An evaluation of behavioural evolution and environmental DNA to inform the management and monitoring of biological invasions

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Abstract

Invasive species are a global phenomenon and the rate of biological invasion is only increasing as human trade and transport, together with climate change, cause the rapid reorganisation of species distributions. The impacts of invasive species can be both severe and wide ranging, adversely affecting native ecosystems, human health, agriculture, and economies. Therefore, understanding how species become invasive and subsequently inflict damage on host environments is imperative. Additionally, once an incursion has occurred, its rapid detection and ongoing monitoring are essential for management measures to be effective. My thesis aimed to further a greater understanding of behavioural evolution in invasive species and advance the application of genetic tools to inform and enhance the prediction, monitoring, and management of invasion events.

In the first analysis (Chapter 2), I reviewed the scientific literature reporting behavioural changes in the traits of invasive species when compared to the mean traits of conspecifics in the native range. In this research, I demonstrated that, while behavioural changes are widely reported across invasive taxa, our understanding of the possible mechanisms enabling these changes – plasticity and adaptive evolution – are poorly understood. In addition, research on the fundamental molecular and genetic processes that underlie measured phenotypic changes is in its infancy. Moving forward, I recommend that opportunities to study new or recent biological invasions should be rapidly exploited such that temporal studies can be used to identify patterns of change that occur during invasion.

In Chapter 3, I investigated the application of environmental DNA (eDNA: genetic material released by organisms into the environment) – an increasingly popular

biomonitoring method that has yet to be optimised for lacustrine environments – to the detection and monitoring of the invasive brown bullhead catfish (*Ameiurus nebulosus*). I sampled lakes across two regions of the North Island of New Zealand and compared the results from different field (filter pore sizes, fyke nets) and laboratory (eDNA assays) protocols. I found that species-specific assays (quantitative PCR and catfish-specific metabarcoding) had the highest rates of catfish detection and further showed that multi-species metabarcoding assays were correlated with catfish capture from fyke nets. This indicates that species-specific assays may be the most effective where detection is the primary goal, while multi-species assays hold some promise for producing semi-quantitative results. Therefore, I recommend the development of standardised, flexible, eDNA protocols that will ensure their sound application and interpretation.

As species continue to move beyond their natural ranges, the need for a better mechanistic understanding of invasion, and for more effective detection and monitoring methods, is increasing. With technological advances and increasing availability of genomic data, more rapidly identifying new incursions and studying invasions in real time will lead to better understanding, monitoring, and management of invasive species.

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Chapter 1. General introduction

1.1 Invasive species

The world is currently experiencing an unprecedented rate of environmental change and biodiversity loss (Vitousek et al. 1997; Dirzo and Raven 2003; Barnosky et al. 2012; WWF International 2016). This global ecological disruption is attributed almost entirely to human activities, resulting in the current time period being named the 'Anthropocene' (Hautier et al. 2015). Prior to human influence, the world's ecosystems existed and evolved within natural boundaries created by barriers, such as waterbodies and mountain ranges, which restricted species' movement and migration. The island nation of Aotearoa New Zealand is a case study of an ecosystem that was isolated for 75-80 million years due to vast areas of surrounding ocean, resulting in a remarkably unique flora and fauna (Atkinson 2001; Gibbs 2009). However, biodiversity is now being reorganised at a global scale (Pecl et al. 2017), with trade and transport having accelerated both the deliberate and unintentional movement of botanical and animal organisms beyond their natural ranges (Mack 2003). As a result, biological invasions have become a common feature of ecosystems worldwide (Ascunce et al. 2011).

An invasive species is defined as one that has been successfully introduced to an area(s) beyond its past or present natural range of distribution, regardless of whether the introduction was direct or indirect, deliberate or inadvertent (Convention on Biological Diversity 2002). However, being non-native is not the only prerequisite to being 'invasive', as alien populations can successfully establish in new habitats without causing adverse effects on native ecosystems. Once a population has expanded and/or caused damage to native ecosystems, economies, and human well-being, the 'non-native' species is generally considered to have become invasive (Blackburn et al. 2011; Schlaepfer et al. 2011). These adverse effects can be devastating and, in some cases, irreversible, disrupting the host environment (Vitousek et al. 1996; Ehrenfeld 2010; Blackburn et al. 2011), native biodiversity (Bellard et al. 2016; Doherty et al. 2016), ecosystem functions and services (Pyšek et al. 2020), human health (Mazza et al. 2014), managed agriculture (Paini et al. 2016), and/or the economy (Vitousek et al. 1997; Diagne et al. 2021). The annual worldwide cost of mitigating and managing invasive species impacts in 2022 was estimated to be at least $US423 billion (IPBES 2023), while in New Zealand, estimated costs of US$69 billion in economic damage and managed expenses were sustained between 1968 and 2020 (Bodey et al. 2022). Such figures are forecast to rise exponentially as the global warming crisis and ongoing trade accelerate the rate of biological invasions (Diagne et al. 2021).

The damage caused by biological invasions underscores the need for further research that will facilitate the prediction of future incursions, map the development of existing invasions, prevent new ones, and mitigate their damaging impacts. Where invasions occur, they also provide opportunities to study the adaptive processes that species undergo as they face environmental change in real time. In the following sections, I discuss the species' requirements for a successful invasion, introduce an invasive fish that is impacting freshwater environments in New Zealand, discuss how invasion biology could benefit from the use of new monitoring tools, and outline the main objectives of my thesis.

1.2 Requirements of successful invasion

For a foreign species to become invasive, it must be transported to a new location, successfully establish, and spread demographically and spatially – i.e., it must successfully complete the invasion process (Figure 1.1). Each stage of this process presents challenges: the translocated individuals must survive the initial journey and, on arrival, navigate the novel physical and biotic features they encounter in the new habitat.

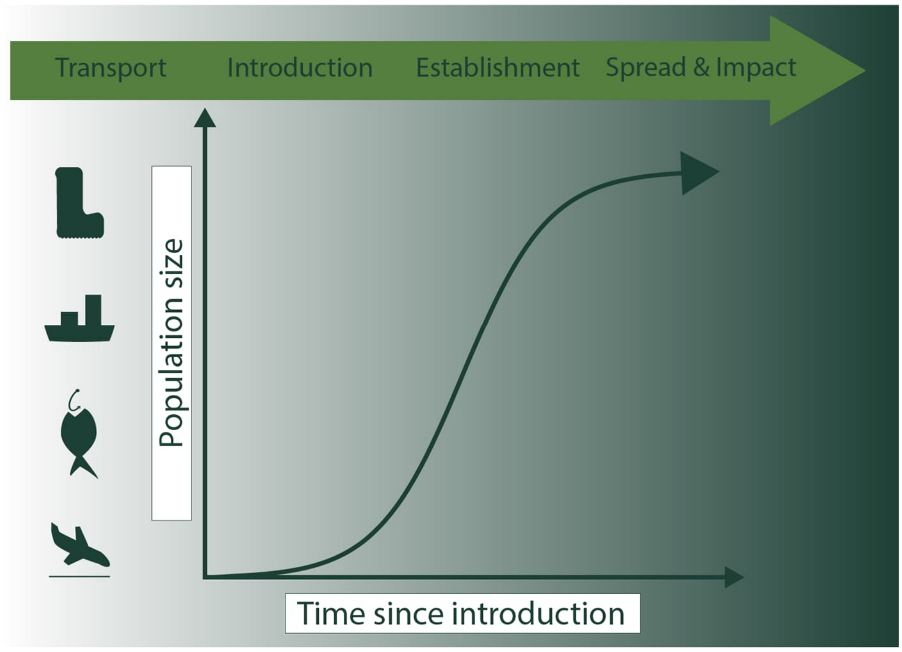

Figure 1.1. The invasion process: transport by an anthropogenic vector (e.g., deliberate capture as a game fish, accidental transport on equipment or vehicles), introduction to the new range, establishment of a viable population, and population spread.

Invasive species may exhibit combinations of certain traits that enable them to overcome these challenges, including large environmental tolerance ranges, small body size, herbivorous feeding habits, high fecundity, high voltinism, short generation times, asexual reproduction, short life spans, rapid development through life stages, behavioural plasticity, and risk-prone animal personalities (Forsyth et al. 2004; Rehage and Sih 2004; Pintor et al. 2008; Short and Petren 2008; Lande 2009; Bates et al. 2013; Capellini et al. 2015; Mahoney et al. 2015; Malange et al. 2016; Baxter-Gilbert et al. 2021; Zhao et al. 2023) (Figure 1.2). Additionally, evolutionary processes operating during the invasion event can result in invasive species developing adaptive characteristics that enable them to thrive in the face of new selection pressures (Carrete et al. 2012; Chapple et al. 2012), ultimately leading to phenotypic and genetic divergence of invasive species from their conspecifics in the natural range (Holway et al. 2002; Pintor and Sih 2009; Blight et al. 2017).

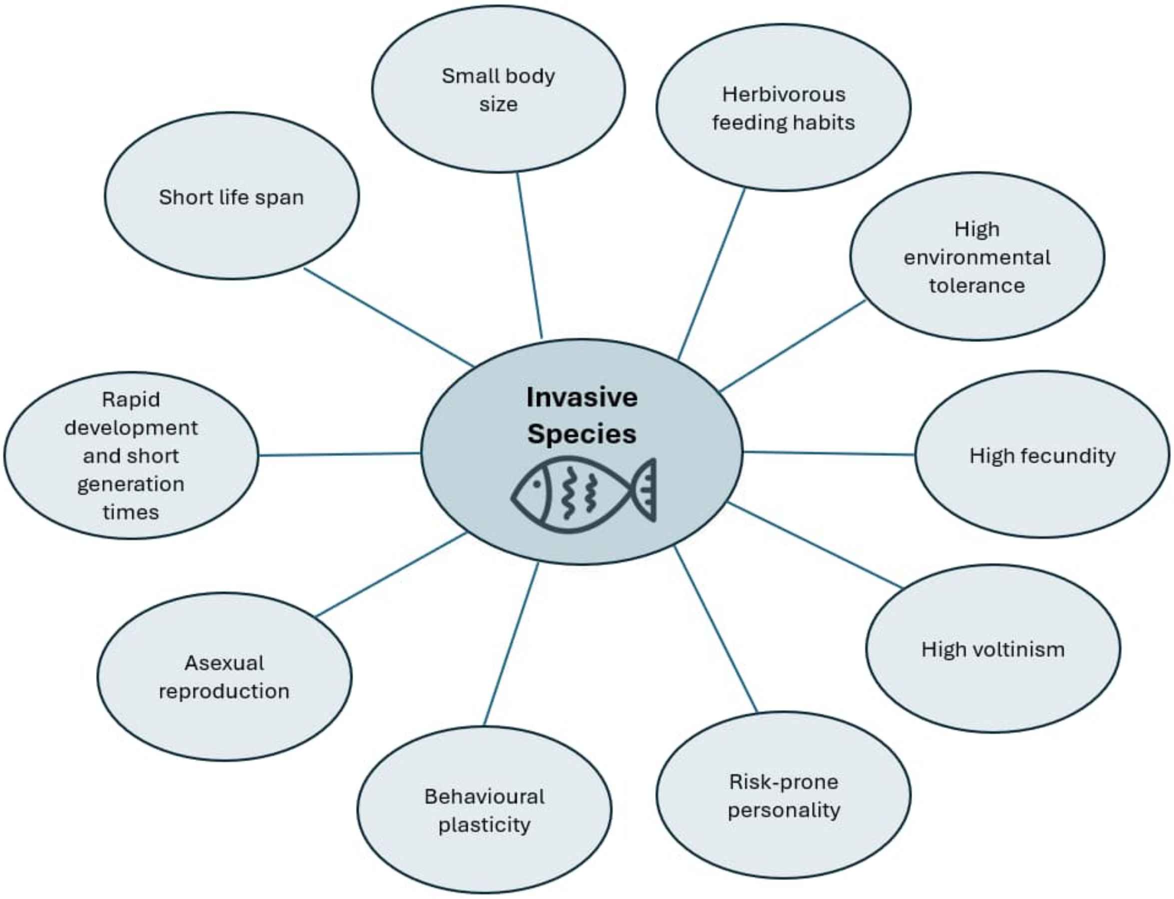

Figure 1.2. Potential characteristics of invasive species.

Among the species traits that are associated with successful biological invasion are certain behavioural characteristics that are thought to be particularly important (Suarez and Phillips 2012). For example, dispersal behaviour is key in determining the rate of spread, with invasive species tending to exhibit behaviours that allow for efficient long-distance dispersal (Barton et al. 2009). Furthermore, invasive species may exhibit an ‘invasive personality’ type that encompasses high levels of aggression, activity (i.e., foraging behaviour), and vigilance (i.e., boldness or antipredator behaviour) (Rehage and Sih 2004; Pintor et al. 2008; Short and

Petren 2008; Malange et al. 2016; Baxter-Gilbert et al. 2021). Such a personality type may afford an invading species an advantage in interspecific competition with native species, and result in an increased survival rate and/or ability to capture and use resources for development and reproduction (Holway and Suarez 1999; Kolar and Lodge 2001; Cote et al. 2010). Importantly, almost all the impacts of invasive species (i.e., predation, competition, parasitism) on native species are likely to be mediated by behaviour (Suarez and Phillips 2012). Therefore, an understanding of invasive behaviour is key to any attempt to map an introduced species' invasion pathway or predict its impacts on the host ecosystem.

Forecasting which species will become invasive when introduced into a new environment has long been an important goal in invasion biology (Elton 1958). However, this is a difficult task due to the many different characteristics that facilitate invasion (Figure 1.2), the complexity of their interactions with each other, and the varying contexts (abiotic and biotic) surrounding invasion events. In addition, it is unclear whether certain characteristics influence invasion success more than others, or how this may vary across taxa. Thus, studies that seek to identify patterns across biological invasions are imperative for advancing our understanding of the invasion process, the common characteristics of invasive species, and the genetic and evolutionary processes associated with invasion, all of which are key to the predictability, prevention, and management of invasive species.

1.3 Methods for the detection and monitoring of invasive species

1.3.1 Brown bullhead catfish

Aquatic ecosystems are ranked among the most threatened systems worldwide (Vörösmarty et al. 2010) and invasive species have been named as one of the key threats to freshwater biodiversity globally (Dudgeon et al. 2006). In this thesis, I study the invasive brown bullhead catfish (*Ameiurus nebulosus*, hereafter catfish Figure 1.3), a member of the Ictaluridae (North American freshwater catfishes) family (Lundberg 1982), which is native to the brackish and freshwaters of North America (McDowall 1990). Catfish have been deliberately introduced to many countries and are now found across the globe, with established populations in Europe, Asia, the Middle East, South America, and the Pacific, including New Zealand (Global Invasive Species Database 2024).

Figure 1.3. Brown bullhead catfish captured in Lake Rotoiti, 2022. Photo credit: N Pyper.

While catfish have generally been introduced to new environments for use as a game fish (Global Invasive Species Database 2024), the motives for their release in New Zealand are unknown. New Zealand's founding population originated from California and comprised 140 individuals released to Auckland's Saint John's Lake in 1877 (McDowall 1990; Collier 2010). Subsequent human-mediated illegal introductions (both accidental and intentional), together with natural range expansion via river networks, have resulted in the species' dispersal to other regions of New Zealand, with catfish populations now established throughout the North Island and high abundances reported in the Waikato region (Lake Taupō, the Waikato River and its tributaries) and, more recently, the Bay of Plenty (Lake Rotoiti and Lake Rotorua; van den Heuvel and Shanley 2017). Isolated populations have also established in the South Island (Barnes and Hicks 2003) (Figure 1.4).

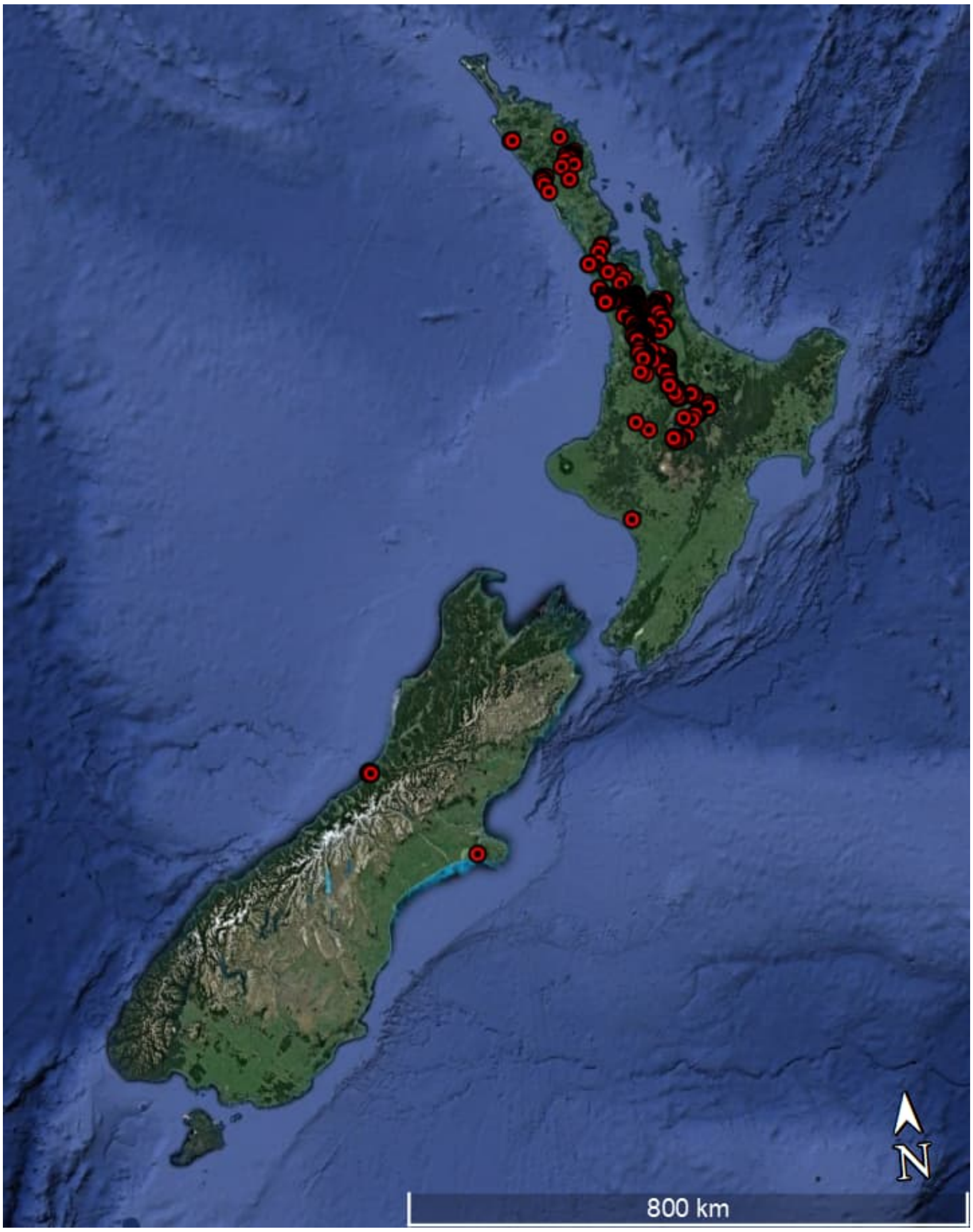

Figure 1.4. Distribution of the brown bullhead catfish across Aotearoa New Zealand. Location information was obtained from the New Zealand Freshwater Fish Database, which is managed by the National Institute of Water and Atmospheric Research.

Catfish typically inhabit lentic environments or slow-flowing, soft-bottomed waterways (Scott and Crossman 1975). They possess several characteristics that promote their invasiveness and facilitate their competition with and/or predation on native aquatic species. For example, catfish have a remarkable tolerance for poor habitat and water quality and are known to thrive in areas where sediment is contaminated with polycyclic hydrocarbons and heavy metals (Lesko et al. 1996). Additionally, catfish are nocturnal feeders that rely on their sense of smell, together with taste sensors on their skin. Therefore, they are not limited by turbidity or low visibility (McDowall 1990). Not only is the species able to tolerate polluted conditions, these habitats have been associated with increased fecundity in female catfish (Lesko et al. 1996) – attributed to reduced competition for food resources and a lack of predators in degraded environments. Moreover, catfish are extremely tolerant of low oxygen conditions (Scott and Crossman 1975) and can survive for lengthy time periods out of water if their skin remains moist (Collier and Grainger 2015). This is particularly problematic as it means they can hitchhike on boat trailers, weed, or algal fragments to enhance their spread. Predation by possible predators, such as piscivorous fish, in their invaded ranges is also limited by the catfish's sharp pectoral and dorsal spines (McDowall 1990), which lock into an upright position upon attack (Scott and Crossman 1975). Finally, the species' reproductive behaviour is thought to significantly increase the rate of survival of their progeny (Blumer 1985), as male and (less so) female catfish continue caring for their young for up to 19 days after the eggs hatch (Blumer 1985).

Catfish are often described as flexible, opportunistic omnivores (Barnes and Hicks 2003), although studies have shown that they obtain more of their nutrition from animal prey than from detritus or algae (Collier et al. 2018). In New Zealand, catfish are known predators of the native freshwater crayfish (*Paranephrops planifrons*, hereafter, kōura) (Barnes and

Hicks 2003) and are thought to be at least partly responsible for the decline of kōura in the Waikato hydro-lakes (Clearwater et al. 2014). In addition, catfish likely compete with native freshwater invertebrates and fish (i.e., the shortfin eel, *Anguilla australis*) for shelter and food resources (Barnes 1996; Collier et al. 2018). As well as impacting native species, catfish alter freshwater habitats through excretion and the resuspension of bottom sediments during feeding (Cline et al. 1994). This can further alter invertebrate communities, reduce water quality, and modify a waterbody's internal processes through the release of nutrients to the water column (Cline et al. 1994; Holmlund and Hammer 1999), further promoting catfish expansion and ecosystem degradation.

Catfish were first confirmed present in the Bay of Plenty region of New Zealand in March 2016 following their accidental discovery during an aquatic weed removal operation. Prior to this, the Bay of Plenty Regional Council (BOPRC) received numerous reports of catfish sightings, but investigation efforts following these sightings repeatedly failed to locate live catfish (Blair and Hicks 2009; BOPRC 2018). Currently, catfish capture and removal occurs using fyke nets in known locations of catfish populations, with nets also regularly set up in catfish-free waterbodies to detect new incursions. However, the delay in detection of catfish presence in Lake Rotoiti highlights the need for new techniques that increase the likelihood of catfish detection during the early stages of invasion following introduction and establishment, such that management responses can be implemented before populations reach problematic densities.

1.3.2 Environmental DNA as a monitoring tool

Environmental DNA (eDNA) is a swiftly-developing, high-sensitivity research tool that has transformed aquatic ecology, conservation, and invasive species management, with several studies demonstrating its ability to support the ongoing detection, monitoring, control, and extermination of invasive fish populations (Jerde et al. 2011; Deiner et al. 2017). eDNA overcomes some of the challenges associated with conventional monitoring (Beng and Corlett 2020) because it does not require physical capture or visual sighting of the target organism. The term eDNA originated in the field of microbiology and refers to traces of genetic material present in the environment as a result of individuals depositing biological material, such as skin cells, gametes, and bodily fluids (Valiere and Taberlet 2000). eDNA sampling denotes the process of collecting genetic material from an environmental sample (e.g., water, soil, air, or sediment) and subsequently extracting, purifying, sequencing, and analysing the DNA to provide information about the species inhabiting that ecosystem (Taberlet et al. 2012; Thomsen and Willerslev 2015; Dickie et al. 2018; Tsuji et al. 2019) (Figure 1.5).

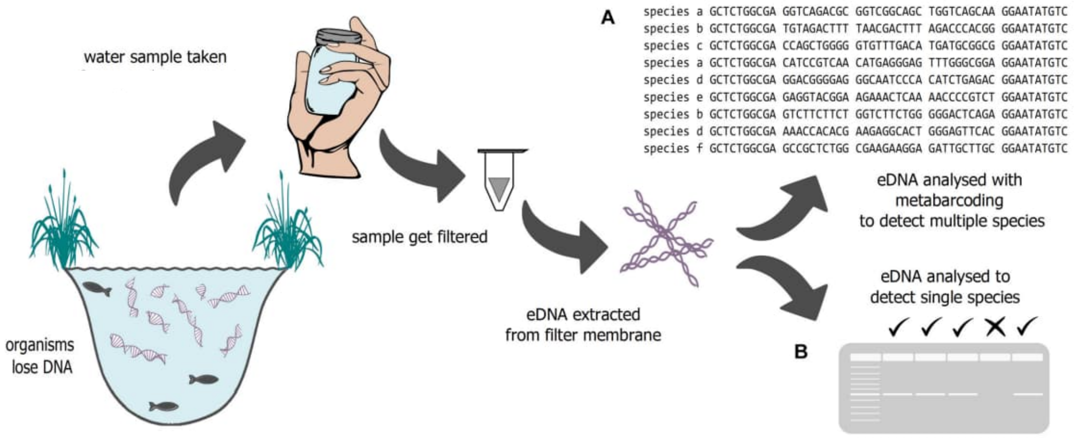

Figure 1.5. eDNA methodology. Typically, water samples are filtered in the field and DNA fragments are extracted from the samples in the laboratory. The fragments are then analysed to provide information on community structure and/or the presence/absence of a target species. Image adapted from Schadewell and Adams (2021).

In aquatic environments, a commonly used method for eDNA collection is the filtration technique. This involves drawing water samples through glass filters in the field, which are then transported to the laboratory for DNA extraction and amplification by the polymerase chain reaction (PCR) (Taberlet et al. 2012) (Figure 1.5). The extracted DNA is then sequenced, either using a species-specific assay (i.e., quantitative PCR (qPCR) or droplet digital PCR (ddPCR)), or a multi-species assay that can detect DNA fragments across a range of taxa (Tsuji et al. 2019; Beng and Corlett 2020) (Figure 1.5).

A number of studies have demonstrated how eDNA can be a powerful tool for detecting fish, including invasive species, both in New Zealand (Hicks and Cursons 2020; Banks et al. 2021; David et al. 2021; Gleeson 2021) and overseas (Jerde et al. 2011; Wu et al. 2023). Early detection of invasive species can significantly improve the likelihood of successfully controlling a new population, because it enables rapid implementation of control methods (Myers et al. 2000; Wittenberg and Cock 2001). This can be particularly important in aquatic environments where, in some cases, early detection can mean efficient extermination. For example, incursions of invasive fish species can occur in discrete habitats, such as small ponds or land-locked lakes (for example, see Thompson and Thompson 2022); and even connected river reaches represent more defined management units than hectares of open, expansive, and undivided terrestrial habitat.

During the initial stage of an incursion, an invasive population can be very limited in size – it may even constitute a single specimen. However, the sampling effort required to capture a few individuals using traditional methods could be significant, and the fish could remain undetected for an indefinite amount of time. This is, perhaps, where eDNA holds the greatest promise as a management tool: having the ability to detect populations when they exist at very low densities. Researchers are continuing to study and test the sensitivity of eDNA technologies, with promising results for various technologies, such as ddPCR (Hicks and Cursons 2020) and targeted metabarcoding (Wu et al. 2023).

However, as with any methodology, there are several challenges and limitations that must be explored and overcome. For example, eDNA results can return false negatives (failures to detect a species of interest when they are present) and false positives (detections of species when they are actually absent, Larson et al. 2020), and imperfect reference sequence databases can limit the methods' taxonomic resolution (Hotaling et al. 2021). Moreover, as eDNA is a relatively recent method for monitoring and detection, standardisation of the procedure is needed, particularly in the context of biomonitoring in lacustrine environments.

1.4 Thesis structure

My thesis aims to explore the evolutionary components of invasion success, with a particular focus on behavioural shifts in invasive species and the use of eDNA as a genetic tool in invasion biology. Following this introductory chapter, there are three remaining chapters in this thesis. Chapter 2 investigates intraspecific behavioural shifts that occur during biological invasion and examines the evolutionary processes associated with these observed behavioural changes. Chapter 3 compares different field and laboratory methods to evaluate eDNA for use as a detection and quantitative tool for monitoring catfish presence/absence in two lakes in the North Island of New Zealand. Chapters 2 and 3 are presented in journal article format, with Chapter 2 submitted to the *New Zealand Journal of Zoology* on 3 December 2023 and Chapter 3 planned for submission to *Environmental DNA* in the near future. Finally, Chapter

4 provides an overall summary of the main findings of Chapters 2 and 3, contextualising them within a broader framework and providing recommendations for future research.

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Chapter 2. Home and away: The role of intraspecific behavioural variation in biological invasion

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2.1 Abstract

Invasive populations can cause adverse impacts on ecosystems, agriculture, and economic activities. Advancing understanding of how populations respond to environmental changes and develop their invasive status will enable improved prediction, prevention, and management of ecological invasions. Here, we review recent literature and find that behavioural shifts within species across native and invasive populations are common during invasion. We collate examples which demonstrate how behaviours associated with invasion can be an outcome of plastic, pre-adaptive, and/or post-adaptive processes. However, we find that research investigating the molecular basis of observed behavioural shifts associated with invasion is limited. Perhaps the most important research (but also the most challenging to conduct), is the long-term study of ongoing invasions that track species' behaviour and genetic change throughout the stages of the invasion process. Additionally, common garden experiments, run alongside genetic studies that track changes at the molecular level, are a key area to target in future research to establish whether behavioural shifts originate from plasticity, microevolutionary processes, or a combination of both.

2.2 Introduction

Ecosystems around the globe are faced with a multitude of challenges, one of the most significant being the establishment and spread of exotic species in areas beyond their natural ranges. Biological invaders are widespread (Vitousek et al. 1997) and present significant threats to agriculture, public health, and other human activities (Hulme 2009). Invasive species also degrade ecosystem services, drive population declines and extinctions, and place considerable stress on native biodiversity through predation, competition for resources, and introduction of foreign diseases (Mooney and Cleland 2001; Clavero and García-Berthou 2005; Bøhn et al. 2008; Mooney et al. 2013; Bellard et al. 2016). Moreover, the risks presented by invasive species are only increasing, as climate change creates opportunities for organisms adapted to warmer temperatures to expand their ranges, and as invading species significantly reduce the resilience of native ecosystems (Mainka and Howard 2010).

Although invasion is common, only a small proportion of species that colonise new environments proliferate and expand their range (Williamson and Brown 1986) – but those that do present major challenges to invasion biologists, biosecurity managers, and environmental protection agencies. Key to the management and prevention of these ecological incursions is the identification of characteristics that correlate with invasiveness (Holway and Suarez 1999; Fogarty et al. 2011; Chapple et al. 2012a). If we can improve our understanding of how species respond to environmental changes and subsequently found and develop as invasive populations, we will be better placed to predict future incursions, map the ongoing development of existing invasions (Chapple et al. 2012a), prevent new ones, forecast their effects, and mitigate their damaging impacts on native communities (Holway and Suarez 1999; Kolar and Lodge 2001; Cassey et al. 2004; Puth and Post 2005).

Studies at the species level have shown that invasive taxa commonly excel at one or more convergent traits, with some invasive species arriving ‘pre-adapted’ to a new area – i.e., in possession of a range of genetic, morphological, life history (e.g., generation time), physiological (e.g., growth rate), and behavioural traits that increase their invasive potential – while others rapidly evolve beneficial characteristics after the invasion event. Among these invasive traits are often behaviours that augment survival, functioning, and dispersal in new habitats, and also increase the invasive species’ environmental impact. These behaviours include increased sociability, boldness/risk-taking, dispersal tendency, aggression, exploration, neophilia, learning ability, conspecific tolerance, foraging activity, and general activity levels (Holldobler and Wilson 1977; Holway and Suarez 1999; Holway et al. 2002; Rehage and Sih 2004; Pintor et al. 2008; Pintor and Sih 2009; Carere and Gherardi 2013; Blight et al. 2017). They are considered important across all the stages of invasion, including uptake onto transport vectors, introduction, establishment, and spread (Figure 2.1). However, data on behaviour is often absent from studies on invasion, having received less consideration than other characteristics that may explain invasion success (Holway and Suarez 1999), likely because such data can be extremely challenging to collect in natural systems.

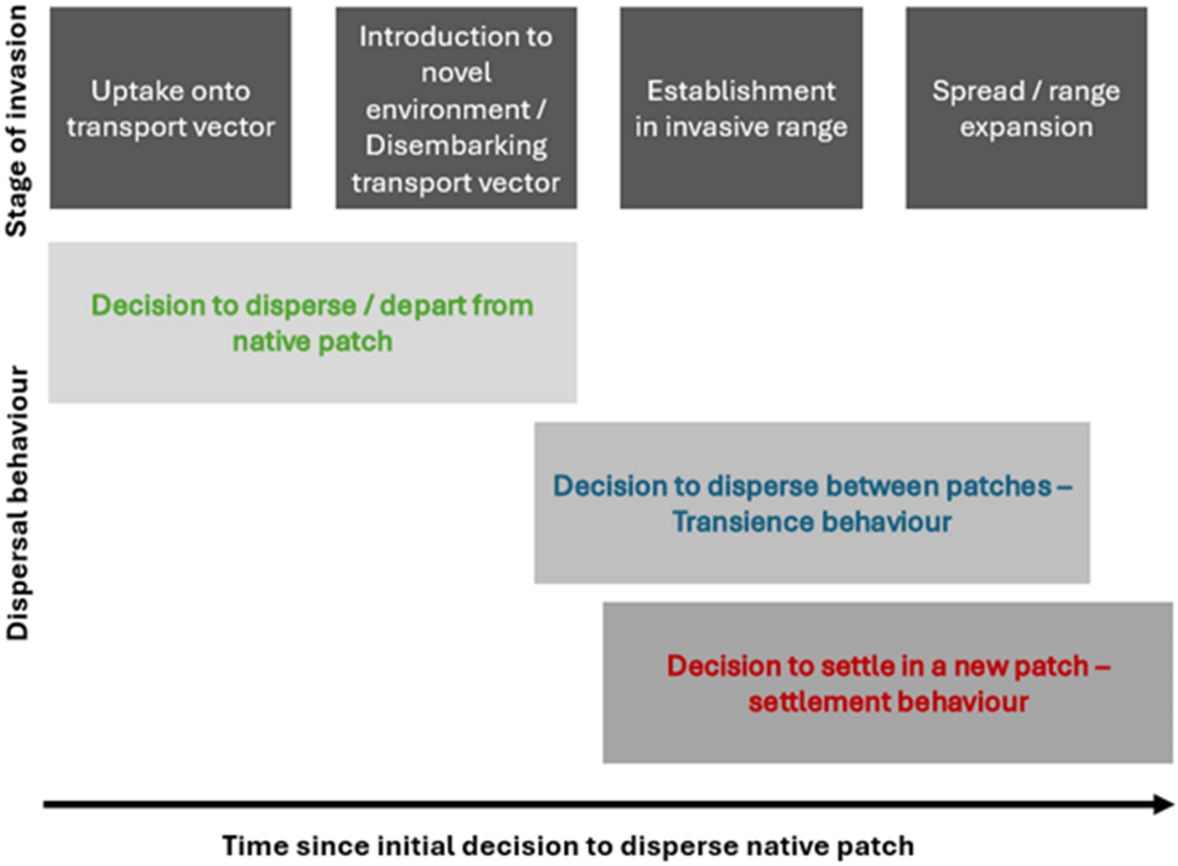

Figure 2.1. The invasion sequence: deliberate (i.e., trapping) or accidental uptake onto a transport vector, transport, introduction to the new range / disembarking from the transport vector, subsequent establishment of a successful population in the new range, and ongoing range expansion and population spread in the invaded environment. Operating in parallel is the behavioural sequence, throughout which an individual makes dispersal decisions (Clobert et al. 2009). An individual's tendency toward certain behaviours (i.e., exploratory behaviour, activity levels, sociability) will affect the dispersal decisions made at each stage.

Where behaviour of invasive taxa has been the subject of research, the scientific literature is dominated by comparisons made at the species level, rather than at the population level, or within an invasive metapopulation. However, different invasive species are likely to possess numerous trait differences aside from those suspected to influence invasion, making it difficult to determine which trait(s) result in greater invasiveness. Drawing intraspecies comparisons (i.e., between native and invasive populations of the same species) can conversely increase our understanding of how behaviour rapidly changes throughout the invasion process as a direct result of the invasion itself.

Here, we review the role of post-invasion behavioural change by synthesising studies that have examined behavioural variation between native and invasive ranges, and between range cores and range edges in the invasive range. To achieve this, we assessed 490 publications that appeared in a Web of Science search using the term "(behavio* OR personalit* OR plastic* OR syndrome*) AND (dispers* OR invas* OR non-invas* OR native OR foreign OR alien OR introduced OR introduction* OR non-native OR 'invasion front' OR 'range edge' OR novel OR 'range expansion') AND (population* OR specie* OR colon* OR evolution)". We combined the results of this search with other recent publications and key studies identified during our wider reading and review of reference lists. We here discuss the mechanisms for behavioural change, highlighting case studies identified in our literature review, summarising common findings and inconsistencies, and detailing directions for future research. This review is limited to the investigation of animal invasions, with studies of invasive plants, microbes, and fungi considered outside the scope.

2.3 Behaviours on the move: Behavioural variation and invasion

A developing body of work shows that behavioural traits can rapidly shift upon introduction to a new environment, and that these post-invasion behavioural modifications could correlate with enhanced invasion success (Chapple et al. 2012b). For example, changes in specific behavioural attributes, such as aggression (Pintor et al. 2008), feeding behaviour (Martin and Fitzgerald 2005; Pintor and Sih 2009; Carbonell et al. 2021), danger responses

(Daniels et al. 2004; Levri et al. 2017), predator avoidance behaviour (Robertson et al. 2011; Levri et al. 2012; Karlsson et al. 2016; Reisinger et al. 2017; Johnston and Smith 2018), dispersal tendency (Levri and Clark 2015), social behaviour (Robertson and Rosenblum 2010), sexual behaviour (Westrick et al. 2019), daily or seasonal activity patterns (Fortes et al. 2019; Poláčková et al. 2022), and neophobia (Candler and Bernal 2015) have all been detected in invasive populations when compared with their native counterparts (Figure 2.2).

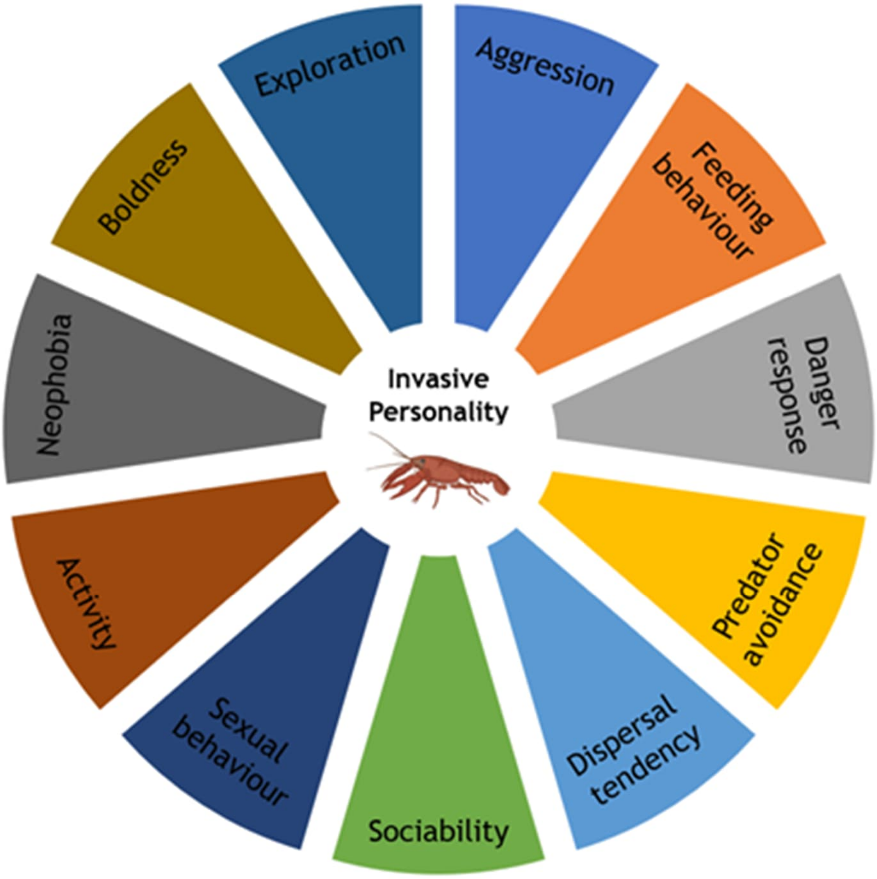

Figure 2.2. Changes in specific behaviours have been recorded in invasive populations when compared with counterparts in the native range. The co-occurrence of changes in multiple behavioural traits are indicative of an 'invasive personality type'.

In some instances, the co-occurrence of changes in multiple invasive behavioural traits have provided evidence for 'invasive personality' types facilitating invasion (Figure 2.2). For example, risk-prone personalities (i.e., a combination of exploratory, active, and bold tendencies) have been shown to be over-represented in invasive populations compared with conspecifics in natural ranges. This is evident in invasive crayfish (Pintor et al. 2008), fish (Rehage and Sih 2004; Myles-Gonzalez et al. 2015; Thorlacius et al. 2015), lizards (Short and Petren 2008; Damas-Moreira et al. 2019), amphibians (Baxter-Gilbert et al. 2021), and rodents (Malange et al. 2016). Similarly, studies investigating the role of personality types in the population dynamics of invasive fishes have suggested that dispersal may be led by individuals exhibiting an asocial personality type. For example, Cote et al. (2010) suggested that the invasion front of the invasive western mosquito fish (*Gambusia affinis*) may be dominated by asocial individuals. Thorlacius et al. (2015) also demonstrated that populations of the invasive round goby (*Neogobius melanostomus*) vary in expression of personality traits, with individuals in newly-established invasive populations shown to be more asocial than older populations.

As well as invasive versus native range comparisons, individuals or populations at the invasion front (range-edge) can be compared to established invasive populations at the range core to provide useful insights into how certain behaviours may be up- or down-regulated at different stages of the invasive process (Duckworth and Badyaev 2007; Groen et al. 2012; Thorlacius et al. 2015). For example, relative to range-core counterparts, range-edge populations of expanding species can differ in dispersal tendency or ability, aggression, boldness, activity, and sociability. One of the consequences of this is that an invasive population can develop a continually accelerating dispersal rate (Sol et al. 2002; Badyaev 2005; Shine et al. 2006; Duckworth and Badyaev 2007; Shine et al. 2011; Hudina et al. 2014; Thorlacius et al. 2015), as has been observed in the Australian cane toad (*Rhinella marina*), whose yearly rate of expansion has increased almost fivefold since first introduction (Shine et al. 2006).

The ecological success of ants has made them globally abundant, yet notable differences in social structure and behaviour of invasive and native populations has been repeatedly recorded across ant species, providing a compelling case study. In the native range, intraspecific competition between colonies generally constrains nest density. However, the formation of large supercolonies that lack intraspecific aggression between sub-colonies has been recorded in numerous invasive ant species (Kjeldgaard et al. 2022). These supercolonies often spread over thousands of kilometres and can contain substantial polydomous (occupying two or more spatially separated nests) and polygynous (males have more than one mate) colonies constructed from connected nests that liberally exchange individuals (Holway et al. 2002; Eyer and Vargo 2021), whereas such associations are generally absent in the native range. This social organisation requires an easing of colony boundaries and no, or significantly reduced, intraspecific aggression among colonies through a loss of nest mate recognition and a lack of aggression toward non-nest mates (Helanterä et al. 2009). Conferring dominance over resources (Tsutsui et al. 2000), and removing costs associated with intraspecific competition and territorial conflicts, this behavioural shift has resulted in populations that are ecologically dominant and outcompete native species, altogether inflating their environmental (Holway et al. 1998; Tsutsui et al. 2000; Holway et al. 2002). It can also work in concert with other behavioural modifications to further promote invasiveness. For example, alongside reduced aggression in the invasive range, Argentine ant (*Linepithema humile*) workers in Europe (introduced range) have a higher tendency to explore novel environments and scatter larvae, are more active and aggressive interspecifically, discover food resources more rapidly, and dominate more space than workers in the native range (Blight et al. 2017).

2.4 Beyond borders: Mechanisms of post-invasion behavioural changes

Despite increasing evidence that behavioural variation occurs between populations in their native and invasive ranges, the underlying mechanisms for these behavioural shifts are not well-understood (Phillips and Suarez 2012). However, there are two broad processes at play: (1) Swift changes in heritable behaviours and associated genotypes driven by the selective pressures imposed by a new environment (i.e., adaptation); and (2) Behavioural plasticity induced by new environments experienced and, perhaps associated with, differences among individuals in regard to past experiences (i.e., learning) (Phillips and Suarez 2012; Gruber et al. 2017b). These two mechanisms likely operate in combination. For example, the capacity for behavioural plasticity may evolve during the process of range expansion or biological invasion (Stamps and Groothuis 2010; Diamond and Martin 2016; Reisinger et al. 2017), and behavioural plasticity may create non-heritable variation in the invading population that become genetically assimilated in later descendant populations (Huey et al. 2003; Badyaev 2005; Ghalambor et al. 2007; Foster 2013).

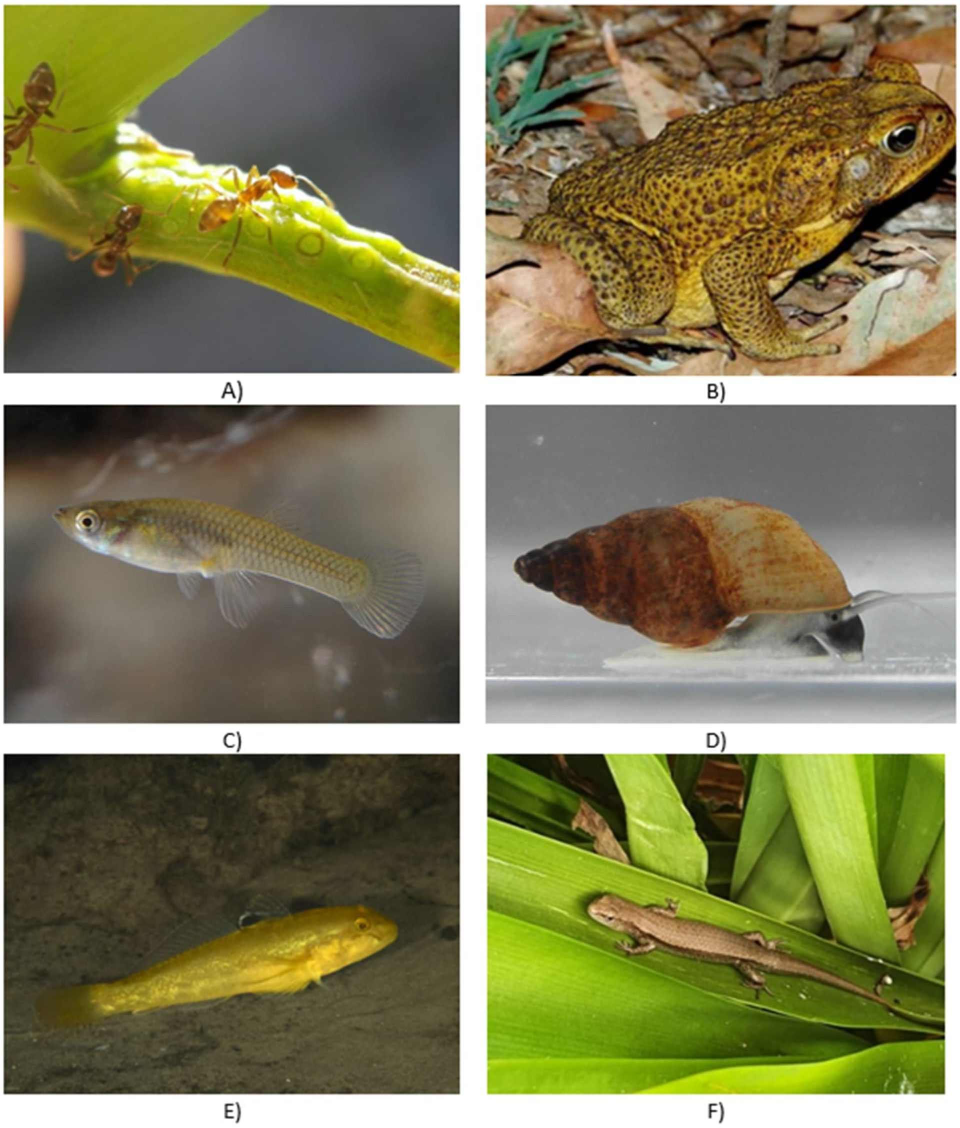

Figure 2.3. Key invasive species that have been the subject of behavioural research: A) Argentine ant, B) cane toad, C) mosquito fish, D) New Zealand mud snail, E) round goby, and F) rainbow skink. Photo credits: A) José Luis Barberán, ArgentiNat, B) Steve Kerr, iNaturalist NZ, C) NOZO, Wikimedia Commons, D) Michal Manas, Wikimedia Commons, E) Rob Dutch, iNaturalist, F) Michael Lambert, iNaturalist NZ.

2.4.1 Variation in dispersal behaviour

Successful biological invasions rely intimately on dispersal behaviour (Phillips and Suarez 2012), encompassing departure from the initial (native) patch, movement between patches (transience), and settlement in a new patch (Bowler and Benton 2005; Clobert et al. 2009) (Figure 2.1). Dispersing individuals often encounter dramatic ecological changes throughout the invasion process. As a result, the benefits conferred from the expression of different behaviours will vary depending on which stage of the invasion process a population is presently experiencing, with each stage potentially acting as a selective filter on traits related to invasion success (Carrete et al. 2012; Chapple et al. 2012a). For example, different behaviours may predispose individuals to capture during transport, or they may enhance dispersal upon arrival to the new habitat.

Dispersal behaviour has been shown to be highly variable among individuals (Clobert et al. 2009), suggesting that dispersers are not a random subsample of a population. In fact, non-random dispersal may be the rule, rather than the exception. Exemplifying this, asocial individuals of the invasive common lizard (*Lacerta vivipara*) are more likely to disperse, particularly when population density is high (Cote and Clobert 2007), and asocial invasive mosquitofish are more likely to disperse further than their social conspecifics (Cote et al. 2010). This sociability-biased dispersal could potentially result in invasive populations that display more asocial behaviours than their native-range counterparts (i.e., socially-biased dispersal acting as a selective filter; Cote et al. 2010; Cote et al. 2011).

Bold, risk-taking, exploratory, active, individuals may be more likely to find their way onto transport vectors (Blackburn and Duncan 2001; Briski et al. 2018; Chapple et al. 2022). Once introduced, these individuals may be more likely to disperse further, more quickly, and discover more appropriate habitats and resources, resulting in an over-representation of such behaviour at the invasion front (Cote et al. 2011; Groen et al. 2012; Liebl and Martin 2012; Chapple et al. 2022). However, it can be difficult to determine whether these behaviours are the result of personality-dependent dispersal, or whether a random set of individuals has dispersed to the new range and those that have a bold, active, and asocial personality are the ones that survive and reproduce. It is also possible that both processes operate and reinforce each other. Regardless, invasive populations often consist of more exploratory, active, and bold individuals than native-range populations (Lapiedra et al. 2017; Chapple et al. 2022), and the higher proportion of these individuals at invasion fronts likely facilitates the further dispersal and rapid expansion of invasive populations (Myles-Gonzalez et al. 2015).

Individuals that are more dispersive also tend to be the first to newly establish a population (Wilson et al. 2001; Hanski et al. 2002; Hanski et al. 2004; Shine et al. 2006; Hughes et al. 2007; Duckworth 2008). When more aggressive, faster-dispersing individuals continue to expand the range front/edge, selection there will likely favour traits that increase dispersal ability further (Phillips et al. 2008; Alford et al. 2009; Léotard et al. 2009) in a process known as "spatial sorting" or the "Olympic Village effect" (i.e., assortative mating or inbreeding of individuals at the range-front) (Shine et al. 2011). As a result, extremely aggressive, highly dispersive characteristics are expected to consistently evolve upwards during range expansion (Alford et al. 2009; Burton et al. 2010), permitting animals at the invasion front to infiltrate uncolonised areas several times faster than in the earlier stages of invasion (Alford et al. 2009).

Finally, disperser-dependent founder effects can occur during invasion, where bolder, more exploratory individuals colonise new areas due to chance. The ongoing expansion of the cane toad range in Australia is considered an example of this, with more bold individuals stochastically dispersing to the range-front to create new founding populations with a bold behavioural phenotype (Gruber et al. 2017b). Similarly, heritable differences in tadpole boldness between mainland and invasive island populations of common frogs (*Rana temporaria*) is thought to have resulted from more exploratory adults by chance being the colonisers of new islands as they rose above sea level approximately 350-800 years ago (Brodin et al. 2013).

Selective pressures on behaviours, such as aggression (Duckworth and Badyaev 2007; Duckworth 2008; Groen et al. 2012; Michelangeli et al. 2017) and tendency to disperse, are expected to differ at the invasion front and range interior (Phillips et al. 2008), as well as between the former and the native range. However, behavioural adaptations for increased dispersal should only exist in a population for a short time after colonisation as the adaptive value of dispersing declines and selection instead favours other trait combinations that confer advantages in higher density populations with increased competition for resources (Thorlacius et al. 2015). This pattern has been observed in the highly successful invasive cane toad (Phillips et al. 2007), where toads at the invasion front show an amplified dispersal ability compared with toads in range-core populations (Urban et al. 2008), but become less exploratory and risk-taking with time (Gruber et al. 2017a). Similarly, in western bluebirds (*Sialia mexicana*), aggressive individuals dominated populations at the invasion front for a few generations, but aggression waned after a competitor (mountain bluebirds, *Sialia currucoides*) had been excluded (Duckworth and Badyaev 2007). These examples demonstrate how selection can favour different traits depending on the invasion stage and associated spatial sorting and population density (Cote et al. 2011; Fogarty et al. 2011).

2.4.2 Evolutionary processes

Evolutionary processes, such as genetic drift (including bottlenecks and founder effects, where demographic changes associated with invasion of a small number of individuals reduces genetic diversity), and adaptive evolution, can operate at multiple stages of the invasion process, and can ultimately lead to genetic divergence from conspecifics in the natural range (Holway et al. 2002; Pintor and Sih 2009; Blight et al. 2017).

2.4.2.1 Genetic drift

Low genetic diversity in the invasive range following population foundation is common (Rollins et al. 2015), with bottlenecks generally predicted to inhibit population growth due to inbreeding depression, increased fixation of deleterious alleles by genetic drift, and reduced evolutionary potential to respond to new selection pressures (Schrieber and Lachmuth 2017). However, many introduced populations do not appear to be challenged by these problems in a phenomenon known as the "genetic paradox of invasion" (Estoup et al. 2016). Theory suggests that such populations may benefit from multiple introduction events from genetically diverse source populations (Facon et al. 2006), while a temporary or permanent release from stress in invaded habitats may also alleviate the problems associated with reduced genetic diversity (i.e., the "enemy release hypothesis"; Roy et al. 2011; Sarabeev et al. 2017; Antonini et al. 2019; Michelan et al. 2023). Moreover, interactions between inbreeding and the novel environment may actually facilitate rapid evolution and contribute to adaptation even under conditions of reduced genetic diversity (Schrieber and Lachmuth 2017).

2.4.2.2 Adaptive selection

Following invasion, populations may be subjected to new selective pressures that result in rapid adaptation of behavioural traits. For example, populations of invasive rainbow skinks (*Lampropholis delicata*) were significantly more exploratory and, in some instances, bolder than their native range counterparts in a manner consistent with selective processes over and above founder effects (Chapple et al. 2022). The authors argued that, under a pure founder effects framework, the directionality of trait differences between native and invasive populations should be random, while they instead discovered consistent differences in exploratory behaviour across three discrete invasive lineages. One of these lineages - the invasive Lord Howe Island population - experienced recurrent introductions but showed no evidence that the associated genetic admixture from divergent source populations had countered or reduced any founder or other drift effects, providing further support that selective forces promoted risk-prone behaviour types in these skinks (Chapple et al. 2022). Other studies, including common garden experiments, have also supported heritable behavioural shifts in invasive populations as the outcome of rapid evolution in the new habitat (e.g., Gruber et al. 2017b; Stuart et al. 2019).

Founder/bottleneck effects and natural selection can of course operate in concert to promote invasion. An excellent example comes from the invasive *Potamopyrgus antipodarum* - a New Zealand mud snail that can reproduce sexually, but for which invasive populations derive from different clones so that individuals within populations are genetically identical but different populations are genetically distinct (Alonso and Castro-Diez 2012). These snails have provided new insights into evolutionary processes associated with behavioural shifts during invasion, with some invasive clonal populations more invasive than others and their associated behaviours differing from each other but matching their relevant source populations, suggesting population, and possibly genotypic, effects on behaviour (Levri and Clark 2015). Levri and Clark (2015) showed that the most invasive clonal population of the snail exhibited behaviours that increased survival, reproduction, and spread, suggesting that particular clones may be more likely to establish and spread as a result of founder effects and adaptive selection operating in concert. Later research showed that two invasive populations of the snail responded behaviourally to the odour of an unfamiliar predator, while a non-invasive American population did not, though it was unclear whether predator avoidance evolved rapidly post-invasion in different populations, or was brought into new habitats by founder individuals and subsequently refined (Levri et al. 2017).

Another example of selection operating rapidly on behavioural traits is seen in the pond slater (*Asellus aquaticus*) following invasion of a novel stonewort habitat. Here, researchers compared the invading population with an ancestral reed population and found that boldness in the two populations diverged, forming two distinct ecotypes (Karlsson et al. 2016). The authors used a combination of common garden experiments and modelling analyses to investigate selection for trait combinations to determine the heritability of these behavioural differences. They showed that genetic correlations among some traits decreased or disappeared following colonisation, while one new correlation arose in the stonewort population. This was taken as evidence that behavioural characteristics had become decoupled in the short time (< 40 years) since colonisation of the new environment (Hargeby et al. 2004). Thus, genetic correlations among behavioural traits can change to optimise fitness even within short time frames, and may reflect adaptive processes post-invasion (Roff and Fairbairn 2012).

2.4.2.3 Pre-adaptation: the AIAI hypothesis

Pre-adaptation is an important feature of invasion success, and recent evidence suggests that urbanisation may facilitate this when species have adapted to urban environments in their native range before being introduced to a similar environment in the invasive range (Hufbauer et al. 2012; Borden and Flory 2021). For example, urban populations in both the native and invasive range of the guttural toad in Durban, South Africa were significantly bolder than their rural counterparts, providing a considerable advantage during invasion of new urban environments in both Mauritius and Réunion Islands (Baxter-Gilbert et al. 2021). Such behavioural modification could occur via behavioural flexibility (Hudson et al. 2017), developmental plasticity (Brodin et al. 2013; Gruber et al. 2017b; Mühlenhaupt et al. 2022), or rapid localised adaptation (Whitney and Gabler 2008; Baxter-Gilbert et al. 2019), and supports the "anthropogenically induced adaptation to invade" (AIAI) hypothesis (that species adapting to human-modified settings can increase their invasive potential through the development of adaptive phenotypes that offer advantages in anthropogenic habitats (Hufbauer et al. 2012). Under the AIAI hypothesis, urban areas can be seen as "sorting grounds" for many invasive species, selecting successful establishers for future invasion (Hufbauer et al. 2012).

While Baxter-Gilbert et al.'s (2021) study provided support for the AIAI hypothesis and showed a clear divergence in behaviour among adult populations of guttural toads, common garden experiments to determine whether the observed behavioural shifts were heritable adaptations or examples of behavioural plasticity were inconclusive (Mühlenhaupt et al. 2022). This highlights the difficulty of conclusively determining the mechanisms behind behavioural change, and many of the studies discussed above were similarly unable to infer the evolutionary processes that may have produced the observed behavioural patterns. Further molecular and common garden experiments are required to elucidate the mechanistic drivers behind behavioural shifts that accompany invasion.

2.4.3 Behavioural plasticity

Behavioural plasticity refers to the capacity of a single genotype to produce different behavioural phenotypes in response to a range of environments and ecological conditions

(Roff 1999). This enables swift responses to environmental change through innovation or social learning of new behaviours. Behavioural plasticity may buffer animals against the novel selection pressures they face within a new range by allowing them to rapidly adjust their behaviour to the new environmental conditions. Therefore, behavioural plasticity is considered particularly important for invasive processes, as it allows immediate within-individual changes in response to new conditions without the requirement for genetic adaptation (Robinson and Holmes 1982; Robinson and Dukas 1999; Ghalambor et al. 2007; Lande 2009). In fact, plasticity may even be a requirement for survival during the initial stage of colonisation, allowing individual modification to occur as a first response before adaptive processes (that may require changes in morphological, physiological, or life history traits can operate; Lande 2009; Chevin et al. 2010; West-Eberhard 2020).

Behavioural plasticity is commonly invoked as a major mechanism that facilitates the expansion of a population’s geographic range and the colonisation of novel environments (Roughgarden 1972; Holway and Suarez 1999; Sexton et al. 2002; Richards et al. 2006; Ghalambor et al. 2007; Caño et al. 2008; Otaki et al. 2010; Davidson et al. 2011; Baldwin 2017; Schrieber and Lachmuth 2017) for two main reasons. First, plasticity may allow more colonising individuals to initially survive and persist so that the population can later adapt to new environmental conditions via directional selection (Ghalambor et al. 2007; Crispo 2008; Foster 2013; Levis and Pfennig 2016). Thus, plasticity may promote evolution by creating non-heritable variation in the invading population that is later genetically assimilated (Huey et al. 2003; Badyaev 2005; Ghalambor et al. 2007; Foster 2013) - a process that may be especially critical in invasive populations that have low genetic diversity (Robinson and Dukas 1999). However, a key condition is that individuals must be able to behaviourally respond to new selective pressures operating on the invading population if new habitats are ecologically, but not evolutionarily, novel (Ghalambor et al. 2007; Crispo 2008; Foster 2013; Levis and Pfennig 2016). This is because lineages may retain the proximate physiological mechanisms that generate adaptive plasticity, even if the environmental factors that induce phenotypic shifts have not occurred in that lineage for extended periods of evolutionary time (West-Eberhard 2020). Invasive populations of guttural toads provide evidence that beneficial behavioural traits can be preserved in populations and re-expressed post-invasion (Baxter-Gilbert et al. 2021). Second, plasticity might evolve rapidly in the initial colonising generations to facilitate local adaptation (Wcislo 1989; Price et al. 2003; Schlichting 2008; Foster 2013). However, despite a large amount of theory, there is no direct evidence that plasticity is positively selected during colonisation (West-Eberhard 2005; Chevin et al. 2010; Lande Russell 2015). Therefore, further research is needed, and native/invasive range comparisons will be critical for assessing relevant hypotheses (Kleunen and Fischer 2005), such as that species become more plastic in their invasive range. Moreover, longitudinal studies will be essential for enhancing knowledge of the role of behavioural plasticity throughout the invasive process; to our knowledge, no such studies currently exist most likely due to the difficulties associated with the rapid collection of genetic and ecological behavioural information once an invasion is detected.

Individuals from invasive populations may adjust the degree to which they express behavioural plasticity in an adaptive manner during the different stages of invasion. This is a key prediction of the “adaptive flexibility hypothesis” (Wright et al. 2010). As it relates to biological invasion, this hypothesis predicts that the expression of behavioural flexibility, and thus the diversity of behaviours observed in a population, will rapidly increase throughout colonisation and the early stages of establishment, due to innovation and the need to shift to a new optimal phenotype in response to new environmental conditions. An associated prediction is that evolution will favour higher plasticity initially in a novel environment, with this then expected to decline during the establishment and growth phases of invasion as a result of social learning of successful behavioural traits (Wright et al. 2010; Lande Russell 2015; Westrick et al. 2019). In particular, Huang et al. (2015) suggested that large costs and minor benefits may constrain the evolution of greater plasticity in the native range, but reduced costs and increased benefits of plasticity may promote such evolution in the introduced range. These authors also suggested that, as invasive populations are often released from natural enemies, they may more easily afford the costs associated with evolving greater plasticity than native populations (Huang et al. 2015). Therefore, behavioural flexibility may promote stability and persistence during the early stages of invasion when invasions are characterised by small populations that are susceptible to environmental and demographic stochasticity, but become disadvantageous as the population becomes established, causing plasticity to decrease towards native-range levels (Lande 2015). As such, behavioural plasticity may itself evolve over time, resulting in changes in the genetic mechanisms underlying the way or degree to which animals respond to environmental cues (Ghalambor et al. 2007; Shaw et al. 2007; Foster 2013; Levis and Pfennig 2016). A fundamental assumption underlying this theory is that there is genetic variation for plasticity, such that selection has the opportunity to act on its expression (Scheiner 1993).

A consequence of fluctuating behavioural plasticity is that the point in the invasion process at which a population is surveyed for plasticity has serious implications for determining whether it is adaptive or not (Agrawal 2001; Pigliucci and Murren 2003). Few empirical studies comparing native and invasive patterns of behavioural plasticity exist (Handelsman et al. 2013). However, female guppies (*Poecilia reticulata*) from introduced populations are less responsive to males when reared without predator cues but return to a high-predation phenotype when reared with predator cues (Westrick et al. 2019). This pattern was evident across introduced populations from different ranges but absent from the native range where females consistently experience high predation pressure, suggesting it is likely to have evolved in the invaded range rather than to have resulted from drift or founder effects and/or adaptive plasticity in the native range (Westrick et al. 2019). The rainbow skinks discussed above provide a second empirical study in support of increased behavioural plasticity in invasive lineages, which show more plastic exploratory behaviour, activity, and boldness than their native counterparts, and the greatest differences in the most recently-established populations (Chapple et al. 2022). Chapple et al. (2022) argued that this increased behavioural plasticity may be one way in which organisms cope with changing environmental conditions during biological invasions, however they could not determine whether it represented a pre- versus post-invasion adaptation (Chapple et al. 2022).

Intriguing research suggests that invasive species may use plastic behavioural responses to kairomones - allelochemicals released by one species that benefit another - to evade predation by new predators (Hazlett et al. 2003). In fact, plasticity in anti-predator responses is assumed to be one of the key ecological forces enabling the survival and rapid evolution of prey that face new predators in the invasive range (Pujol-Buxó et al. 2013). However, empirical evidence in support of behavioural plasticity to kairomones in invasive populations is weak. For example, Levri et al.'s (2017) experiments on New Zealand mud snail clones revealed that genetically distinct invasive clonal populations responded differently to novel predators (though this may reflect founder effects and selective forces as discussed above).

Closely related to behavioural plasticity is the ability to alter behaviour in response to learned patterns, and this is likely a contributing factor to the success of invasive species. For example, invasive cane toads use spatial memory of experiences with an environment to alter their exploratory and foraging behaviours in order to increase foraging efficiency (Miller et al. 2018). This is consistent with anecdotal information on cane toad foraging behaviour in urban environments (Alexander 1965) and aligns with similar research on colonising rats, which decrease their exploratory behaviour over time to stay close to known areas (Russell et al. 2010). Similar to behavioural plasticity, Miller et al. (2018) noted that the adaptive value of spatial learning is likely highest at the initial stages of introduction and is expected to decrease as individuals spread.

2.5 The molecular basis for behavioural change

One of the main objectives in current animal personality research is to identify the molecular basis of measured behavioural variation (Laine and van Oers 2017). While many quantitative genetic studies of animal personality have reported moderate to high estimates of heritability (Stirling et al. 2002; Dochtermann et al. 2015; Dochtermann et al. 2019), few studies have investigated the molecular basis for this observed heritability, and research on the relationship between genes and behaviour is in its infancy. Recently, Laine and van Oers (2017) provided an overview of quantitative and molecular genetics approaches in the field of animal personality and Bubac et al. (2020) reviewed the scientific literature investigating the molecular basis of behavioural traits in natural populations. Both reviews revealed that few studies have attempted to determine the genetic basis of behavioural phenotypes in natural populations and there is a need to integrate molecular and quantitative genetic methods. Similarly, many of the studies discussed so far have used behavioural assays, common garden experiments, and the tracking of behavioural patterns to theorise the mechanisms behind behavioural shifts in invasive species. However, the molecular mechanisms that underpin these behavioural changes are less-studied and poorly understood despite the fact that elucidating their genetic basis is a key step towards understanding the evolutionary potential and limitations of the invader.

Fortunately, high-throughput nucleotide sequencing and other technological advances are now increasing our ability to examine the genomic basis for the functional shifts associated with biological invasions (Rius et al. 2015). In a notable study, Mueller et al. (2014) identified SNPs associated with neophiliac behaviour and activity level in two invasive populations of the yellow-crowned bishop (*Euplectes afer*) and proposed that these alleles were likely introduced from the native population. The short invasion history (c. 30 years) and assumed small original population size(s) of the birds were used to suggest that the behaviour-associated SNPs were unlikely to have established from new mutations that arose after the invasion. However, the study lacked a comparison of allele frequencies between the invasive and native populations and did not compare invasive populations of different ages to track genetic changes over time (Carrete et al. 2012; Edelaar and Bolnick 2012). More recently, Yagound et al. (2022) used RNA sequencing to investigate brain transcriptomes of cane toads from Hawai'i (the native population) and nine invasive populations along an east to west transect across the Australian invasive range that reflects the timeline of the cane toad invasion. These authors found extensive differences in brain gene expression profiles between Hawai'i and Australian populations for key genes with functions putatively associated with dispersal behaviour, while more modest differences were seen across the Australian transect (Brown et al. 2015). These genes may therefore play an important role in the behavioural changes associated with the introduction of invasive Australian cane toads

(Yagound et al. 2022). However, teasing apart the effects of rapid evolution and environmentally-induced variation (i.e., phenotypic plasticity), or the adaptive evolution of phenotypic plasticity itself, is difficult (Ghalambor et al. 2007) and common garden experiments that measure dispersal-related behaviour alongside gene expression in the identified genes will be required to elucidate the heritability, and thus evolvability, of the observed behavioural and transcriptomic changes (Yagound et al. 2022).

As noted above, ants provide a compelling case study for behavioural studies. The most touted theory explaining the lack of, or limited aggression among, invasive ants is that individuals in invasive colonies are genetically alike (via a founder effect) and thus have similar or identical heritable recognition cues (Tsutsui et al. 2000; 2003). In supercolonies of invasive Argentine ants, many populations exhibit low levels of genetic variation, even at a transcontinental scale, though others exhibit considerable genetic variation between populations (Tsutsui et al. 2000; Sunamura et al. 2009). Reduced variation in loci relating to cuticular hydrocarbon production ('CHC') - chemicals used in kin mate recognition in ants (Pirk et al. 2001; Giraud et al. 2002) has garnered much interest. In fact, supercoloniality appears to be coupled with a reduced number of, or low variability in, these recognition-related compounds for several invasive ant species (Errard et al. 2005; Cremer et al. 2008; Brandt et al. 2009), suggesting a potential mechanism for the rise of supercoloniality whereby a loss of polymorphism at CHC loci may have decreased the ability of workers to discriminate between nest mates and non-nest mates (Tsutsui et al. 2000; Giraud et al. 2002; Tsutsui et al. 2003). To gain further insight into the molecular basis of variation in the Argentine ant's behaviour across its native and introduced ranges, Felden et al. (2019) measured variation in the expression of genes associated with key biogenic amine neural pathways. Various behavioural traits (such as foraging and inter- and intraspecific aggression) are regulated by biogenic amines in social insects (Liang et al. 2012; Kamhi and Traniello 2013; Kamhi et al. 2017), thus Felden et al. (2019) theorised that range-specific behavioural differences would also display range-specific expression profiles at the molecular level. Indeed, these authors found that ten of 14 gene receptors were expressed at significantly higher levels in the native versus introduced range (Felden et al. 2019), providing evidence for variation in the molecular basis of behavioural differences between the ranges. Further clarification of the underlying causes of variation in gene expression between native and introduced ranges will require additional studies that quantify genetic variation in functional or regulatory loci related to the relevant signaling pathways for long-term replicate populations from independent introduction events.

2.6 New horizons: Conclusions and future research

With global invasions rates escalating (Huang et al. 2015), much work remains to explain intraspecific behavioural variability between native and invasive populations, and between range edges and range cores in the invaded environment. Indeed, we found an overall paucity of information on this topic, despite its key implications for the effective management of invasive species - including the potential for better identification of threats prior to establishment and for enhanced understanding of dispersal behaviours and adaptive shifts during the invasive process.

Based on our review, future research should prioritise increasing our understanding of how behavioural changes among frontier populations arise, as it is at the range edge that “invasiveness” (e.g., dispersal tendency), accelerates to expand a species range and worsen its impact (Figure 2.4). Another marked gap we noted is the lack of research that uncovers the evolutionary (including genetic and molecular) processes that underlie behavioural shifts in invasive species. While the theories concerning the role and evolution of behavioural plasticity in biological invasion are well-developed, empirical studies are necessary to determine which processes are important and how they may interact (Figure 2.4). Common garden experiments run alongside genetic studies that track changes at the molecular level, are a key area to target to establish whether behavioural shifts originate from plasticity, microevolutionary processes, or a combination of both (Figure 2.4).

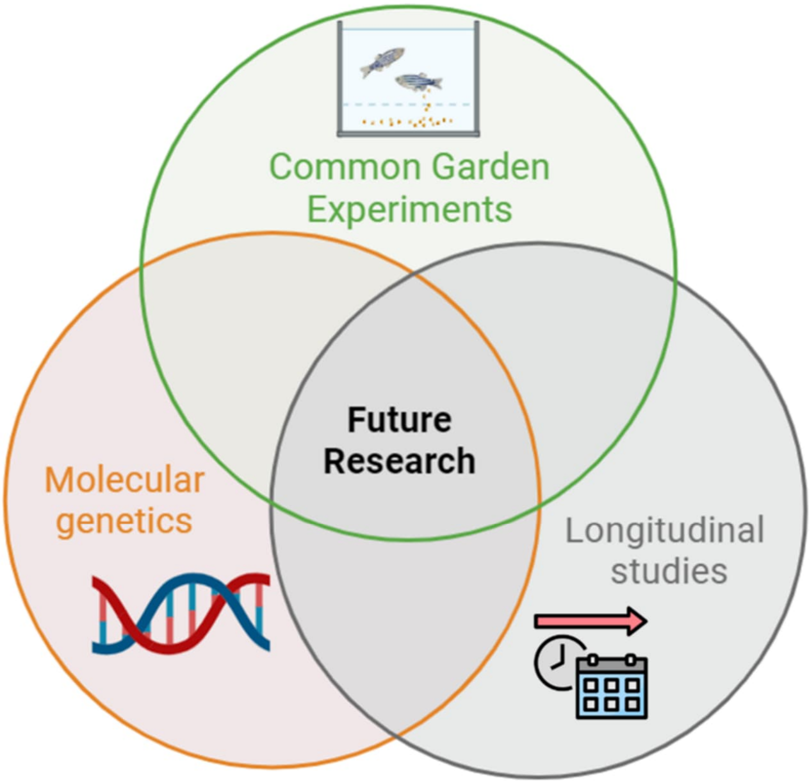

Figure 2.4. Recommendations for future studies of invasive behaviour. Future research should prioritise common garden experiments that are run alongside molecular genetics studies. Where possible, these studies should be carried out over time to map behavioural and genetic changes through the invasion process.

Finally, perhaps the most important research, but also the most challenging to conduct, are long-term studies (Figure 2.4) of ongoing invasions that track species' behaviour and genetics throughout the stages of the invasion process. This will require systems and processes to be established that enable the rapid mobilisation of funding and resources as soon as incursions are detected to forecast range-expansion trajectories and best inform invasion management.

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Chapter 3. Environmental DNA as a detection and quantitative tool for brown bullhead catfish: A comparison with the traditional netting method

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Contributions: NP and AM conceived the research project to align with the goals of Bay of Plenty Regional Council. NP carried out the traditional surveys and eDNA sampling, with Laura Hunter, Mark Law, Rebecca Law, and Steven Law helping with the former, and Starsha Bird helping with the latter. NP analysed the data with occasional assistance from AM, and created all figures. NP wrote the first draft of the manuscript and all authors provided feedback, with NP leading the subsequent revision of the final manuscript.

3.1 Abstract

eDNA methods have significant potential for use in pest fish management in New Zealand. However, questions remain regarding how eDNA compares with traditional sampling surveys, whether there is a relationship between eDNA data and population density, and how the detection capabilities of different field and laboratory eDNA techniques compare to each other. Here, we addressed these questions using invasive catfish (*Ameiurus nebulosus*) as a study species.

We collected eDNA samples alongside biomass and count data from fyke nets set at two lakes in the Bay of Plenty and Waikato regions of the North Island of New Zealand. We investigated the effects of filter pore size (1.2 µm, 5 µm, dacron) and laboratory assay type (qPCR, ddPCR, catfish-specific metabarcoding, multi-species metabarcoding) on catfish eDNA detection and compared our eDNA data to contemporary and historic abundance records.

All three filter types were similarly successful at detecting catfish presence when a minimum of five replicates were taken from each sampling site, while the qPCR and catfish-specific metabarcoding assays outperformed the ddPCR and multi-species metabarcoding assays in detection rate. All methods returned some false negatives, while we identified a significant correlation between contemporary abundance and the multi-species metabarcoding assay for 1.2 µm samples from Lake Rotoiti.

Our results indicate that biosecurity surveillance efforts should employ qPCR or species-specific metabarcoding assays when aiming to detect a single species. However, multi-species metabarcoding assays have potential to be a semi-quantitative tool for measuring catfish abundance and warrant further investigation in this context.

3.2 Introduction

As human populations have expanded and technology has advanced, our ability to travel, trade, and migrate across the globe has increased. Coupled with this amplified human mobility are increases in both inadvertent and deliberate transportation of animals, plants, and microbes. Regardless of the purpose or intention behind these alien introductions, approximately 10% of introduced species establish in the new environment, and 10% of those go on to become invasive (Williamson et al. 1986), often causing catastrophic ecological and economic impacts in the invaded range.

The remote island nation of New Zealand is a small landmass with a disproportionately large concentration of freshwater lakes and rivers. New Zealand's aquatic environments support unique species and ecosystems treasured for their commercial, intrinsic, and cultural values (Ministry for the Environment and Stats NZ 2023). However, New Zealand is also one of six global hotspots for non-native freshwater fish introductions, with over a quarter of all resident freshwater fish species of alien origin (Leprieur et al. 2008). These introduced fish compete with and predate on native species (Collier and Grainger 2015; Francis 2019), while also modifying the habitat and water quality of the environments in which they reside (Rowe 2007). Moreover, climate change is currently expanding the range of habitats within New Zealand that are vulnerable to invasion by alien species (Reid et al. 2019), further increasing the rate of species introductions together with their adverse impacts on the physical and biological environment (Rowe 2007; Collier and Grainger 2015; Francis 2019; Moore 2020).

Avoiding new introductions and managing the containment and elimination of existing invasives is a complex task. Invasive freshwater fish are highly adaptable, tricky to detect, and extremely hard to eradicate. Generally speaking, methods and tools to detect and control invasive fish are poorly developed and limited to visual or capture-based approaches, such as spotlight surveys, electrofishing, traps, and nets – with some sites (e.g., deep, swift rivers) very challenging to sample (Joy et al. 2013; Grainger 2015). In addition, these biomonitoring methods can be costly and labour-intensive. New options are desperately needed, and environmental DNA ('eDNA' – the non-invasive isolation of genetic material from biological material that has been shed into the environment) is a promising tool that is starting to support the ongoing monitoring, control, and eradication of invasive fish populations (Jerde et al. 2011; Deiner et al. 2017).

A number of studies have demonstrated the effectiveness of eDNA methods for detecting and monitoring freshwater fish, including invasive populations in New Zealand (Hicks and Cursons 2020; Banks et al. 2021; David et al. 2021; Gleeson 2021) and overseas (Jerde et al. 2011; Wu et al. 2023). The ability of eDNA to potentially detect invasive species early has significant implications for improving the likelihood of controlling new populations because early detection allows for a more rapid implementation of control methods (Myers et al. 2000; Wittenberg and Cock 2001). This can be particularly important in aquatic environments, where early detection can sometimes mean that extermination is achievable (e.g., when incursions occur in discrete aquatic habitats, such as small ponds or land-locked lakes) (Thompson and Thompson 2022).

During the early stages of an incursion, an invasive population can be very limited in number – perhaps consisting of a single gravid female. At low densities, detection rates of conventional methods can be low, impossible, or may necessitate a huge sampling effort. Therefore, the potential for eDNA sampling to detect populations at very low abundance thresholds is a key advantage and an active area of research. So far, promising results have been reported for various species-specific eDNA technologies, such as droplet digital polymerase chain reaction (ddPCR; a refinement of traditional PCR that uses nanodroplet sample partitioning to allow for highly sensitive DNA quantification without the use of standard curves) (Banks et al. 2021) and targeted metabarcoding (Wu et al. 2023) – a hybrid approach that utilises high-sensitivity PCR primers and high-throughput sequencing. However, while eDNA methods have significant potential for pest fish management in New Zealand, there remain questions regarding how eDNA survey results compare with traditional sampling surveys, whether there is a relationship between eDNA results and population density, and how the detection capabilities of the various field and laboratory eDNA techniques currently in use compare to each other.

The brown bullhead catfish (*Ameiurus nebulosus*; catfish hereafter) is an invasive species that has adverse effects on aquatic communities in New Zealand. Introduced in the late 1800s, the catfish poses a serious risk to New Zealand lake ecosystems, with the potential to modify lake ecosystem processes, nutrient cycling, lake water quality, and benthic invertebrate communities due to its benthic feeding activity, which stirs up bottom sediments (Cline et al. 1994; Hicks et al. 2001). Additionally, as catfish are omnivorous, they likely compete with native carnivorous fish, such as eels (*Anguilla spp.*), and predate on other natives (Collier and Grainger 2015) – for example, negatively impacting kōura (*Paranephrops spp.*) populations (Clearwater et al. 2014; Francis 2019). Like most invasive fish species in New Zealand, catfish are currently monitored by electrofishing or passive netting surveys (Grainger 2015), with fyke nets being the most effective for capturing these benthivorous fish.

Here, I aimed to determine how eDNA sampling and conventional biomonitoring methods (fyke netting) compare to each other when applied to catfish in lacustrine environments. In the field, we tested different eDNA filter sizes and compared the results of filtering different volumes of lake water. In the laboratory, we compared four different eDNA assays (qPCR, ddPCR, catfish-specific metabarcoding, multi-species metabarcoding). We also compared the resulting eDNA data to historic and contemporary netting data. Our aims were to: (i) identify which eDNA assay methods were the most sensitive and, therefore, likely to be most effective in detecting populations of invasive catfish; and (ii) assess whether eDNA has the potential to contribute to semi-quantitative monitoring of invasive catfish in lacustrine environments.

3.3 Methods

3.3.1 Site selection

Sampling was carried out over two years and across two regions. Six sites were sampled in the Bay of Plenty over three weeks in May and June of 2022, and a single site was sampled in the Waikato over two days in late April 2023 (Figure 3.1, Table 3.1).

In the Bay of Plenty, five sites in Lake Rotoiti were selected. The selected sites represented locations where catfish had previously been recorded at low, medium, or high abundance based on 14 months (April 2020 – June 2021) of fyke net data provided by the Bay of Plenty Regional Council (BOPRC). In addition, a single site in Lake Okareka was chosen as a negative control, as regular monitoring by BOPRC indicated that catfish did not inhabit this lake (Figure 3.1, Table 3.1). Lake Rotoiti and Lake Okareka are large lakes (3,430 ha and 350 ha, respectively) with ‘fair’ water quality (Land Air Water Aotearoa 2023).

A)


Otamarae Boat Ramp
Te Weta Bay
Ohau Channel Mouth
Okawa Bay
Delta Boat Ramp
Lake Okareka - Control
8 km
N

B)


5
4
3
2
1
100 m
N

Figure 3.1. Sampling sites in A) Lake Rotoiti, Bay of Plenty (Inset: an adult catfish specimen); and B) Oranga Lake, Waikato.

In the Waikato , a small (1.7 ha), closed system (i.e., no inlet or outlet) of constructed, interconnected lakes (Oranga Lake and Knighton Lake, hereafter referred to collectively as 'Oranga Lake'), was sampled at five sites along the eastern margin (Figure 3.1, Table 3.1). Catfish had previously been reported in this lake system as existing at high densities (Hicks and Bryant 2002).

3.3.2 Netting

At each site, fine mesh (4 mm) fyke nets were set overnight after eDNA samples had been collected (see below). One net was set at each site in Oranga Lake in the Waikato. Two nets were set at each site in the Bay of Plenty (Table 3.1). All captured fish were anaesthetised (2-phenoxyethanol) before being identified to species level and measured for total and forked length (where applicable). All catfish individuals were weighed, and individuals of other species were weighed if they exceeded >1 g. Where more than 50 individuals of one species were captured, a random subset of 50 fish was weighed and measured, and the remaining fish were counted.

3.3.3 eDNA

3.3.3.1 Sample collection

eDNA samples were collected by filtering lake water. Negative controls were also filtered; these consisted of store-bought bottled water. Sterile, single-use eDNA syringe kits (Wilderlab, Wellington, New Zealand, see https://www.wilderlab.co.nz/order) were used at all sites, with 1.2 µm pore (hereafter 'fine') and 5 µm pore (hereafter 'coarse') filters tested at each sampling site. Each syringe included a pre-attached cellulose acetate hydrophilic filter

(33 mm in diameter) with a polypropylene prefilter (Membrane Solutions) for catching DNA. One litre of water was pushed through each of the filters, unless the filter clogged, in which case the total volume able to be filtered was recorded. In addition, a semi-quantitative dacron filter was tested at each site in the Bay of Plenty. Ryobi ONE+ 18V transfer pump was used to push a maximum of 100 L of water through each dacron filter. Due to the amount of water required per sample, no negative controls were filtered using the dacron filter type.

Once filtering of water was complete, each filter was preserved in 300 µl of DNA/RNA Shield preservative (Zymo Research Cat No. R1200-125). Samples were kept at ambient temperature following the DNA/RNA Shield standard protocol (_r1100-50_r1100-250_r1200-25_r1100-125_dna_rna_shield.pdf; zymoresearch.com) and couriered to Wilderlab New Zealand Ltd (Wellington) for DNA extraction.

For all syringe samples, buckets were used to collect lake water from just below the lake surface. When the motorised pump was used, water was filtered directly from the lake. Samples were collected either from the lake edge or from a jetty (if available) to minimise the risk of sample contamination. In addition, at each site, a new bucket was used for lake water collection. Latex or nitrile gloves were worn, and gloves and buckets were decontaminated with a 10% bleach solution prior to sampling. All eDNA sampling gear was kept separate from fishing gear to minimise the risk of cross-contamination. On all occasions, eDNA sampling was carried out prior to netting to minimise cross-contamination risk and to avoid any effect that netting may have had on fish activity and the DNA concentration in the lake water column. At each sampling site, water was collected from the same location for all replicates to minimise variation between replicates.

3.3.3.2 DNA extraction and sequencing

eDNA analysis followed the protocol of Wilkinson et al. (in prep.). In brief, lysates were obtained from the sampled filters and stored at -20ºC until DNA extraction. DNA extraction and purification used 200 µl of each sample lysate. Samples were then processed using a Genolution Nextractor NX-48S system using the standard extraction settings. Extracted DNA was quantified before adapter-fusion, indexing, and amplification steps as part of a single-step quantitative PCR process using an Applied Biosystems QuantStudio 1 qPCR instrument.

3.3.3.3 Quantitative PCR

A master mix was prepared, comprising 5 µl SensiFAST LoRox 2 X Probe Mix (Bioline), 0.9 µl of primers (forward, GTGCAGAAGCGGACATACG; Reverse, CGCGTGGGAGCTTTATTTTCT; Probe, CAACTACGTCAAGAACCCAGTTAAACT), 0.5 µl BSA, and 3.6 µl of template DNA. Reactions were run with the following reagent conditions: an initial step of 95ºC for 2 min, followed by 45 cycles of 95ºC for 10 s and 60ºC for 30 s. The assay targets the mitochondrial 16S ribosomal RNA gene of catfish. All PCR reactions were carried out in quadruplicate.

3.3.3.4 Digital droplet PCR (ddPCR)

ddPCR was carried out following the methodology outlined in Pearman et al. (2022). In brief, ddPCR was conducted using an automated droplet generator (QX200 Droplet Digital PCR System), which partitioned the reaction mixture into nandroplets on a PCR plate. The plate was then analysed on the QX200 instrument. The primers and probe sequences used for the qPCR assay were also used for the ddPCR assay. All samples were run in triplicate.

3.3.3.5 Metabarcoding

Both species-specific and multi-species metabarcoding assays were performed. For the multi-species metabarcoding assay, DNA extracts were amplified using eight fusion-tag mitochondrial and nuclear RNA assays designed for detecting vertebrate, invertebrate, plant, microeukaryote, and microbial DNA. For the catfish-specific metabarcoding assay, the reverse primer CAAGATCAACTACGTCAAGAACCC was used.

Sequencing libraries for metabarcoding used these same primers and were pooled at approximately equimolar concentration. The final pooled library was run on an Illumina iSeq 100 instrument (150 PE). Following this, amplicon sequence variants were generated and identified to the lowest possible taxonomic rank.

3.3.4 Data analysis

Netting data was converted to Catch Per Unit Effort (CPUE) and Biomass Per Unit Effort (BPUE) to account for any discrepancies in the time each net was set for. For the Lake Rotoiti dataset, the data was averaged across the multiple nets set at each site.

For the qPCR results, quadruplicate cycle threshold (CT) values were averaged per sample. In addition, average CT values for the replicate samples collected at each site were calculated. For the ddPCR results, average DNA copy number per µl per sample was calculated across replicates. For the metabarcoding results, eDNA reads for replicate samples were averaged. In addition, each assay result (i.e., CT value, eDNA reads, or average DNA copy number per µl) was converted to a per litre value to account for the different quantities of lake water filtered per sample.

To examine the correlation among the different assay types, averaged results for both overall and per litre datasets were used to conduct correlation analyses in R v. 4.2.2 (R Core Team 2022). To investigate differences in detection rate between filter sizes and laboratory assays, the number of 'hits' per site and per method was calculated. eDNA 'hits' per site are the number of replicate samples at a site in which catfish DNA was detected (i.e., the presence/absence of catfish DNA within each replicate sample) (David et al. 2021). These data were assessed for normality using a Shapiro-Wilk test in R. As the data were not normally distributed, the non-parametric Kruskal Wallis rank sum test was used in R to test for differences between the number of hits per site for the different filter types and eDNA assay methods. Following this, pairwise comparisons were undertaken using Wilcoxon rank sum tests in R.

For the multi-species metabarcoding assay, additional correlation analyses were undertaken to compare the eDNA reads per fine filter sample with the netting data for additional species captured at the Rotoiti sites. This included common bully (*Gobiomorphus cotidianus*), common smelt (*Retropinna retropinna*), goldfish (*Carassius auratus*), and kōaro (*Galaxias brevipinnis*).

Table 3.1. Sampling information for catfish collected in the Bay of Plenty (2022) and Waikato (2023).

Sample Site	Region	Latitude	Longitude	Historic catfish abundance	No. of reps, 1.2 µm filters	No. of reps, 5 µm filters	No. of reps, Dacron filter	Net	Comments
Lake Okareka	Bay of Plenty	-38.176182	176.348054	Negative control	5 + 1 control	5 + 1 control	5	2 x fykes	Set overnight
Otamarae, Lake Rotoiti	Bay of Plenty	-38.012327	176.368976	Low	5 + 1 control	5 + 1 control	5	2 x fykes	Set overnight
Delta Boat Ramp, Lake Rotoiti	Bay of Plenty	-38.051758	176.338377	Medium	5 + 1 control	5 + 1 control	5	2 x fykes	Set overnight
Okawa Bay, Lake Rotoiti	Bay of Plenty	-38.047700	176.331618	Medium	5 + 1 control	5 + 1 control	5	2 x fykes	Set overnight
Ohau Channel mouth, Lake Rotoiti	Bay of Plenty	-38.037884	176.335566	High	5 + 1 control	5 + 1 control	5	2 x fykes	Set overnight
Te Weta Bay, Lake Rotoiti	Bay of Plenty	-38.031800	176.351359	High	5 + 1 control	5 + 1 control	5	2 x fykes	Set overnight
Oranga Lake 1	Waikato	-37.787030	175.316777	NA	6	6	Nil	1 x fyke	Fyke nets set overnight; 2 controls (1.2 and 5 µm) taken on day of eDNA sampling
Oranga Lake 2	Waikato	-37.786759	175.316477	NA	6	6	Nil	1 x fyke	
Oranga Lake 3	Waikato	-37.786318	175.316091	NA	6	6	Nil	1 x fyke	
Oranga Lake 4	Waikato	-37.785792	175.315769	NA	6	6	Nil	1 x fyke	
Oranga Lake 5	Waikato	-37.785606	175.315061	NA	6	6	Nil	1 x fyke	

3.4 Results

3.4.1 Filter sizes and sample volumes

The volume of lake water filtered per sample varied across filter types, sites, and lakes. In Lake Rotoiti, the majority of the samples taken using the fine filter comprised 1 L of filtered lake water. However, 12 out of a total of 30 filters clogged before 1 L of lake water could be filtered, with the minimum volume of water filtered using the fine filter at Lake Rotoiti being 0.75 L per sample. All samples collected using the coarse filter at Lake Rotoiti comprised 1 L of filtered lake water. Lastly, the samples collected using a motorised pump and the dacron filter all comprised 100 L, excluding those collected at the Okawa Bay site, where the water was more turbid and only 50 L was filtered per sample.

In contrast, Oranga Lake (Waikato) was highly turbid and filtering water using handheld syringes was challenging. Sample volumes ranged from 0.05 L to 0.55 L for the fine filter samples, and from 0.265 L to 1 L for the coarse filter samples.

3.4.2 Detection of catfish

All four laboratory assays detected catfish DNA at every site where catfish were also captured in fyke nets, with two exceptions: the catfish-specific metabarcoding assay and the multi-species metabarcoding assay did not detect catfish DNA in the samples taken using the dacron filter at the Delta Boat Ramp site in Lake Rotoiti (Appendix A3.1, A3.2). Additionally, catfish DNA was detected at one site in Lake Rotoiti, Otamarae (using all four eDNA assays and all three filter sizes) where catfish were not captured in the fyke nets. However, historic netting data confirmed that catfish had previously been recorded at Otamarae in low densities (Table 3.1).

There was no disagreement among the four eDNA assays, with each detecting the presence of catfish DNA in at least one replicate sample from each site. However, in total there were 55 false negatives (i.e., catfish not detected when it should have been) out of a total of 540 eDNA assays (Figure 3.2). The multi-species metabarcoding assay had the highest number of false negatives (n=19), and ddPCR the second highest number of false negatives (n=17). The catfish-specific metabarcoding and qPCR assays recorded seven and five false negatives, respectively (Figure 3.2).

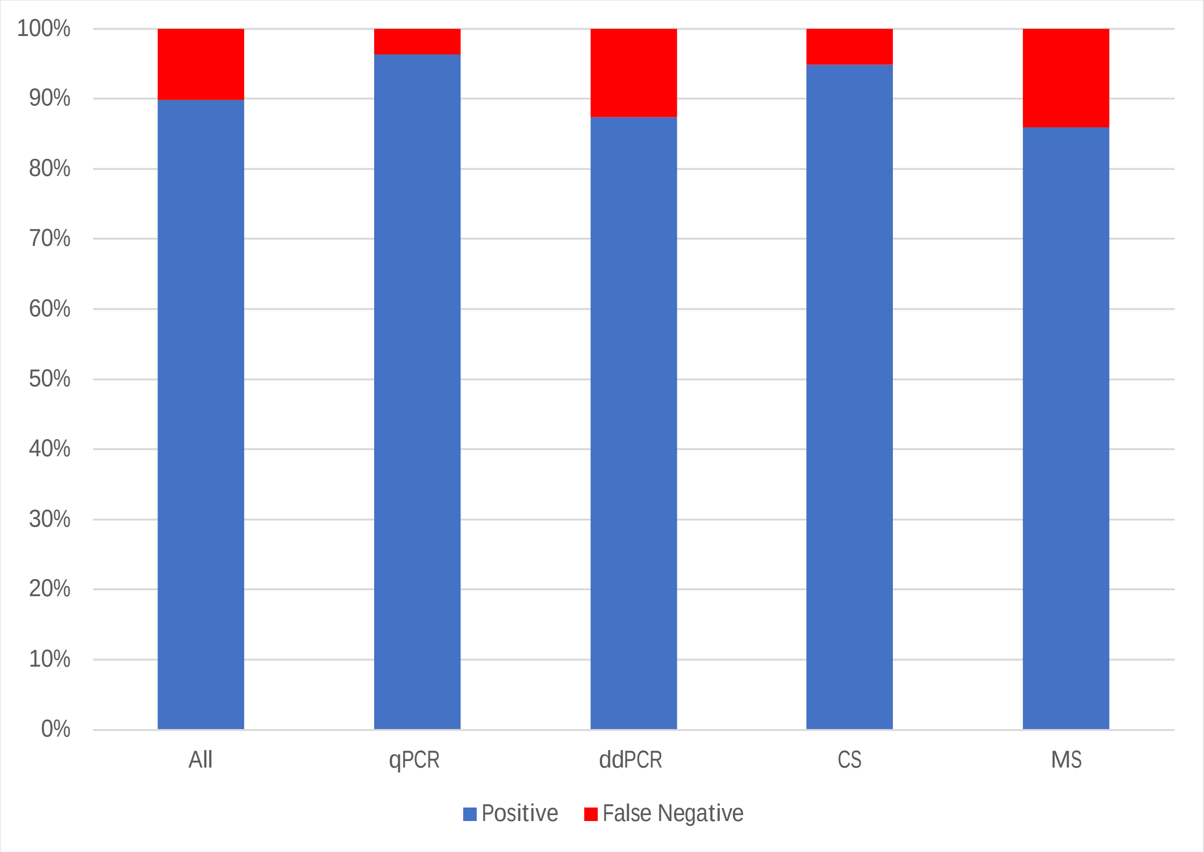


Figure 3.2. Detection rates of the four assays across all sites sampled in 2022 (Lake Rotoiti) and 2023 (Oranga Lake). CS = catfish specific metabarcoding assay; MS = multi-species metabarcoding assay.

A Kruskal Wallis test indicated that the number of positive hits per site across all combinations of eDNA assay and filter size for the Lake Rotoiti dataset was significantly different (*p* = 0.047). However, while this test indicated significant differences across the entire model, Wilcox pairwise comparisons revealed that none of the individual pairwise tests were significant, indicating a more complicated contrast than what was considered by the pairwise tests. Therefore, two additional Kruskal Wallis tests were conducted. The first was undertaken to compare the number of positive hits between different field methodologies (i.e., filter sizes) only, and the second was undertaken to compare the number of positive hits per eDNA assay. The first test indicated that there was no significant difference (*p* = 0.13) in detection rate between filter types; this included a single site (Delta Boat Ramp) where only two out of the 20 total eDNA assays (i.e., four assays with five replicates each) run on dacron filter samples resulted in a positive detection. The dacron filter was therefore removed from the survey design for the 2023 Oranga Lake sampling. The second Kruskal Wallis test revealed a significant difference (*p* = 0.003) in detection rate (hit number) between the four eDNA assays. Wilcox pairwise comparisons revealed that the hit rates for the qPCR and catfish-specific metabarcoding assays were significantly higher (*p* = 0.018 and *p* = 0.024, respectively) than those for the multi-species metabarcoding and ddPCR assays. However, there was no difference in detection rate between the qPCR and catfish-specific metabarcoding assays (*p* = 0.725), or between the multi-species metabarcoding and ddPCR assays (*p* = 0.760).

All eDNA assays had a detection rate of 5/5 at every Oranga Lake sample site, excluding ddPCR (where, at site Oranga Lake 1, catfish were detected in 4/5 fine and 4/5 coarse filter samples). A Kruskal Wallis test confirmed that there were no significant differences (*p* = 0.525) in detection rates across eDNA assays conducted on Oranga Lake samples in 2023 (*p* = 0.525).

3.4.3 Correlation analyses

Analysis of the Lake Rotoiti dataset revealed several significant associations across both the laboratory and field methodologies (Figure 3.3). Overall, eDNA reads and eDNA concentration were considerably higher in the Oranga samples when compared to the Lake Rotoiti samples (see Supplementary info); however, very few significant correlations were detected in the Oranga dataset, with only two significant relationships evident after eDNA results had been adjusted for volume filtered per sample. In the Oranga dataset, the coarse and fine filter eDNA results were correlated for both the catfish specific metabarcoding (r = 0.9; *p* = 0.037) and ddPCR (r = 1; *p* = 0.037) assays (Figure 3.3). Due to the few positive correlations identified in the Oranga dataset, the correlations identified below focus on the Rotoiti dataset.

Correlation analyses revealed that there were several significant associations across different filter sizes. Results from the 2022 Rotoiti dataset recorded a greater number of significant correlations between eDNA results from the fine and coarse filter samples than the dacron-filtered samples. The results from the samples filtered using dacron pores produced only three significant correlations with other sample results, when results were adjusted for volume filtered. These positive correlations all applied to samples processed utilising the catfish-specific metabarcoding assay (Figure 3.3). In contrast, eight and seven correlations were recorded for eDNA samples filtered with the fine and coarse filters, respectively, in the 2022 Rotoiti dataset. Additionally, correlations between sample results for both the fine and coarse filters were detected across all eDNA assays, except for the multi-species metabarcoding assay.

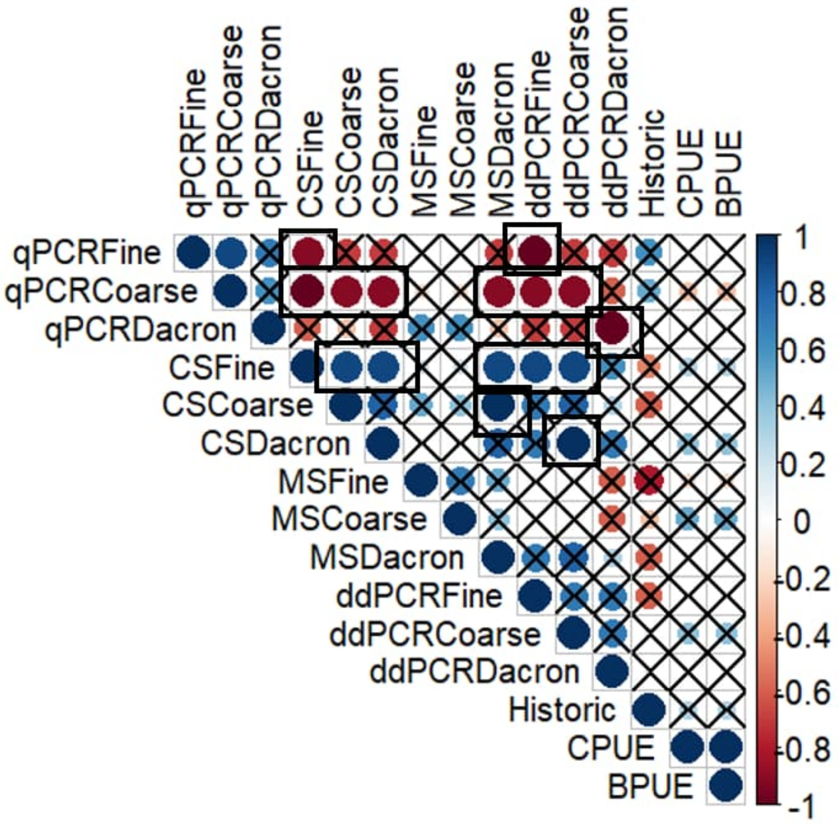
Comparisons between eDNA assays also revealed several significant correlations across methods (Figure 3.3). In the Rotoiti dataset, after adjusting for volume filtered, strong

($r \geq 0.9$) and significant correlations were identified between samples processed using the catfish specific assay and all the other assays, regardless of which filter size was used. The multi-species metabarcoding results were also correlated with all the other assays, but, interestingly, this only applied to the samples filtered using the dacron filter. In addition, the ddPCR results were positively correlated with the results of other methods, but this did not apply to any of the samples taken with the dacron filter.

Overall, samples collected using the coarse filter and analysed by qPCR displayed the largest number of significant associations with other methodologies, being strongly correlated with the results of all the catfish specific metabarcoding results ($p < 0.04$ in all cases), the ddPCR results from the fine and coarse filter samples ($p = 0.037$), and the multi-species metabarcoding results from the dacron filters ($p = 0.037$).

There were no significant correlations between eDNA and netting data for Lake Oranga, 2023 (Spearman, $p > 0.05$ for all possible associations (Figure 3.3), or for Lake Rotoiti dataset, with one exception: the fine filter eDNA samples analysed using the multi-species metabarcoding assay ($r = 0.9$; $p = 0.037$ for both CPUE and BPUE), following adjustment for volume of water filtered (Figure 3.3).

A)


qPCRFine
qPCRCoarse
qPCRDacron
CSFine
CSCoarse
CSDacron
MSFine
MSCoarse
MSDacron
ddPCRFine
ddPCRCoarse
ddPCRDacron
Historic
CPUE
BPUE
1
0.8
0.6
0.4
0.2
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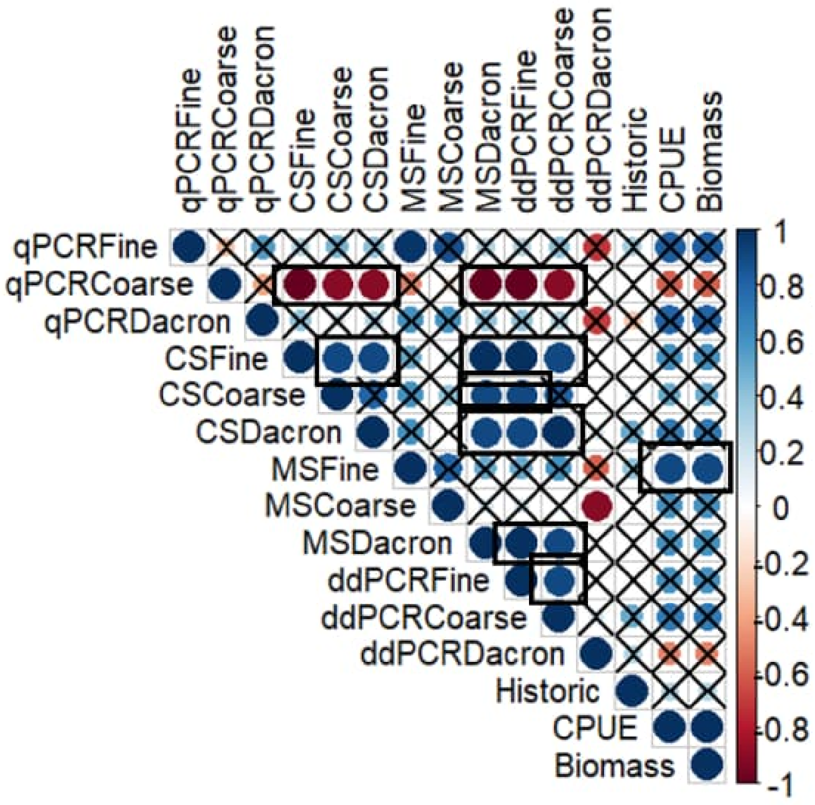
-0.2
-0.4
-0.6
-0.8
-1

B)


qPCRFine
qPCRCoarse
qPCRDacron
CSFine
CSCoarse
CSDacron
MSFine
MSCoarse
MSDacron
ddPCRFine
ddPCRCoarse
ddPCRDacron
Historic
CPUE
Biomass
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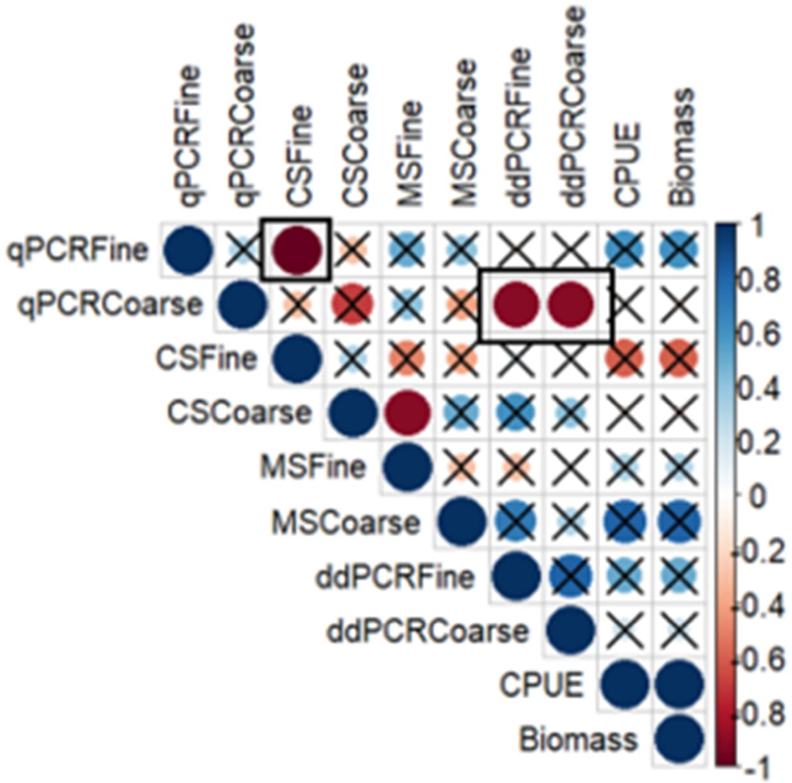
-0.2
-0.4
-0.6
-0.8
-1

C)


qPCRFine
qPCRCoarse
CSFine
CSCoarse
MSFine
MSCoarse
ddPCRFine
ddPCRCoarse
CPUE
Biomass
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-1

D)

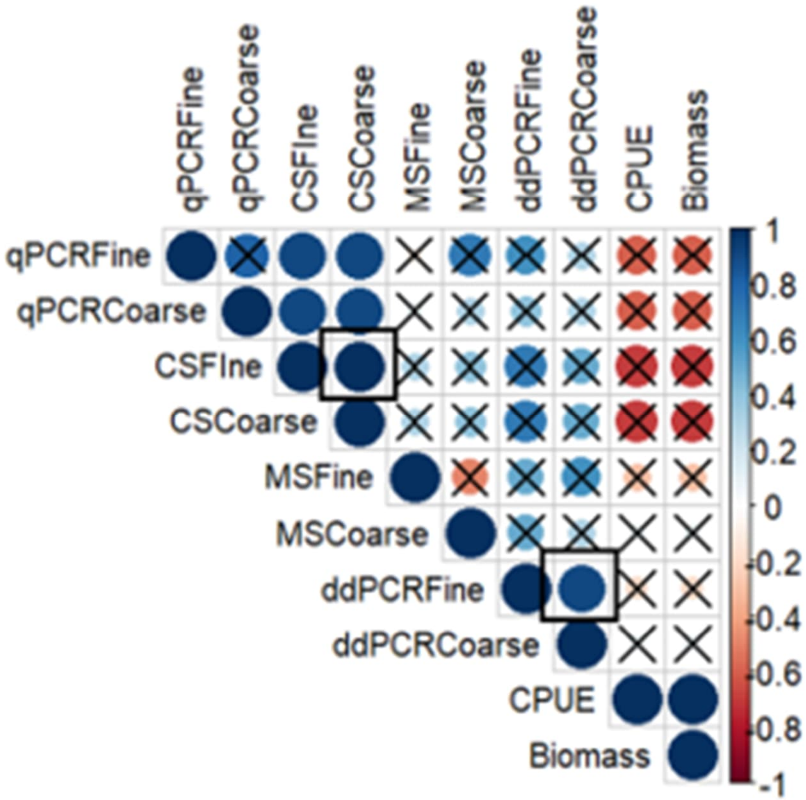
qPCRFine
qPCRCoarse
CSFIne
CSCoarse
MSFine
MSCoarse
ddPCRFine
ddPCRCoarse
CPUE
Biomass
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

Figure 3.3. Results of the correlation analysis comparing eDNA sample results, historic netting data, and contemporary netting data (i.e., samples taken immediately after eDNA sampling had occurred). Relationships that are not significant have been crossed out and significant relationships (Spearman, $p < 0.05$) are highlighted with black boxes, with the colours indicative of the correlation strength as per the colour scale to the right of each correlogram. Fine = 1.2 µm filter; Coarse = 5 µm; CS = catfish specific metabarcoding assay; MS = multi-species metabarcoding assay. Note that only an inverse (negative) relationship is considered significant between netting data and qPCR data (CT values). A) Lake Rotoiti, B) Lake Rotoiti, adjusted for volume filtered, C) Oranga Lake, D) Oranga Lake, adjusted for volume filtered.

Visualising the eDNA read data produced by the multi-species metabarcoding assay alongside BPUE to display the relationship between the two datasets highlighted Okawa Bay as an outlier, with a very high CPUE and BPUE but a relatively weak eDNA signal (Figure 3.4). However, the relative strength of the eDNA signal and the relative abundance detected by the netting results were similar across the remaining four sites (Figure 3.4).

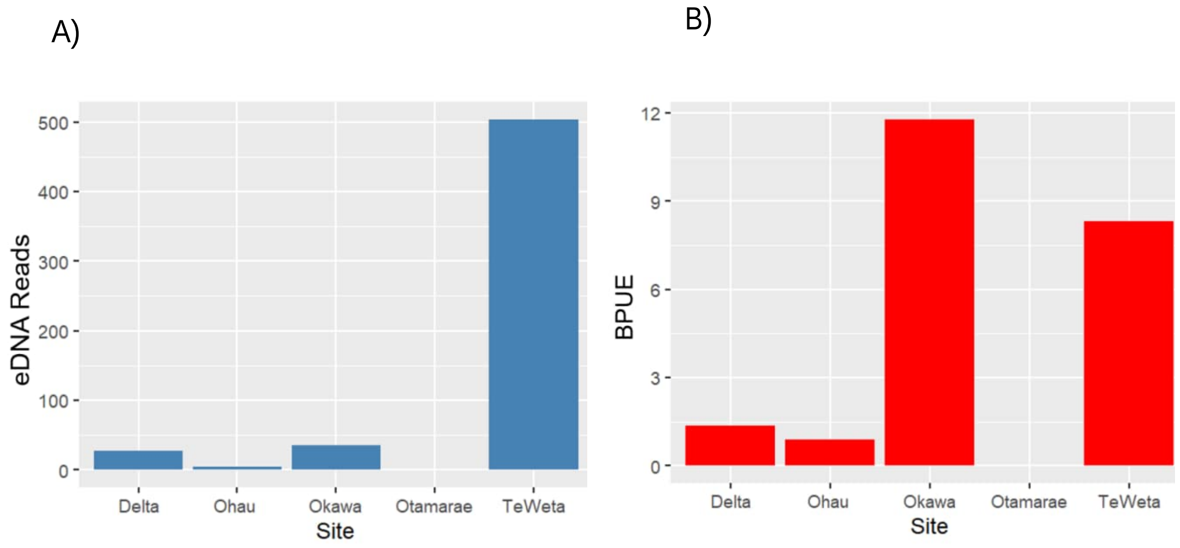

Figure 3.4. Comparing eDNA and traditional methods at Lake Rotoiti (2022). A) eDNA reads per site per L from the fine filter samples analysed with the multi-species metabarcoding assay; B) Biomass Per Unit Effort (BPUE) (g/hr) per site.

No significant correlations (Spearman, $p > 0.05$) were detected between the eDNA reads obtained from fine filter samples and the netting data for the non-catfish species detected in the multi-species metabarcoding assays at Rotoiti. Of note, two non-catfish species – the native invertebrate, kōura (*Paranephrops planifrons*), and the native giant bully (*Gobiomorphus gobioides*) – were captured in fyke nets but not detected by the multi-species metabarcoding assay for the fine filter samples (Table A3).

3.5 Discussion

We sampled three lakes in the North Island of New Zealand to investigate biomonitoring techniques for catfish and found that eDNA and traditional sampling methods produced dissimilar results. For eDNA, all three filter types were similarly successful at detecting catfish from five replicate samples, with the qPCR and catfish-specific metabarcoding assays having the highest detection rate and all methods returning some false negatives. The relationship between catch and eDNA data was limited, although we identified a significant correlation between contemporary abundance and the multi-species metabarcoding assay for 1.2 µm samples from Lake Rotoiti.

3.5.1 Environmental DNA for detecting catfish presence/absence

A key factor in whether eDNA can be successfully used as a biomonitoring tool is its ability to determine the presence (and absence) of a species with high accuracy (Erickson et al. 2019). Here, we found that all four eDNA assays produced multiple false negatives, emphasising the importance of collecting replicate samples from each site to ensure that the patchy distribution of eDNA in the environment does not lead to failed detection where invasive species are present. Current sampling guidelines report that three independent replicates per site is generally considered the minimum level of replication (Pawlowski et al. 2020). Hhowever our results suggested that – especially for those assays with lower detection rates (i.e., ddPCR and multi-species metabarcoding) – five replicates was a sufficient number to detect catfish where the species had previously been confirmed as present.

Filter size can have important impacts on species detection, due to different pore sizes allowing different-sized particles. For example, Toshiaki et al. (2020) found that filters with larger pores increased the capture of longer – and, therefore, less degraded – fragments of eDNA from water samples without reducing the yield of eDNA. Here, we found no consistent differences in the sensitivity of the three filter sizes for detecting catfish DNA. However, at one site in Lake Rotoiti (Delta Boat Ramp), the dacron filter performed poorly – with only 2/20 assays resulting in a positive detection, while the 1.2 µm and 5 µm filters detected catfish in 16/20 and 14/20 assays, respectively. Species detection can also be affected by the water volume that is filtered per sample, which is generally related to site water quality. As expected

(Thomas et al. 2018; Banks et al. 2021), we found that larger volumes of water could be filtered through larger pore sizes. However, we found no evidence that detection rate was affected by pore size/water volume filtered: at least one positive detection occurred in five (Lake Rotoiti) or six (Lake Oranga) replicate samples in all but the two instances with the dacron filter at the Delta Boat Ramp. This included samples from Oranga Lake where as little as 50 mL of water was filtered for some replicates. Thus, filtering a sufficient lake water volume may be less of an issue when the density of the target species is sufficiently high.

All of our eDNA laboratory assays detected catfish DNA in at least one replicate sample per site. Moreover, at one site (Otamarae), all four eDNA assays and all three filter sizes detected catfish when they were not captured by fyke nets, aligning with historic netting surveys that had recorded catfish at this site and providing an instance in which eDNA offered more effective detection than traditional netting. However, we found consistent differences in the sensitivity of the four laboratory assays, with the qPCR and catfish-specific metabarcoding assays detecting the highest number of positive catfish hits, while the ddPCR and the multi-species metabarcoding assays produced the greatest number of false negatives and lowest rates of detection. The multi-species metabarcoding assay also produced false negatives for other species that we captured in fyke nets, such as kōura and giant bully.

We anticipated that the multi-species metabarcoding assay would be less effective at detecting the presence of catfish, consistent with previous studies (Wood et al. 2019; McColl-Gausden et al. 2023). However, we did not expect ddPCR to be less effective than the other three assays, as the nanodroplet sample partitioning associated with this assay is designed to be especially sensitive and previous comparative studies have shown ddPCR to be a superior detection tool when compared to metabarcoding and qPCR (Doi et al. 2015; Wood et al.

2019). Our qPCR and metabarcoding assays were carried out in the same laboratory where the DNA was extracted. However, the extracted DNA was then transported on ice to the laboratory where the ddPCR assay was performed. Thus, sample quality may have deteriorated during storage or transport, resulting in the lower ddPCR detection rate found here. Future research into the sensitivity of the different methods to sample storage/transport following DNA extraction would help to confirm whether this explains our ddPCR detection results.

Overall, though there was no significant difference in detection rate between the catfish specific metabarcoding and qPCR assays in our study, the qPCR assay produced the lowest number of false negatives of all the assays and may therefore be the best choice for detecting catfish in New Zealand's lacustrine environments. However, future work should explore more sites where catfish abundance is known to be low, as well as the effects of seasonal sampling. While our study consisted of sites with a known variation in catfish density in Lake Rotoiti (low – high density), we were unable to include sites on the eastern shores of Lake Rotoiti, where catfish are thought to have recently invaded and thus densities are expected to be low; these additional sites would be useful for further eDNA research. Moreover, the rate of false negatives is likely to vary in relation to various factors, including seasonal activity levels (Troth et al. 2021). Our study was carried out at the end of autumn and beginning of winter in both sampling years – a time when catfish move towards the deeper areas of lakes and are less active (Dedual 2017). While it is encouraging that detection rates remained high across the sampling times employed in our study, eDNA sampling at other times of the year might yield different detection rates and should be investigated.

3.5.2 Semiquantitative aspects: Correlation between netting data and eDNA data

A key objective of eDNA research is to determine whether eDNA counts can serve as a proxy for semi-quantitative measures of relative species abundance (Rees et al. 2014). This has proven challenging to date because eDNA is affected by variables that hinder the interpretation of the strength of the eDNA signal as an estimate of relative abundance. For example, if an animal moves away from a sample site shortly before sample collection, its DNA signal will persevere at that site, which may confuse any correlation between eDNA signal and physical animal counts. Likewise, if target DNA is carried by water currents to a sample site, positive eDNA detection could occur without a corresponding physical detection (Thomsen et al. 2012). The strength of correlation between biomass and eDNA signal is also influenced by site conditions, such as local water quality (Rees et al. 2014).

Nevertheless, several studies have identified significant positive correlations between measured animal biomass and eDNA concentration (Takahara et al. 2012; Thomsen et al. 2012; Pilliod et al. 2013; Klymus et al. 2015; Banks et al. 2021; Plante et al. 2021; Everts et al. 2022), though others have shown that this relationship is variable in the natural environment and across different species (Biggs et al. 2015; Spear et al. 2015; Goldberg et al. 2016; Sanchez et al. 2022). In our study, a single significant relationship between biomass and eDNA estimates was detected for the fine filter samples processed using the multi-species metabarcoding assay from the Lake Rotoiti dataset. This aligns with previous research on carp, where the slope of the regression line between gene copy number and biomass was greater when filters with smaller pores were used (Eichmiller et al. 2016), and may indicate some potential for eDNA as a tool to estimate catfish population densities. However, the multi-species metabarcoding assay was the least sensitive assay in our study, supporting the idea that lower detection rates may promote the ability of the multi-species metabarcoding assay to detect variation in abundance, while saturation via detection of high amounts of target DNA may prevent estimation of biomass for the other eDNA assays (Di Bernardo et al. 2007). This would also explain the absence of significant relationships between biomass and eDNA results from Oranga Lake, which produced very high eDNA signals across all sampling sites in the current study. Further studies are needed to fine-tune and validate the interpretation of multi-species metabarcoding results as an estimate of relative abundance in natural freshwater environments, so that researchers can best understand the trade-offs for detection and semi-quantitative outcomes arising from species-specific versus community eDNA-based approaches.

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Chapter 4. Thesis discussion

4.1 General overview

The overarching aims of my thesis were to investigate behavioural evolution in invasive species and the application of genetic tools to invasion biology to together inform and enhance the prediction, monitoring, and management of invasion events. Chapter 2 shed light on the intraspecific behavioural shifts that occur during biological invasion, with emphasis on phenotypic plasticity and the evolutionary processes associated with observed behavioural changes. A key finding was the gap in research on the evolutionary, genetic, and molecular processes that underlie behavioural shifts in invasive species and the need to exploit contemporary invasion events as opportunities for longitudinal studies of the invasive process. In Chapter 3, I identified the eDNA assays that offer potential for the detection for invasive catfish populations in lentic environments and found that multi-species metabarcoding assays may have the potential to produce semi-quantitative estimates of abundance. These findings enabled recommendations to be made that will facilitate the future development and application of an effective, standardised methodology for bio-surveillance and monitoring of aquatic communities. In this current chapter, I conclude my thesis by highlighting outstanding knowledge gaps and providing recommendations for future research direction.

4.2 Recommendations and future work

4.2.1 The role of behavioural studies in invasion biology

There are three factors that are considered key for predicting the consequences that follow the arrival of an alien species in a new environment: (i) which species are likely to be successful invaders; (ii) the likely distribution of invaders in the new habitat; and (iii) the effects the invaders will have on the recipient community (Griffen et al. 2021). The third component has been an important focus of invasion biology, with several methods developed to predict the impacts of invasive species following their introduction. These methods generally forecast impacts using the invasion history of a species (Ricciardi 2003), data on the per capita impact of a species on prey (Lohrer and Whitlatch 2002), and/or a calculation of the anticipated impact of the invasive relative to the resident native species (Dick et al. 2017). Thus, these models typically rely on the track record of a species in its native range or in previously invaded ranges, with limited consideration of the complex interactions between an invasive species and its recent or hypothetical new context.

Almost all the effects inflicted by invasive species upon the native community are likely to be mediated by behaviour (Suarez and Phillips 2012), including those that differentiate a species from its native and invasive range and those that occur during the interaction of native and invasive species (e.g., predation, parasitism, competition). For example, the rate of spread of an invasive species and, consequently, the scale of its impact, is heavily influenced by dispersal behaviour (Suarez and Phillips 2012), and behaviour during the early stages of invasion can allow for individual modification to occur as a first response before adaptive processes initiate changes in morphological, physiological, or life history traits (Lande 2009; Chevin et al. 2010; West-Eberhard 2020). In Chapter 2, I referenced multiple examples of invasive populations exhibiting shifts in behavioural trait means compared to their native conspecifics, with these often increasing the invasive population's ability to capitalise on novel resources (Tsutsui et al. 2000; Blight et al. 2017; Chapple et al. 2022).

An understanding of behavioural evolution during invasion allows for a more effective identification of threats prior to the establishment and spread of a potential invasive species.

However, as far as I am aware, a model has yet to be developed that utilises behaviour as a predictive variable in forecasting the impact of an invasive species. In the literature review documented in Chapter 2, I found a general paucity of published research on invasive behaviour within the wider field of invasion biology, despite its key management implications. This is likely due to a lack of appreciation among environmental managers and funding agencies of the value of animal behaviour in invasive species management. Additional barriers to conducting behavioural studies on invasive species - such as the ethics approvals and special permissions required to keep captive individuals of invasive species – are also almost certainly an issue, alongside the technical expertise and time/labour expense involved. Thus, a key recommendation for future work is to overcome these barriers in order to gain a greater understanding of the behavioural changes that facilitate invasion both within and across species. Identification of common patterns and trends will lead to better prediction of invasive behaviour, potentially leading to better management of invasive species and their impacts. In Chapter 2, I also highlighted how an understanding of behavioural changes at frontier populations should be a key focus of future research, as it is at the range edge that "invasiveness" (e.g., dispersal tendency), accelerates to expand a species range and worsen its impact.

4.3 Evolutionary and genetic change during invasion

Genetic techniques help us understand the role of adaptation in invasion success (McCartney et al. 2019), facilitating an understanding of evolutionary change during invasion and the conditions under which invasiveness is enhanced or suppressed (Olazcuaga et al. 2020). Technological advances are advancing our ability to study and examine the genomic basis for the phenotypic shifts associated with biological invasions (Rius et al. 2015). However, in

Chapter 2 I noted a marked gap in research on the evolutionary, genetic, and molecular processes that underlie behavioural shifts in invasive species. While phenotypic changes are widely reported in invasive populations, and theories regarding the role of evolution and plasticity in these shifts abound, there is a significant lack of empirical research that investigates the underlying processes. I speculate this is partly due to inherent challenges associated with studying the invasion process and associated adaptive and genetic processes in real time. Many invasive populations established in the past, incursions are often not detected until after an invasive population has reached to a certain size or density, and new invasions (though increasing) can still be relatively infrequent in the face of intensive efforts to prevent them. Thus, it is imperative that opportunities to study invasions in real time are exploited when they do occur.

In Chapter 2, I emphasised the need for longitudinal studies that follow the invasion process as it occurs. These will help to map an invasive's spread and to characterise the adaptive processes and underlying genetic changes that are operating in parallel. However, this requires agile agencies and funders that are able to rapidly mobilise resources to initiate research as soon as a potential invasive is detected in a new environment. A contemporary example is that of the very recent (May 2023) detection of the freshwater gold clam (*Corbicula fluminea*) in the Waikato River of New Zealand. While unfortunate, such incursions represent fascinating examples of wild experiments that can be exploited to better study the invasion process and associated evolutionary and molecular processes in real time. I recommend the rapid initiation of a long-term study of this, and other new New Zealand introductions to inform their rapid management and advance our general understanding of the invasive process.

4.4 The application of eDNA methodology to bio-surveillance and biomonitoring

The field of eDNA is no longer in its infancy, with validation of the methodology and demonstration of its potential resulting in accelerated uptake and use of the tool over the last 5-10 years. New Zealand has been relatively active in developing and adapting eDNA methodology, and the commercial availability of testing kits and laboratory processing has resulted in broad application of the tool by local government agencies and environmental consultants, especially in aquatic environments.

However, unlike conventional monitoring, eDNA has yet to be recognised as a validated approach to routine monitoring or ecological surveys. For example, the National Policy Statement for Freshwater Management 2020 (NPS-FM) produced by the New Zealand Ministry for the Environment (MfE) guides regional and territorial authorities to carry out their State of the Environment (SOE) monitoring following the methodologies published in the *New Zealand Freshwater Fish Sampling Protocols* (Joy et al. 2013), which include electrofishing, spotlighting, and trapping/netting methods. These protocols document standardised approaches that have remained largely unchanged for several decades, if not longer, and that allow for comparison of data across locations and years and provide a minimum standard for the collection, comparison, and interpretation of reliable data. In contrast, eDNA is rapidly developing, with ongoing methodological advancements and emerging technologies, such as nanopore sequencing (Egeter et al. 2021). Thus, a challenge with eDNA is to standardise sample collection methods, statistical processes for data analysis, and standards for data interpretation and reporting – including how results can be interpreted alongside the existing body of conventional monitoring methods, which eDNA complements but cannot currently (or perhaps ever) replace.

To facilitate the uptake and application of eDNA, there is thus a need for standardised protocols. In Chapter 3, I presented a study that represents a step towards this for eDNA sampling in lake environments in New Zealand. In this chapter, I provide recommendations for the number of sample collection replicates and for the types of laboratory eDNA assays that should be used to detect invasive catfish. In particular, I found that the species-specific qPCR and catfish-specific metabarcoding assays outperformed the multi-species metabarcoding assay in their catfish detection ability.

However, a key caveat is that these results are not an indication that qPCR or species-specific metabarcoding approaches are necessarily the most appropriate choice for every study. The right tool for the job depends on the aim of the work. For example, if surveillance or detection of a specific invasive species, such as the freshwater gold clam, is the chief aim of a study, a species-specific assay is recommended, as this will increase the likelihood of detection at low densities. However, where community characterisation is the goal, or surveillance is being conducted for a broad set of potential invasives (or for a future unknown objective, such as the retrospective detection of an invasive species), the multi-species metabarcoding approach may be more cost effective. Such trade-offs between a study's current and potential future motivation require careful consideration, particularly as rates of invasion are increasing. There is also a clear movement in biodiversity (and other) research fields towards reusability of data for biomonitoring and other applications (Mons 2018).

In New Zealand eDNA methods have been enthusiastically adopted. However, those who use and apply the methodology do not always have a comprehensive understanding of its underlying mechanisms and limitations, or of how to accurately interpret the results, which are often in the form of raw datasets from commercial service providers. Therefore, I recommend the development of a framework that will guide environmental agencies and consultants to apply the most appropriate sampling design for their specific contexts and study questions. Given the complexity and range of potential biosecurity-related management questions and monitoring purposes, creating a single standardised protocol will be a nuanced task, made more challenging by the fact that the eDNA field is constantly advancing. However, a universal, flexible protocol that ensures detection in varying habitats, and for an array of purposes, could be developed through consultation with scientists and stakeholders working in this field nationally.

One of the current limitations of eDNA is that results cannot be reliably interpreted as estimates of species density or abundance. However, encouragingly, in Chapter 3, my results indicated that there is potential for the multi-species metabarcoding assay to be applied as an estimate of relative abundance. While this is an exciting result, it requires further investigation and testing across different species and environments. This is an active area of research in the field, and it is likely that future studies will better elucidate these outstanding questions, as well as those relating to the use of eDNA to determine population distributions and demographics. In the meantime, bio-surveillance and biodiversity programmes should continue to integrate complementary approaches in their biomonitoring protocols.

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Appendix A3

Supplementary information: Chapter 3

Table A3.1 *Ameiurus nebulosus* eDNA results for each replicate environmental sample from four laboratory assays (qPCR, ddPCR, catfish specific metabarcoding, and multi-species metabarcoding) and netting data (catch per unit effort, CPUE; and biomass per unit effort, BPUE) at Lake Rotoiti, 2022.

Site	Filter pore size	Average eDNA result	Standard Deviation	Standard Error of the Mean	Positive detection rate	CPUE (count/hr)	BPUE (g/hr)
qPCR		Average CT					
Ohau Channel Mouth	1.2 µm	37.39	2.35	0.63	5/5	0.11	0.88
	5 µm	35.80	0.88	0.28	5/5		
	Dacron	35.38	1.19	0.31	5/5		
Okawa Bay	1.2 µm	34.78	1.74	0.39	5/5	1.37	11.77
	5 µm	34.06	0.93	0.23	4/5		
	Dacron	32.78	0.88	0.20	5/5		
Delta Boat Ramp	1.2 µm	37.92	2.06	0.69	5/5	0.77	1.37

	5 µm	35.92	1.29	0.92	5/5		
	Dacron	36.76	0.00	0.00	1/5		
Te Weta Bay	1.2 µm	35.33	1.76	0.39	5/5	0.23	8.32
	5 µm	35.66	1.12	0.27	5/5		
	Dacron	36.34	0.73	0.30	5/5		
Otamarae	1.2 µm	34.95	1.25	0.28	5/5	0.00	0.00
	5 µm	36.74	2.21	0.64	5/5		
	Dacron	35.92	0.86	0.26	5/5		
ddPCR		Average DNA copy number / L					
Ohau Channel Mouth	1.2 µm	0.08	0.04	0.02	5/5	0.11	0.88
	5 µm	0.06	0.06	0.03	4/5		
	Dacron	0.15	0.11	0.05	4/5		
Okawa Bay	1.2 µm	0.48	0.22	0.10	5/5	1.37	11.77
	5 µm	0.48	0.24	0.11	5/5		
	Dacron	1.22	0.77	0.35	5/5		
Delta Boat Ramp	1.2 µm	0.04	0.06	0.03	2/5	0.77	1.37
	5 µm	0.01	0.01	0.00	2/5		

	Dacron	0.00	0.01	0.00	1/5		
Te Weta Bay	1.2 µm	0.27	0.13	0.06	5/5	0.23	8.32
	5 µm	0.09	0.10	0.04	3/5		
	Dacron	0.02	0.01	0.00	4/5		
Otamarae	1.2 µm	0.32	0.23	0.10	5/5	0	0
	5 µm	0.05	0.03	0.01	5/5		
	Dacron	0.12	0.11	0.05	5/5		
Catfish specific metabarcoding assay		Average reads					
Ohau Channel Mouth	1.2 µm	1019.20	498.17	222.79	5/5	0.11	0.88
	5 µm	762.80	439.90	196.73	5/5		
	Dacron	2812.60	1429.84	639.45	5/5		
Okawa Bay	1.2 µm	4295.60	744.27	332.85	5/5	1.37	11.77
	5 µm	4196.00	810.88	362.64	5/5		
	Dacron	13473.20	4718.72	2110.27	5/5		
Delta Boat Ramp	1.2 µm	711.20	542.21	242.48	4/5	0.77	1.37
	5 µm	316.40	211.01	94.37	5/5		
	Dacron	0.00	0.00	0.00	0/5		

Te Weta Bay	1.2 µm	4150.80	2649.37	1184.83	5/5	0.23	8.32
	5 µm	5151.80	3252.26	1454.45	5/5		
	Dacron	3146.20	1549.07	692.76	5/5		
Otamarae	1.2 µm	2159.60	815.73	364.81	5/5	0.00	0.00
	5 µm	2207.00	1424.63	637.11	5/5		
	Dacron	530.40	353.98	158.31	5/5		
Multi-species metabarcoding assay		Average reads					
Ohau Channel Mouth	1.2 µm	4.00	3.74	1.67	3/5	0.11	0.88
	5 µm	1.00	3.80	1.60	2/5		
	Dacron	100.00	13.21	5.91	4/5		
Okawa Bay	1.2 µm	27.00	16.69	7.46	5/5	1.37	11.77
	5 µm	26.20	14.60	6.53	5/5		
	Dacron	154.00	82.55	36.92	5/5		
Delta Boat Ramp	1.2 µm	27.40	14.86	6.65	5/5	0.77	1.37
	5 µm	74.40	140.58	62.87	2/5		
	Dacron	0.00	0.00	0.00	0/5		
Te Weta Bay	1.2 µm	377.40	343.92	153.80	5/5	0.23	8.32

	5 µm	119.80	95.82	42.85	5/5		
	Dacron	222.20	195.59	87.47	4/5		
Otamarae	1.2 µm	37.00	38.37	17.16	4/5	0	0
	5 µm	15.40	7.91	3.54	4/5		
	Dacron	29.40	51.66	23.10	3/5		

Table A3.2 *Ameiurus nebulosus* eDNA results for each replicate environmental sample from four laboratory assays (qPCR, ddPCR, catfish specific metabarcoding, and multi-species metabarcoding) and netting data (catch per unit effort, CPUE; and biomass per unit effort, BPUE) at Oranga Lake, 2023.

Site	Filter pore size	Average eDNA result	Standard Deviation	Standard Error of the Mean	Positive detection rate	CPUE	BPUE (g)
qPCR		Average CT					
Oranga Lake 1	1.2 µm	31.15	0.54	0.22	5/5	0.26	2.56
	5 µm	31.56	0.55	0.22	5/5		
Oranga Lake 2	1.2 µm	33.75	1.22	0.50	5/5	0.92	7.32
	5 µm	30.37	1.48	0.60	5/5		
Oranga Lake 3	1.2 µm	35.40	1.45	0.59	5/5	8.38	34.73
	5 µm	31.01	0.52	0.21	5/5		
Oranga Lake 4	1.2 µm	30.34	1.35	0.55	5/5	0.35	2.98
	5 µm	28.68	1.19	0.49	5/5		
Oranga Lake 5	1.2 µm	32.26	1.07	0.44	5/5	0.13	0.90
	5 µm	30.51	0.70	0.29	5/5		

ddPCR		Average DNA copy number / L					
Oranga Lake 1	1.2 µm	2.43	2.20	0.90	4/5	0.26	2.56
	5 µm	0.27	0.26	0.11	4/5		
Oranga Lake 2	1.2 µm	16.06	26.45	10.80	5/5	0.92	7.32
	5 µm	4.03	2.48	1.01	5/5		
Oranga Lake 3	1.2 µm	10.82	14.05	5.73	5/5	8.38	34.73
	5 µm	0.45	0.32	0.13	5/5		
Oranga Lake 4	1.2 µm	175.47	129.95	53.05	5/5	0.35	2.98
	5 µm	5.35	1.75	0.71	5/5		
Oranga Lake 5	1.2 µm	24.85	1.18	7.11	5/5	0.13	0.90
	5 µm	3.34	1.18	0.48	5/5		
Catfish specific metabarcoding assay		Average reads					
Oranga Lake 1	1.2 µm	9,676.17	1,589.45	648.89	5/5	0.26	2.56
	5 µm	6,597.00	3,453.02	1,409.69	5/5		
Oranga Lake 2	1.2 µm	4,321.50	2,697.92	1,101.42	5/5	0.92	7.32
	5 µm	9,255.17	4,071.99	1,662.38	5/5		

Oranga Lake 3	1.2 µm	3,068.50	3,140.30	1,282.02	5/5	8.38	34.73
	5 µm	9,404.17	4,317.37	1,762.56	5/5		
Oranga Lake 4	1.2 µm	22,668.83	5,894.93	2,406.59	5/5	0.35	2.98
	5 µm	27,917.00	5,650.49	2,306.80	5/5		
Oranga Lake 5	1.2 µm	7,246.50	5,438.05	2,220.08	4/5	0.13	0.90
	5 µm	20,201.30	5,215.96	2,129.41	5/5		
Multi-species metabarcoding assay		Average reads					
Oranga Lake 1	1.2 µm	438.67	234.33	95.66	5/5	0.26	2.56
	5 µm	373.50	145.79	59.52	5/5		
Oranga Lake 2	1.2 µm	722.00	487.60	199.06	5/5	0.92	7.32
	5 µm	580.00	105.81	43.20	5/5		
Oranga Lake 3	1.2 µm	288.33	291.05	118.82	3/5	8.38	34.73
	5 µm	1,320.00	882.86	360.43	5/5		
Oranga Lake 4	1.2 µm	103.50	71.47	29.18	5/5	0.35	2.98
	5 µm	609.67	197.02	80.43	5/5		
Oranga Lake 5	1.2 µm	161.67	115.73	47.24	5/5	0.13	0.90
	5 µm	485.50	293.75	119.93	5/5		

Table A3.3 Fyke net data for Lake Rotoiti sample sites (2022), and Oranga Lake sample sites (2023).

Year	Site	Species	Count
2022	Ohau Channel Mouth	Brown bullhead catfish (*Ameiurus nebulosus*)	2
		Common bully (*Gobiomorphus cotidianus*)	~1,000
		Common smelt (*Retropinna retropinna*)	>300
		Giant bully (*Gobiomorphus gobioides*)	16
		Kōura / freshwater crayfish (*Paranephrops planifrons*)	4
	Okawa Bay	Brown bullhead catfish (*Ameiurus nebulosus*)	27
		Common bully (*Gobiomorphus cotidianus*)	759
		Giant bully (*Gobiomorphus gobioides*)	1
		Goldfish (*Carassius auratus*)	3
	Delta Boat Ramp	Brown bullhead catfish (*Ameiurus nebulosus*)	16
		Common bully (*Gobiomorphus cotidianus*)	~1,500
		Common smelt (*Retropinna retropinna*)	20
		Giant bully (*Gobiomorphus gobioides*)	7
		Goldfish (*Carassius auratus*)	36
		Kōaro (*Galaxias brevipinnis*)	4
		Kōura / freshwater crayfish (*Paranephrops planifrons*)	4

	Te Weta Bay	Brown bullhead catfish (*Ameiurus nebulosus*)	3
		Common bully (*Gobiomorphus cotidianus*)	433
		Common smelt (*Retropinna retropinna*)	11
		Giant bully (*Gobiomorphus gobioides*)	5
		Goldfish (*Carassius auratus*)	25
		Kōaro (*Galaxias brevipinnis*)	1
	Otamarae	Common bully (*Gobiomorphus cotidianus*)	319
		Common smelt (*Retropinna retropinna*)	2
		Giant bully (*Gobiomorphus gobioides*)	5
		Kōaro (*Galaxias brevipinnis*)	4
2023	Oranga Lake 1	Brown bullhead catfish (*Ameiurus nebulosus*)	5
		Common bully (*Gobiomorphus cotidianus*)	11
		Gambusia affinis	158
		Shortfin eel (*Anguilla australis*)	6
	Oranga Lake 2	Brown bullhead catfish (*Ameiurus nebulosus*)	17
		Common bully (*Gobiomorphus cotidianus*)	41
		Gambusia affinis	1
		Shortfin eel (*Anguilla australis*)	4

	Oranga Lake 3	Brown bullhead catfish (*Ameiurus nebulosus*)	147
		Common bully (*Gobiomorphus cotidianus*)	316
		Gambusia affinis	223
		Goldfish (*Carassius auratus*	2
	Oranga Lake 4	Brown bullhead catfish (*Ameiurus nebulosus*)	6
		Common bully (*Gobiomorphus cotidianus*)	6
		Gambusia affinis	166
		Shortfin eel (*Anguilla australis*)	3
	Oranga Lake 5	Brown bullhead catfish (*Ameiurus nebulosus*)	2
		Common bully (*Gobiomorphus cotidianus*)	114
		Gambusia affinis	385
		Shortfin eel (*Anguilla australis*)	21