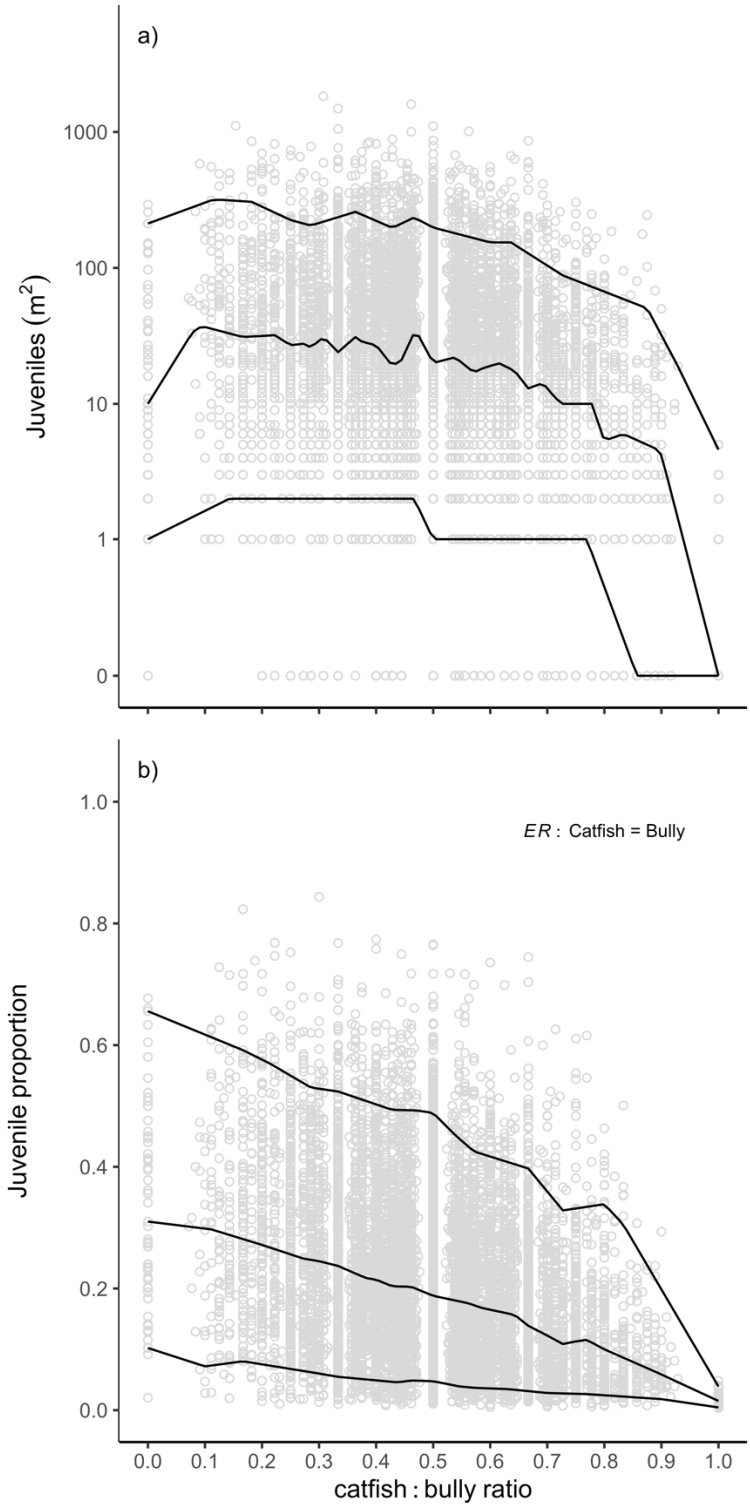


Figure 7-10: Modelled juveniles excystment in total (a) and as a proportion of total excystment (b) across a gradient of invasion intensity expressed as the ratio of catfish to common bully. Encounter rate (*ER*) was specified as equivalent (0.001) for catfish and common bully (see text). Black lines display the 5th,50th, and 95th percentiles fitted using additive quantile regression smoothing.