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Using genetic techniques to improve understanding of source-sink dynamics of the invasive mosquitofish, *Gambusia affinis*

A thesis

submitted in fulfilment

of the requirements for the degree

of

Master of Science (Research) in Ecology and Biodiversity

at

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by

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Abstract

Biodiversity loss is more prominent in freshwater versus terrestrial or marine ecosystems, with temperate estuaries believed to be the most degraded of all ecosystems globally. This is due, in part, to the proliferation of invasive non-native species and the inadequacies of current biomonitoring practices for monitoring population trends over sufficient spatiotemporal scales to inform management decisions. Environmental DNA (eDNA; genetic material shed to the surrounding environment by organisms) is an increasingly popular, non-invasive biomonitoring method that provides the opportunity to increase the scale at which population trends can be monitored, while also having the potential as a sampling method for population genetic studies. My thesis aimed to use a variety of methods to understand source-sink population dynamics at varying spatial and temporal scales and explore the value genetic techniques can provide to species management, using the invasive pest fish, *Gambuis affinis*, as a test case.

Chapter 2 used traditional netting techniques to survey occupancy patterns of *G. affinis* to understand the species' physical dispersal drivers within a large coastal ecosystem in the South Island of New Zealand. I demonstrated a decrease in abundance and likelihood of *G. affinis* presence as tide height increased, and a decrease in abundance of *G. affinis* at a specific site as rainfall increased. These findings show that monitoring of occupancy patterns of freshwater fish is often done at too small a spatiotemporal scale and that future work should incorporate additional methods to increase the scale at which monitoring is performed.

Chapter 3 analysed mitochondrial DNA collected across New Zealand from both tissue and water samples to explore the efficacy of eDNA as a sampling tool for determining mitochondrial variation at large spatiotemporal scales. I showed that >99% of the *G. affinis* mitochondrial assemblage was shared by both tissue and water samples, but the remaining detected genetic variation was unique to either tissue or water only. These findings show that, while that eDNA holds great promise for biomonitoring in freshwater environments, careful consideration of study design is required for its use in assessing the drivers of biodiversity differentiation.

Together, my thesis demonstrates the utility of eDNA metabarcoding techniques as a complement to traditional survey methods to increase the spatiotemporal scale at which biomonitoring is undertaken. Continued application of a combination of these methods will facilitate improved knowledge of occupancy and dispersal patterns for *G.* affinis and, as such, greater protection of our native ecosystems and species.

Acknowledgements

By submitting this thesis, I can proudly close this chapter of both personal and professional development – one that has been the culmination of years of work and interest. I would not have been able to complete this without the amazing support I have received by a number of people.

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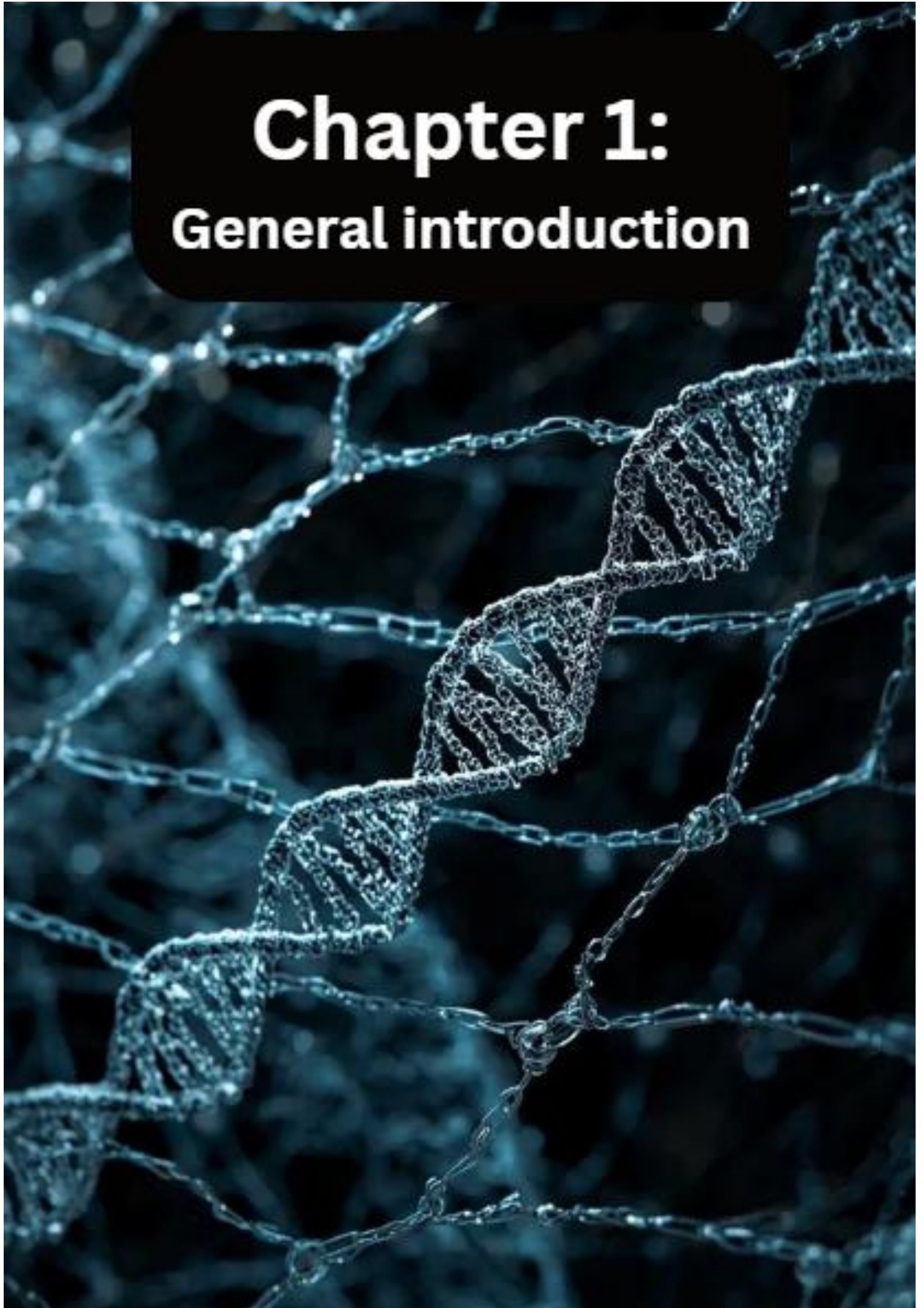
Chapter 1:
General introduction

1.1 Introduction

The concept of ecosystem services reflects the core idea that ecosystems contribute to human well-being (Kandziora et al. 2013; Jax et al. 2013), and are commonly classified into three broad categories: regulating, such as pollination and erosion control; provisioning, such as crop harvests and mineral resources; and culture, such as recreation, tourism, and landscape aesthetics (Kandziora et al. 2013). However, achieving nature protection while providing for ecosystem 'services' is complex, and management at the human-environment interface often does not include a clear linkage between the two (Kandziora et al. 2013; Jax et al. 2013; Ruiz-Frau et al. 2013). Thus, services derived from extractive relationships (generally provisioning) in the human-environment system undisputably contribute to biodiversity degradation (Ruiz-Frau et al. 2013), and as the demand and value of these relationships increases, the permanence and resilience of ecosystems and species reduce. Knozowski et al. (2023) explains this paradigm through analysis of the relationship between an increasing value of crop harvests and biodiversity loss in grasslands of Western Europe, while Pérez-Fleitas et al. (2023) mention the value of the global trade for crocodilian meat and skin irrespective of global declines in natural populations. Despite attempts to protect natural environments and the ecosystem services they provide (Geange et al. 2019), it is clear that the human-environment system is unbalanced in favour of anthropogenic benefits, and ecosystem services are generally provided for at the detriment of nature protection.

Biodiversity loss is more pronounced in freshwater ecosystems than terrestrial or marine (MacNeil et al. 2024), with temperate estuaries believed to be the most degraded of all ecosystems globally (Tweedley et al. 2017). As just under half of the world's population lives within 100 km of the ocean (Bishop et al. 2017), threats relating to extractive, anthropogenic relationships, such as habitat destruction (Pérez-Fleitas et al. 2023; Walker et al. 2006), fragmentation (Gillet 2008; Walker et al. 2008), climate change, over-exploitation, and pollution (Lanzén et al. 2021), are naturally more prevalent in these ecosystems. In addition, the spread of invasive non-native species (INNS) - either deliberately via non-extractive, anthropogenic relationships, such as recreation and tourism, or accidentally as a component of human well-being, such as via employment and transport - is attributed to significant decline in species persistence and ecosystem integrity across all ecosystems (Mayfield III et al. 2021; Meyer et al. 2021).

1.2 Invasion and freshwater ecosystems

A non-native species (NNS) is one that has established outside its natural range through natural or human-mediated processes, while an INNS is an NNS that poses a specific adverse impact to human well-being (Paini et al. 2016), or ecosystem functioning (MacNeil et al. 2024; Blackburn et al. 2004). Not all NNS become INNS, as some lack the necessary resistance to biotic and abiotic factors in the established range, including habitat generality and adaptability, to survive and spread. Simberloff and Von Holle (1999) describes this phenomenon as biotic resistance but does not go as far as to incorporate abiotic consideration, such as habitat favourability or differing climates. Bajer et al. (2015) postulate that understanding of both biotic and abiotic processes is needed to understand invasion success (both initial establishment and ongoing spread).

INNS are recognised as a major threat to ecosystem function and a key driver of global biodiversity loss (MacNeil et al. 2024; Xiong et al. 2019). In New Zealand, in association with habitat degradation, competition for food, predation, and degradation to ecosystem processes, INNS "pose the greatest single threat to our remaining natural ecosystems and habitats and threatened native species." (Department of Conservation 2000). The Ministry for the Environment (1997) further decrees INNS as an army that is 'our second historical legacy… which now threatens our remaining natural habitats."

Freshwater ecosystems are more prone to biological invasions than other ecosystems (MacNeil et al. 2024) and introduced freshwater species have a wide variety of direct and indirect ecological impacts on native freshwater species and ecosystems (for

examples of major reviews see Gozlan et al. 2010; Cucherousset and Olden 2011; Bernery et al. 2022; Britton 2022). Direct impacts include competition for resources, such as food and habitat (theorised to be the main source of extinction; Simberloff and Von Holle 1999), predation, and alteration of ecosystem dynamics - including the structure and function of food-webs, with cascading effects on ecosystem processes (Gozlan et al. 2010; Cucherousset and Olden 2011; Bernery et al. 2022; Britton 2022). Indirect impacts include alteration of physical and chemical water quality through bioturbation, changes in nutrient cycling, and the introduction of novel diseases and parasites (Bernery et al. 2022; Britton 2022).

In New Zealand, 73% of all native freshwater fish are classified as either Threatened or At-Risk of extinction, and introduced fish account for 29% of the total species diversity in natural systems (Dunn et al. 2018). As a result, there exists a fundamental necessity to understand the tangible impacts of INNS to native species and ecosystems.

1.3 Source-sink population dynamics

The persistence, and hence success, of a species is reliant on recruitment (King et al. 2011). In isolated populations, as is often the case with new introductions of NNS, this is merited to the number of births exceeding the number of deaths. In open populations that are interconnected over a larger geographic area, recruitment in areas of unfavourable habitat is aided by immigration from more productive source populations (Andreasen et al. 2012; van Klinken and Pichancourt 2015; Dauphinais et al. 2018).

The existence of source-sink dynamics facilitates greater resilience to fine- and coarse-scale pressures present within the ecological context of a given area (King et al. 2011) (*Figure 1.1*). Dauphinais et al. (2018) discuss the persistence of the invasive carp *(Cyprinus carpio*) in the Upper Mississippi Basin lakes to be a result of source populations in interconnected shallow, predator-free ponds. Meanwhile, Lee and Perry (2019) provide evidence that the extinction of the New Zealand grayling (*Prototroctes oxyrhynchus*) is a result of anthropogenic environmental change-induced sink populations causing a 'dilution' effect on source populations in pristine habitats.

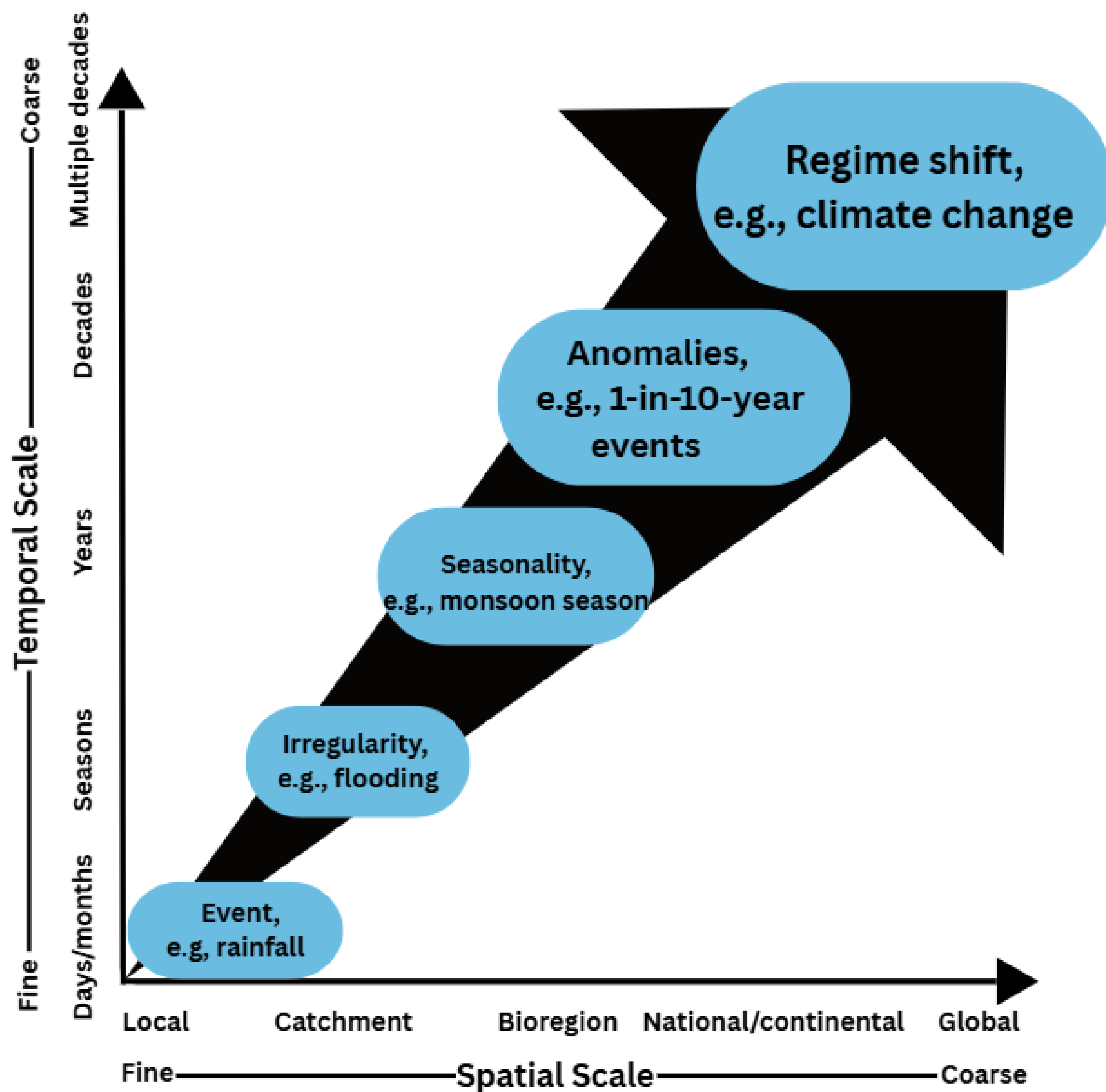

Figure 1. 1. A visualisation of fine and coarse-scale pressures, using rainfall as an example, within an ecological context of space and time. Figure created in CANVA.

Understanding the complex interaction between population dynamics and fine- and coarse-scale habitat conditions underpins successful management of both native and invasive species (King et al. 2011; Andreasen et al. 2012; Draheim et al. 2016; Dauphinais et al. 2018). Despite this, Bajer et al. (2015) highlight that fish invasions are typically investigated at scales too small to consider source-sink population dynamics

across large geographic regions, rendering management of INNS at too-small a scale for effective nature protection.

Physical habitat parameters, such as rainfall, are projected to change as a result of anthropogenic climate change (van Klinken and Pichancourt 2015). Such changes have the potential to drastically alter dispersal rates and patterns between source and sink populations of a species. With implications to both native and INNS alike, tools and methodologies for monitoring and managing freshwater species need to be applied at larger geographic and temporal scales.

1.4 eDNA for biodiversity monitoring

Biodiversity monitoring, herein referred to as biomonitoring, enables assessment of the impact of environmental stressors (Cuff et al. 2025) and anthropogenic activities (Lanzén et al. 2021) on individuals and ecosystems, and the management of conservation threats (Pérez-Fleitas et al. 2023). Traditional methods can require considerable resourcing (Pérez-Fleitas et al. 2023), raise ethical issues (Duval et al. 2021), or have low detection probabilities (Gillet 2008; Rose et al., 2019; McColl-Gausden et al. 2021).

Emerging molecular approaches to biomonitoring involve sampling genetic data (DNA) released into the environment, known as environmental or eDNA, to detect the presence of species (Uchii et al. 2016; Duval et al. 2021) (*Figure 1.2*). Compared to traditional survey methods, eDNA biomonitoring typically enables assessment at wider spatiotemporal scales (Duval et al. 2021; Lanzén et al. 2021; McColl-Gausden et al. 2021; Pérez-Fleitas et al. 2023) at relatively lower cost (Lanzén et al. 2021; Pawlowski et al. 2021), and can be more effective at identifying certain species, such as those that are rare or cryptic (Gillet 2008; McColl-Gausden et al. 2021). For example, Hallam et al. (2021) detected 25% more freshwater fish species across nine sites in the River Thames with eDNA compared to electrofishing surveys. Furthermore, sampling is non-invasive and doesn’t require taxonomic expertise to identify species. Despite these benefits, there are some outstanding limitations to the wider use of eDNA as a biomonitoring tool, and the field is currently working towards development of optimised and validated

sampling and processing protocols, alongside improved genetic sequence databases (Pawlowski et al. 2021).

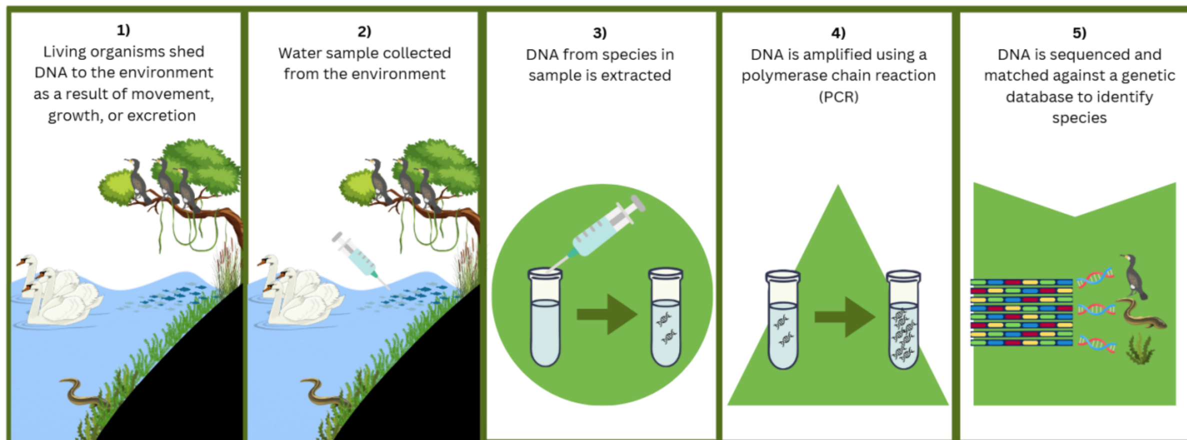

Figure 1. 2. The steps required to obtain species identification from eDNA samples, using water as an example DNA substrate. Figure created in CANVA.

Alongside species detection, eDNA methods are being explored for their potential to provide population genetic level data that could be used to answer questions about evolutionary processes, including hybridisation, inbreeding, migration, and population decline (Grummer et al. 2019). For example, genetic diversity can decrease as a result of founder effects associated with colonisation and invasion, while peaks in diversity can be observed in areas of high connectivity and gene flow between multiple divergent lineages (Ruis and Darling 2014). Although only few studies have assessed this potential to date, results look promising. For example, Sigsgaard et al. (2016) demonstrated that components of the mitochondrial genome can be extracted from eDNA samples in the marine environment and used to determine population haplotype diversity and structure from data similar in quality to that obtained using invasive tissue-based samples. Similarly, Uchii et al. (2016) demonstrated that similar markers

of the mitochondrial genome can be extracted from eDNA samples in freshwater ecosystems.

1.5 Gambusia affinis

Gambusia affinis is a small-bodied, viviparous freshwater fish intentionally spread from its native range in South-Eastern North America to waters around the world (Sloterdijk et al. 2015), predominantly for the control of mosquito larvae (Ling 2004; Pyke 2005). Now present in fresh and saline aquatic environments in all continents except Antarctica (Xiong et al. 2019), *G. affinis* is considered the most widely distributed fish in the world (Pyke 2005) and is one of the world's top 100 worst invasive alien species (Lowe et al. 2000) - primarily due to its negative impacts on the host ecosystems and species with which it interacts (Pyke 2005). For example, *G. affinis* can cause declines in native species abundance and distribution (Rowe et al. 2007), and reductions in their growth and overall condition (Ling and Willis 2005).

Figure 1. 3. Gambusia affinis individuals; top, adult female; middle, adult male; bottom left and right, juveniles. Photo: P Hale, 2025.

Gambusia affinis was deliberately introduced into a constructed waterbody in Auckland Domain in New Zealand from Texas via Hawai'i in 1930 (Ling 2004; K. M. Purcell et al.

2012; Kevin M. Purcell and Stockwell 2015), after two failed attempts (Walton et al. 2012). It is now distributed throughout lowland, coastal waters of the North Island (Ling 2004; Rowe et al. 2007), with fewer known populations in the lower North Island and the Nelson-Tasman area of the South Island.

The species has a general preference for warm, shallow, still, or slow-moving waters, where dense aquatic vegetation occurs (Pyke 2008), but can also tolerate a wide range of environmental conditions (Ling 2004; Pyke 2008; Lee et al. 2017), such as water temperature (0◦C to 45◦C) and salinity (0 to 41ppt) (Pyke 2005). It is also capable of persisting in various ecosystem types, including ponds and wetlands (Casterlin and Reynolds 1977; Pyke 2008), rivers (Walton et al. 2012), mangrove and saltmarsh habitats in estuaries, and oxygen-depleted habitats, where it gulps oxygen from the surface of the water (Pyke 2005).

Its local abundance and widespread distribution in open, coastal systems, alongside its well-known invasive properties and global invasion history makes *G. affinis* an ideal study organism to improve our understanding of source-sink population dynamics and the applicability of eDNA methods as a tool for population assessments.

1.6 Thesis structure

My thesis uses ecological and genetic methods to better understand source-sink population dynamics of freshwater fish, with a particular focus on eDNA metabarcoding as a tool for population assessment. Following this introductory chapter, **Chapter 2** investigates the metapopulation structure of *G. affinis* using traditional biomonitoring methods. **Chapter 3** compares DNA sequencing data derived from both eDNA and tissue samples and examines variation in overall biodiversity in eDNA samples collected from eight sites across New Zealand. Finally, **Chapter 4** discusses the broader implications of the thesis, including limitations and suggestions for future research.

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Chapter 2:

Physical dispersal drivers of *Gambusia affinis* in the South Island of Aotearoa, New Zealand

2.1 Abstract

Species occupancy (i.e., dispersal) patterns are driven by the ability of an individual to navigate a complex network of biotic and abiotic interactions. While the ornamental species trade is one of the main sources of non-native species to freshwater ecosystems, dispersal mechanisms within an ecosystem remain poorly understood, complicating management of these species.

We sampled infestations of the invasive fish, *Gambusia affinis*, within interconnected freshwater streams in the southernmost infestation in New Zealand (Waimea Inlet, Tasman) to determine physical dispersal drivers and understand source-sink population dynamics of *G. affinis*.

We found rainfall (mm) and tide height (m) to have significant impacts on the abundance of *G. affinis*, measured in Catch Per Unit Effort (CPUE), while the latter also impacted the likelihood of species' presence at the stream level. Additionally, we caught multiple individuals of *G. affinis* in a stream that had no prior record of *G. affinis* presence.

Overall, this study demonstrates the importance of monitoring occupancy patterns of freshwater fish over large spatiotemporal scales when assessing source-sink population dynamics.

2.2 Introduction

Species persistence depends on individuals navigating complex spatiotemporal variability within the environment to seek favourable conditions that provide for basic ecological needs (Barry et al. 2016) and enhance fitness (McPeek et al. 2024; Kaylor et al. 2025) and survival. As such, habitat use and occupancy patterns reflect a complex network of biotic (e.g., predator-prey; Choi et al. 2015; McPeek et al. 2024), and abiotic (e.g., environmental parameters; Bašič et al. 2019) interactions. Shifts in habitat use and occupancy, referred to herein as dispersal, in response to unfavourable conditions of a given habitat is a fundamental process for population growth, expansion, and/or survival.

Spatiotemporal shifts demonstrate important information about the ecological requirements of a species and how it responds to resource fluctuations - particularly for fish (Barry et al. 2016; Bašič et al. 2019). For example, Kaylor et al. (2025) observed that recently emerged salmon juveniles that disperse from highly dense spawning grounds to areas of lower densities benefit from greater growth and survival rates. However, not all dispersal events are natural responses to environmental stochasticity but instead represent naturalisation to novel ecosystems (Hulme et al. 2008), or the intentional or unintentional spread of species. In the latter case, this can occur when the species is commoditised (e.g., agricultural species or the aquaria trade) or is dependent on a commodity (e.g., pathogens or virus), or when it is inadvertently displaced - as a result of transportation of goods or people (e.g., ballast tanks of ships

and boats), or via new dispersal corridors facilitated by infrastructural developments (e.g., water races or railroad corridors).

The ornamental species trade is one of the main sources of commoditised species to non-native ecosystems, due to the subsequent release or escape of individuals from aquaria (Early et al. 2016; Atalah et al. 2022). In New Zealand, almost 300,000 aquatic ornamental individuals across 865 taxa are imported per annum, 98% of which consist of freshwater fish from predominantly tropical environments (Atalah et al. 2022). Among the 21 catalogued species of naturalised freshwater fish in New Zealand, 28% are known to have arrived through the aquarium trade, with the remainder arriving for the establishment of a sports fishery culture (McQueen and Morris 2013), or as a form of biocontrol.

Although rarely observed, those species that are successfully introduced have the potential to proliferate and become 'invasive' (Adrian-Kalchhauser et al. 2020), defined by the threat they pose to local biodiversity, economy, and/or human wellbeing (Early et al. 2016; MacNeil et al. 2024). Of the 21 freshwater fish species mentioned above, only three species are legislatively recognised nation-wide as 'pest species' in New Zealand (Freshwater Fisheries Regulations 1983, r 65; Biosecurity Act 1993, s 52). This includes the invasive mosquitofish, *Gambusia affinis*.

Gambusia affinis populations in the South Island weren't discovered until 2000 (Shaw and Studholme 2001), where it is thought to have been deliberately introduced by anglers as a food source for exotic coarse fishery species, such as perch, trout, and rudd (Grainger, pers. comms., 2024). Though widely distributed throughout New

Zealand's North Island, with hotspots in Northland, Auckland, and Waikato, *G. affinis* currently remains contained to the Waimea, Moutere, and Riuwaka watersheds in the South Island (*Figure 2.1*). The lack of spread to adjacent catchments in the South since initial delimitation of the infestation is accredited in part to the prohibition of coarse fisheries in the region (Angler Notice for Fish and Game Regions 2025 (1 October 2025) New Zealand Gazette No 2025-gs3978). However, total eradication of these populations has been deemed unfeasible due to a lack of knowledge around dispersal mechanisms between infestations, meaning reinvasion cannot be ruled out (Falleiros 2022).

Figure 2. 1. Distribution of *Gambusia affinis* in the South Island (inset), with each red dot representing an infestation.

Successfully mitigating the current and future impact of *G. affinis* requires a better understanding of human-mediated introduction pathways, but also an extensive understanding of how it may respond to biotic and abiotic variables that may or may not

exist in the infestation area. Here, we characterise current dispersal of *G. affinis* in the Tasman Bay coastal ecosystems to help understand its future dispersal potential. We hypothesised that *G. affinis* dispersal (measurable as changes in temporal abundance in catch per unit effort, CPUE) between coastal freshwater habitats occurs infrequently based on the lack of spread post-original infestation. However, we predicted that infestations of *G. affinis* would be scattered throughout coastal freshwater habitats, with higher CPUE in warmer, slow-flowing, shallow habitats upstream of regular tidal influence. We also predicted that physical habitat parameters, such as higher-than-normal tides and greater levels of rainfall, would result in higher CPUE in lower reaches of coastal freshwater habitats. This is because species in the genus *Gambusia* are poor swimmers (Rehage and Sih 2004), and as a result, their dispersal is catalysed by abiotic interactions. Addressing these hypotheses will enable greater preparedness and response for future invasions and inform management actions to reduce the future impact of *G. affinis*.

2.3 Methods

Study area

The Waimea Inlet (1,400 ha; located within the administrative boundaries of Tasman District and Nelson City Councils) is characterised by large, shallow, well-flushed tidal lagoon-type estuaries that are fed by several small streams and rivers. This estuary drains into Te Tai o Aorere Tasman Bay, with a residence time of less than one day (Stevens et al. 2020). Based on data stored in the New Zealand Freshwater Fish Database and annual surveillance data held by the local Department of Conservation Operations Motueka Office, a total of six streams in the area recorded to have *G. affinis* present were selected as infestation sites, alongside one control stream (Orchard Creek) from which *G. affinis* had been previously eradicated and, since, a dispersal barrier has been installed (*Figure 2.2*).

Sampling methods

Initial surveys took place in March 2024, with repeated surveys occurring at 12-weekly intervals in June, September, and December 2024, and in March 2025.

At each stream, sampling took place at 100 m intervals (sample sites) between the confluence with the Waimea inlet and up to the first 1,500 m of stream (*Figure 2.2*).

Figure 2. 2. Study sampling sited (red) and control site (yellow) in the Waimea Plains Inlet, Tasman District, New Zealand.

Dipnet sampling has been found to be the most effective method to catch *G. affinis* (Howell et al. 2013; Cheng et al. 2018). Thus, at each 100 m interval, five samples were taken using a dipnet (2 mm mesh size, 20 cm opening diameter). A 'sample' was defined as three scoops at the surface of the water body, with strokes of 2 m in length that did not overlap, i.e., sampling a total of 6 m^2 per sample, and 30m^2 at each 100 m interval. Areas of low flow with emergent and/or submergent macrophyte growth were targeted for sampling as this is well-documented as preferred habitat for *G. affinis* (Ling et al. 2011). All caught individuals were euthanised using Aqui-S, and sexed, while non-target

species were recorded and returned to the sample site post-sampling to avoid recapture.

Physical parameters, such as water temperature (∘C), salinity (ppt), pH, depth (cm), distance from estuary (cm), habitat, substrate type (see: Harding, et al., 2009), duration of time until next full tide (hrs), and tide height (m) were recorded for each site before sampling for *G. affinis* took place. Total rainfall for the calendar month was also extracted from the Earth Sciences New Zealand (ESNZ) database on 11 April 2025.

Data analysis

Microhabitat surveys

Recorded counts of each life stage were pooled and used to investigate the difference in relative abundance between sampling sites, measured in CPUE.

To assess the relationship between physical habitat parameters and both presence and abundance of *G. affinis*, the “glmmTMB” package version (Brooks et al. 2017) in R 4.5.1 (R Core Team 2024) was used to fit a Hurdle Negative Binomial Generalised Linear Mixed-Effects model to the data, accounting for excess zeros, overdispersion and the hierarchical nature of the dataset. Diagnostic Plots for Hierarchical Regression Models (DHARMa) were also created to assess the fit and assumptions of the model and improve the overall reliability of results (*Figure A1.1*).

Dispersal surveys

To understand the relationship between *G. affinis* presence in a specific catchment and variables that were assessed to have the greatest likelihood to passively disperse individuals (i.e., rainfall, time until next high tide, and height of next high tide) the “MASS” package version (Venables and Ripley 2002) in R was used to fit a Negative Binomial Generalised Linear Mixed-effects Model, which accounts for overdispersion of count data. This model was conditioned on “*stream”* to account for the baseline difference of *G. affinis* abundance between streams. This analysis was chosen as no stable infestations of *G. affinis* were found within the zone of tidal influence, and as such the data contained a high proportion of zero detections in these sample sites. This enabled *G. affinis* presence to be used as a proxy for dispersal, as individuals must have arrived either from the Waimea Inlet or further upstream in the freshwater system.

To assess the quality of the fitted model and validate the assumptions, we analysed the simulated residuals using the “DHARMa” package v0.4.7 (Hartig 2025). The core assumptions of the Negative Binomial Generalised Linear Mixed-effects Model were checked by comparing the observed data to 1,000 simulated datasets generated from the simulated model, and assessments of uniformity of residuals, dispersion, and residual patterns were undertaken to confirm model assumptions were met prior to interpretation (*Figure A1.2*).

2.4 Results

Microhabitat surveys

The Conditional Generalised Linear Mixed-effects Hurdle Model (*Table 2.1*) predicted the relationship between *G. affinis* abundance (in CPUE) and the seven physical habitat parameters at the sample sites, given that CPUE was greater than zero. Of the seven parameters, the model indicated a statistically significant relationship only with rainfall ($p=0.022$), and tide height ($p< 0.001$). The model demonstrated that a one standard deviation increase in rainfall was associated with a decrease (-0.516) in CPUE of *G. affinis*, while a one standard deviation increase in tide height corresponded to a strong decrease (-1.042) in CPUE of *G. affinis*.

Meanwhile, the Zero-inflated Generalised Linear Mixed-effects Hurdle Model (*Table 2.1*) predicted the log-odds of observing a CPUE of exactly zero at a sample site. This model highlighted a statistically significant relationship between CPUE of *G. affinis* and water depth ($p=0.043$), and time until the next high tide ($p=0.008$), but not between CPUE and tide height ($p=0.964$). Specifically, a one standard deviation increase in water depth was associated with a decrease in the log-odds (i.e., a lower probability) of observing a CPUE of zero at a sample site, while a one standard deviation increase in time until the next high tide was associated with an increase in the log-odds of observing a CPUE of zero at a sample site.

Table 2. 1. Coefficient estimates from the Negative Binomial Generalised Linear Mixed-effects Hurdle Model for the correlative relationship between physical habitat predictors and *Gambusia affinis* abundance at the site level. All variables have been standardised. Values of significance are presented in bold.

Term	**Estimate**	**Std. error**	**Z value**	**P value**	**95% CI Lower**	**95% CI Upper**
Conditional Model						
(Intercept)	-0.004	0.904	-0.005	0.996	-1.776	1.768
Water temperature (◦C)	0.055	0.182	0.303	0.762	-0.302	0.413
pH	0.954	1.255	0.760	0.447	-1.506	3.145
Salinity	-2.338	1.287	-1.816	0.069	-4.861	0.185
Water depth (cm)	-0.043	0.373	-0.128	0.898	-0.780	0.684
Rainfall	-0.516	0.226	-2.0285	**0.022**	-0.959	-0.730
Next High Tide	0.347	0.267	1.300	0.194	-0.176	0.869
Tide Height (m)	-1.042	0.237	-4.398	**<0.001**	-1.506	-0.578

Zero-inflation Model (Log-Odds)						
Intercept	3.010	0.254	11.833	**<0.001**	2.511	3.508
Water Depth (cm)	-0.387	0.191	-2.025	**0.430**	-0.762	-0.012
Next High Tide	0.549	0.208	2.638	**0.008**	0.141	0.956
Tide Height (m)	0.010	0.218	0.045	0.964	-0.418	0.437

Dispersal surveys

The Conditional Negative Binomial Generalised Linear Mixed-effects Model (*Table 2.2*) indicated a statistically insignificant relationship likelihood of *G. affinis* CPUE being greater than zero, i.e., presence of *G. affinis*, as a result of time until the next high tide (p=0.933) and rainfall (p=0.212). However, the model demonstrated a statistically significant relationship between presence of *G. affinis* and tide height (p=0.048), with a one unit increase in tide height was associated with a decrease in the likelihood of *G. affinis* presence (p=0.048) at the stream level.

The Random Effects Negative Binomial Generalised Linear Mixed-effects Model (*Table 2.2*) estimated a standard deviation of 5.223, indicating significant heterogeneity in the presence of *G. affinis* across the different streams sampled, even after accounting for the fixed effects of the habitat parameters sampled. This suggests that stream-specific factors were highly influential in determining presence of *G. affinis*.

Table 2. 2. Coefficients and Random Effects from Negative Binomial Generalised Linear Mixed-effects Model for the correlative relationship between habitat predictors and presence of *Gambusia affinis* at the stream level. All variables are expressed in long form and values of significance are presented in bold font.

Term	**Estimate**	**Std. error**	**Z value**	**P value**	**95% CI Lower**	**95% CI Upper**
Conditional Model						
Rainfall	-0.433	0.346	-1.249	0.212	-1.112	0.246
Next High Tide	-0.038	0.455	-0.084	0.933	-0.929	0.852
Tide Height	-0.824	0.417	-1.974	**0.048**	-1.641	-0.006
Random effects						
Intercept (for stream)	5.223	N/A	N/A	N/A	N/A	N/A

2.5 Discussion

This research sought to identify changes in temporal abundance of *G. affinis*, measured as CPUE, to determine which physical habitat parameters facilitated dispersal between streams that were hydrologically interconnected through a large, intertidal ecosystem (the Waimea Inlet). Of all the physical habitat parameters measured, we found that tidal components influenced *G. affinis* presence, while tide and water depth both influenced *G. affinis* abundance.

Spatial patterns of species dispersal and occupation are governed, in part, by habitat preferences that are underpinned by an organism's assessment of resource availability, and environmental suitability, which combine to express a species' theoretical distribution (Wizs et al. 2013). In this study, we found no specific preference of *G. affinis* for water temperature, pH, or salinity, but a notable preference for deeper water habitats. This generally contravenes an extensive body of literature that describes microhabitat preference and selection by *G. affinis*, particularly with respect to water temperature (Shaw and Studholme 2001; Pyke 2008; Walton et al. 2012), water depth (Shaw and Studholme 2001; Pyke 2008), and pH (Pyke 2008; Walton et al. 2012). Described as habitat generalists (Pyke 2008), *G. affinis* tends to prefer warmer, shallower waters, and a neutral to slightly alkaline pH ranges (Pyke 2008; Walton et al. 2012), however our results suggest there is no threshold of these variables that indicates a higher likelihood of greater abundance of *G. affinis* except for water depth, where patterns documented in the literature completely contravene the findings of this study. In particular, previous work has shown greater occupancy of *G. affinis* in water

that is shallow, with well-vegetated, shallow edges preferred in waterbodies that are moderately deep (Pyke 2008). Our contrasting findings could be due to the physical habitat parameters being too similar in our study, resulting in limited environmental variability across sites. For example, the preference we found for deeper habitats could reflect an over-representation of habitats in our dataset that were too shallow for *G. affinis*, or other, nested variables not considered in this analysis, such as habitat type (e.g., pool versus run). Thus, future work should focus on greater spatial scales across geographically isolated regions, so as to include greater variable representation of physical habitat parameters. This should also include infestations outside of the Tasman area, which is the southern extent of the range of *G. affinis*.

Other biotic and abiotic factors, e.g., tide height, can influence the likelihood of presence of a species which, combined with habitat preferences, are expressed as a species realised distribution (Wizs et al. 2013). As *G. affinis* is considered a poor swimmer (Rehage and Sih 2004), dispersal occurs slowly, often impeded by barriers (e.g., channel morphology; Chapman and Waburton 2006). Consistent with this, our results highlighted a negative influence of tide height on the likelihood of presence of *G. affinis* at the catchment/stream level. This suggests that greater tide heights, and hence salinity, of the lower reaches of the sample sites presented a dispersal barrier more so than a mechanism for dispersal throughout the course of this study. These results also align with recorded rainfall across sampling events. The overall catchment experienced very dry conditions, with less than normal levels of rainfall during the sampling period, confounding the ability of our study to consider the full impact of parameters such as rainfall on abundance and rates of dispersal of *G. affinis*. However, following a series of

extreme rainfall events in the Nelson-Tasman area, we resampled the Borck 0900 site on 11/08/2025 to contrast the study sampling event from 20/08/2024 when rainfall was much lower. We caught a total of four adults in 2025, compared to one adult and four juveniles in 2024. This variation in size-class structure, with juveniles completely absent from samples following the heavy rainfall event in 2025, suggests that ontogenetic variation in ecological niches and tolerances exists between adult and juvenile *G. affinis*. Ontogenetic niche shifts have been described by Stoffels and Humphries (2003) in floodplain ponds, where adult *G. affinis* individuals were found to be associated with the bottom of littoral macrophyte beds, while juvenile individuals were associated with the surface of macrohptye beds or the limnetic zone, which could make them more susceptible to displacement from abiotic variables, e.g., rainfall and associated flood events. To confirm these findings, future work should monitor infestations of *G. affinis* on a larger temporal scale that spans multiple years to encompass greater variation in rainfall as a result of natural stochasticity and multi-year climatic cycles, e.g., the El Niño-Southern Oscillation cycle.

We found evidence for potential local dispersal driven by other abiotic interactions. For example, temporal abundance between sites within Borck Creek (the only catchment with a permanent population of *G. affinis*), was variable, with likelihood of presence more notable across sampling events in dense macrophyte beds or in small, isolated ponds on the margins of the creek when compared to flowing areas without macrophyte growth. These observations suggest a complex spatial distribution of *G. affinis* within the sampled streams, with source populations residing in isolated pools, connected to the watercourse only during flooding events. During these episodes,

individuals are likely dispersed into sink habitats (macrophyte beds) in the watercourse or completely dislodged downstream into estuarine habitats. Notably, we found four adult males at site Swamp 0100, which was the only other stream at which occurrence of *G. affinis* was recorded throughout the total study period. While no permanent population of *G. affinis* was found in this catchment, the repeated detection of individuals may indicate their dispersal from higher stream reaches rather than as new arrivals via the Waimea Inlet. Indeed, while anecdotal evidence suggests that heavy rainfall events can passively disperse individuals, there is no evidence to suggest they can be swept out to the Waimea Inlet and then enter adjoining creeks – either actively via swimming or passively by way of tidal influence; thus, further research is needed to validate this.

Our study highlighted some difficulties using traditional netting methods to determine dispersal drivers of *G. affinis* over small spatial and temporal scales. Indeed, occupancy patterns were demonstrated to result from a combination of biotic and abiotic interactions that require surveying across larger spatiotemporal scales. Without sampling regimes sufficient in size to account for environmental stochasticity at such a scale, the models generated appear to inadequately determine absolute dispersal drivers and, as such, further investigation is needed. Future work should focus on assessing the efficacy of supplementary methods to monitor occupancy patterns of invasive fish over wider spatiotemporal scales, such as tagging of individuals with Visible Implant Elastomers (VIEs). VIE tagging has not been shown to impair survival of individuals (Chapman and Waburton 2006; Jungwirth et al. 2019) or adversely alter social behaviour between tagged and non-tagged individuals (Jungwirth et al. 2019) and

may enable move effective monitoring of movements of individuals over a larger spatial and temporal scale using a netting regime similar to that employed here.

Concluding remarks

We found that stream-specific factors were highly influential in determining presence of *G. affinis* but careful consideration of study design to encompass the adequate environmental context (i.e., spatiotemporal scales) is required to best assess dispersal drivers for this species. A key component we were unable to achieve was an assessment of physical habitat parameters over greater thresholds. Finally, our assessment of traditional netting (i.e., dipnet) surveys to determine dispersal drivers suggests that supplementary methods should be investigated and used together, particularly for species that are located at the extent of their invaded range.

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Chapter 3:

Analysis of environmental and tissue mitochondrial DNA for the invasive pest, *Gambusia affinis,* in Aotearoa, New Zealand

3.1 Abstract

Traditional biomonitoring is often resource-intensive and fails to capture coarse-scale spatiotemporal population dynamics. Environmental DNA (eDNA) is increasingly used as a tool for assessing community diversity as it is sensitive, non-invasive, and less resource intensive. However, the efficacy of eDNA for inferring population-level genetic variation – particularly for invasive species – requires further validation against tissue-based methods.

We investigated the utility of eDNA metabarcoding, focusing on the mitochondrial cytochrome c oxidase subunit I (COI) gene, to assess biodiversity and population genetic variation of the invasive pest fish *Gambusia affinis* across eight catchments in New Zealand. We found that biodiversity patterns were driven primarily by habitat type, with significant variation between lotic systems. In terms of genetic variation, a single variant accounted for >99% of total *G. affinis* sequence reads across both tissue and water (eDNA) samples. There were an additional 88 variants detected, 51 of which were only found in tissue samples and 37 of which were only found in eDNA samples.

Overall, this study demonstrates the utility of eDNA for biomonitoring in freshwater environments. However, technical issues still limit the potential of eDNA for population genetic inference, particularly for recent biological invasions in which only a single or small number of high-frequency haplotype(s) dominates the sequencing pool.

3.2 Introduction

A species' distribution and general population trends result from processes that occur at multiple, large scales (Jones 2011) that are costly to survey with traditional survey methods (Gerber et al. 1999; Manly et al. 2004; Jones 2011; White 2019). As a result, monitoring data likely fails to accurately describe coarse-scale spatial and temporal shifts in population trends due to inaccurate sampling methodologies or data interpretation (Czeglédi et al. 2022). White (2019) suggested that most datasets are not temporally broad enough to understand population dynamics, while Noon et al. (2012) explained how the costs of traditional survey methods can promote a coarse-filter approach to landscape-scale monitoring, resulting in an oversimplified understanding of habitat use and population trends and impacting the efficacy of management decisions.

Dispersal between subpopulations is an important ecological process that impacts the persistence of a species in a landscape (Jones 2011). For example, in a recent review, Czeglédi et al. (2022) showed that the majority of 307 publications on freshwater fish assemblage cited environmental stochasticity (e.g., flooding or drought conditions) as the main driver for variability in occupation patterns over time, particularly in rivers. This suggests a naturally complex framework of occupancy dynamics over fine and coarse spatial scales for freshwater fish, and a need for monitoring data that spans dynamic, multi-scale matrices to effectively influence management decisions. Indeed, greater understanding of population dynamics at both fine and coarse spatiotemporal scales is required to successfully manage biodiversity loss (Anderson 2018) and the risk of

invasion alike (King et al. 2011; Andreasen et al. 2012; Draheim et al. 2016; Dauphinais et al. 2018). To facilitate this, novel, resource-sensitive approaches to monitoring and data interpretation are required.

One such approach is that of environmental DNA (eDNA) monitoring, which consists of collecting and analysing genetic material shed to the surrounding environment by organisms to infer a species' recent interaction with that habitat. First used by Ficotela et al. (2008) to detect invasive American bullfrogs (*Lithobates catesbeianus*), eDNA is a robust biomonitoring tool of increasing utility that is now widely used to assess the diversity of animal communities (Hallam et al. 2021), with sampling and processing protocols most advanced and well established for freshwater habitats (Pawlowski et al. 2021). eDNA offers many advantages for biomonitoring, as a result of fewer resource requirements (Rees et al. 2014; Eva E. Sigsgaard et al. 2020; Fonseca et al. 2021), greater sensitivity (Rees et al. 2014; Peixoto et al. 2020; Yamahara et al. 2025), and an ability for more comprehensive surveys of biodiversity. As such, it offers an exciting approach for exploring population dynamics over coarse spatial and temporal scales when compared to traditional survey methods (i.e., netting or electrofishing) (Blackman et al. 2020; Yamahara et al. 2025). However, outstanding challenges include limitations with: methodological development in certain ecosystems (such as estuaries; Hallam et al. 2021), understanding of abiotic influences on the lifespan of eDNA (Rees et al. 2014; Fonseca et al. 2021), data utility (i.e., difficulties inferencing microhabitat variation, life-history and size-class of surveyed populations; Rees et al. 2014), and accuracy (i.e., false positives and negatives; Fonseca et al. 2021). Each of these can reduce

confidence in the information collected, thus eDNA tools are best used in conjunction with traditional methods to gain complementary insights (Adams et al. 2019).

While eDNA sampling has predominantly been used to detect absence or presence of a target species (Barnes and Turner 2015), more recent applications have shown potential for obtaining population genetic information (Eva E. Sigsgaard et al. 2020; Andres et al. 2023). For example, Sigsgaard et al. (2016) demonstrated that components of the mitochondrial genome can be extracted from eDNA samples in the marine environment to determine haplotype diversity and population structure from data similar in quality to that obtained using tissue-based analyses. Similarly, Uchii et al. (2016) demonstrated that similar markers of the mitochondrial genome can be extracted from eDNA samples in freshwater ecosystems, for example with koi carp (*Cyprinus carpio*). Genomic applications of eDNA sampling may therefore offer a non-invasive, cost-effective method (Adams et al. 2019) for furthering our understanding of dispersal mechanisms, coarse-scale habitat occupancy, and metapopulation dynamics by facilitating spatiotemporal analyses of gene flow and distributions in a target population.

In this study, we explored the utility of eDNA sampling for mitochondrial DNA analysis through a comparative investigation with tissue samples. We focused on one of the most widespread freshwater fish species (K. M. Purcell et al. 2012; Kevin M. Purcell and Stockwell 2015), the pest fish *Gambusia affinis*, for which previous work has shown eDNA to be an effective tool for species detection (Banks et al., 2021). Assessments of the genetic diversity and structure of the closely related *Gambusia holbrooki* in both Australia (Ayres et al. 2012) and Spain (Díez-del-Molino et al. 2013) suggest that gene

flow among recently established populations is a result of natural and human-mediated dispersal. Meanwhile in New Zealand, upper North Island samples of *G. affinis* fall into three genetically distinct population clusters, two of which overlap geographically, alluding to repeated human-assisted colonisations in these areas (Kevin M. Purcell and Stockwell 2015). Now widespread throughout the upper North Island of New Zealand since its introduction to Auckland in the 1930s, infestations of *G. affinis* are restricted in the South Island to only a few confirmed catchments since 2000 (Shaw and Studholme 2001). The broad timeline of invasion history, coupled with evidence of genetic variation across a large geographical scale, presents an ideal opportunity to explore eDNA-based biodiversity patterns for *G. affinis* and compare the efficacy of eDNA and tissue samples for mitochondrial DNA assessments and monitoring.

We hypothesised that overall beta-level biodiversity eDNA patterns would be more similar for similar habitat types (e.g., creek, pond) and would become more dissimilar among catchments as their geographical proximity decreased. Further, we expected that eDNA samples would reveal a greater assemblage of *G. affinis* mitochondrial variation compared to tissue samples due to their broader environment sampling.

3.3 Methods

Study area

Specimens and eDNA samples were collected between 11 December 2024 and 20 January 2025 from eight locations distributed throughout the known infestation area in New Zealand, being the North Island and the Nelson-Tasman region in the South Island (*Figure 3.1*). Collection sites were selected to reflect the diverse biotic and abiotic characteristics across different hydrological units *G. affinis* has invaded (*Table 3.1*).

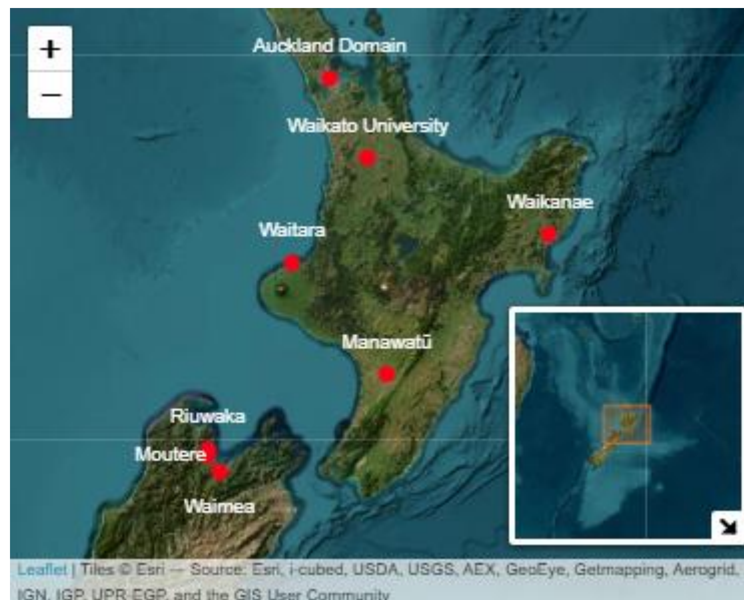

Figure 3. 1. Locations, in red, of *Gambusia affinis* specimens and eDNA collection sites across New Zealand.

Sites were chosen on the basis of hydrological isolation from other sites (e.g., separate catchments), geographical distance, coastal proximity, condition (e.g., constructed vs. natural), and the type of waterbody (e.g., pond) (*Table 3.1*). For example, the Auckland Domain collection site is characterised as an inland, constructed pond with no known hydrological connections and is of particular interest, as it was the first release site of

G. affinis in New Zealand (Purcell, et al., 2012). South Island collection sites were categorised as separate sites due to their hydrological isolation; however uncertainty exists as to whether these are in fact a single metapopulation connected by the Tasman Bay during flooding events (H. McCaughan, pers. comms 2025).

Table 3. 1. Summary of all specimens and eDNA collection sites in New Zealand.

Location/ Catchment	**Latitude**	**Longitude**	**Coastal proximity**	**Condition**	**Habitat type**	**First recorded population**
Riuwaka	-41.0780	172.9973	Coastal	Constructed	Drain	2000 (Shaw and Studholme 2001)
Moutere	-41.1491	172.9933	Coastal	Natural	River	2000 (Shaw and Studholme 2001)
Waimea	-41.3289	173.1698	Coastal	Highly modified	Stream	2000 (Shaw and Studholme 2001)
Manawatū	-40.2521	175.5950	Inland	Constructed	Drain	2001 (Stoffels 2022)*
Waitara	-39.0002	174.2220	Inland	Constructed	Pond	2004 (Stoffels 2022)*
Waikanae	-38.6623	177.9843	Coastal	Highly modified	Stream	2002 (Stoffels 2022)*
Waikato University	-37.7865	175.3161	Inland	Constructed	Pond	1985 (Stoffels 2022)*
Auckland Domain	-38.8596	174.7727	Inland	Constructed	Pond	1930 (K. M. Purcell et al. 2012)

*The New Zealand Freshwater Fish Database (Stoffels 2022) has been running since 1977, and records may not accurately reflect the invasion history of *Gambusia affinis* at a particular site. The oldest record within a 5 km radius from the site is displayed.

eDNA collection

To eliminate the risk of cross contamination between sites, or from previous fieldwork, all field equipment was wiped down with 10% bleach and left to sit for 5 mins, before being rinsed with water from the sample site on a grassy surface at least 10 m away from the edge of the waterbody. At each site, methodology described by PlaceGroup Ltd. (2022) was modified to take a composite sample: 500 mL sub-samples (n = 14) from a 10 m stretch of water were collected into a bucket; the bucket was then left to sit for 5 mins to allow any sediment to settle before water was filtered through a 1.2 μm active syringe sampler (standard eDNA kits; Wilderlab New Zealand Ltd.) until either 1 L of water had been filtered per sample or no more water could be passed through the filter (*Table A2.1*). A preservative agent was then added to the filter before storage at room temperature until DNA extraction. A total of six sample replicates were collected per site.

Specimen collection

Fish specimens were sampled using a dipnet (2 mm mesh size and 20 cm in opening diameter), with five sub-samples collected into a bucket of cool water from the same water body. Each dipnet 'sample' was defined as three scoops taken at the surface of the water body, with strokes of 2 m in length that did not overlap, i.e., sampling a total of 6 m^2.

All dipnet-caught *G. affinis* were euthanised using Aqui-S, while any non-target species were returned to the sample site. A total of ten euthanised individuals, consisting of a

minimum of three females, three males, and three juveniles each, were immediately preserved in 90% ethanol and stored in a refrigerator until DNA extraction. Female *G. affinis* were identified due to their larger size and the presence of a large, black blotch behind their operculum, while males were identified due to the presence of the gonopodium, a modified anal fin used in reproduction. Juveniles were identified due to their small size and the absence either a black blotch or a gonopodium (*Figure 3.2*).

Figure 3. 2. Female *Gambusia affinis*, above, with black blotch behind its operculum demonstrated with an orange arrow; male *Gambusia affinis* below, with its gonopodium demonstrated with the red arrow. Photo: P Hale, 2025.

DNA extraction and sequencing

DNA was extracted from muscle tissue at the base of the caudal fin from six specimen samples per site following the DNeasy Blood and Tissue DNA extraction protocol (Qiagen). DNA was extracted from the preservative fluid in each eDNA filter following the same protocol but substituting the tissue sample for the preservative fluid.

Extracted DNA was PCR-amplified using Illumina-tailed primers for the detection of invertebrates, vertebrates, and plants, using the cytochrome c oxidase subunit I (COI) gene region. PCR amplification was undertaken using the mlCOIintF (GGWACWGGWTGAACWGTWTAYCCYCC; Leray et al., 2013) and jgHCO2198 (TAAACTTCAGGGTGACCAAAAAATCA; Geller et al., 2013) primers.

Each 25 µL PCR reaction was comprised of 5 µL of MyFi© 5× reaction buffer, 1 µL each of 10 µM forward and reverse primers, 1 µL of 20 mg/mL bovine serum albumin (BSA), 1 µL of 2 U/µL MyFi© DNA polymerase, 15 µL of nuclease-free water, and 2 µL of extracted DNA. These reactions were subjected to thermal cycling conditions that included an initial denaturing step at 95 °C for 4 mins, followed by 40 cycles consisting of denaturation at 95 °C for 20 s, annealing at 46 °C for 45 s, and extension at 72 °C for 45 s, with a final extension step at 72 °C for 5 mins. Negative controls were included in all amplification runs to monitor quality and potential contamination of samples.

All PCR products were visualised using an agarose gel (1%) and quantified using a Qubit fluorometer (Thermo Fisher Scientific New Zealand Ltd). Four samples had faint gel bands, indicating low concentrations of PCR product, and were re-amplified using the same conditions. Quantified PCR products were then sent to SeQuench Ltd. (Nelson, New Zealand) for sequencing on an Illumina MiSeq v2 (500-cycle kit; 2 x 251 bp) using

Nexterta XT v2 Index Kit Set C dual indices. A sequencing control was included in the sequencing carried out by SeQeunch.

Data analysis

DNA sequence reads were analysed using DADA2 v1.34.0 (Callahan et al. 2016) in R v4.2.1 (R core team 2024). Primer sequences were removed from sequence reads, then trimmed in accordance with the quality plot profile (trimming up to 30 bp from either end of the sequence based on quality, Q score). Reads with 1 or more ambiguous bases and >2 expected errors (maxN=0 and maxEE=c(2,2), respectively) were filtered out of the dataset. Subsequently, error rates were learned from each amplicon, and reads were de-replicated and denoised before merging and chimeras *de novo* removed to produced amplicon sequence variants (ASVs).

ASVs were assigned taxonomic information using a bespoke DNA database created on the 17th of March 2025 using CRABS v.1.7.6 (Jeunen et al. 2023), following the tutorial steps at: https://github.com/gjeunen/reference_database_creator (Abarenkov et al., 2023). This database was created using National Center for Biotechnology Information (NCBI) and the query 'COI[All Fields] OR COXI[All Fields] OR CO1[All Fields] OR COX1[All Fields] OR cytochrome oxidase subunit i[All Fields] OR cytochrome oxidase subunit 1[All Fields] OR cytochrome oxidase subunit I[All Fields] AND (mitochondrion[filter] AND ("200"[SLEN] : "50000"[SLEN]))'. The database was then imported to CRABS format using the --import command. Next, amplicon regions were extracted from each database, pairwise global alignment was performed to retrieve amplicons without primer regions, and the database was de-replicated and filtered

using default settings before being exported to DADA2 format. ASVs with unassigned taxonomy were further annotated to species level using BLAST searches in the nucleotide database of NCBI based on e-values < $1e^{-50}$, with the top hit then determined based on the bitscore.

Per-sample ASV counts, final taxonomic assignment, and the associated metadata were read into R v4.4.2 for analysis and visualisation of ASV data using phyloseq v1.50.0 (McMurdie and Holmes 2013). The number of raw reads ranged from 6,002-198,786 (*Table A2.1*), and initial ASVs totaled 8,677. Raw reads were filtered to first remove potential contaminants (e.g., Hominidae), and second to retain phyla considered to be ecologically impacted by *G. affinis* as a result of predation. The final filtered dataset therefore included the following phyla: Athropoda, Chlorophyta, Haptophyta, Platyhelminthes, Tardigrada, Annelida, Bryozoa, Chordata, Cnidaria, Nematoda, Gastrotricha, Mollusca, Nemertea, Placozoa, Streptophyta.

Data exploration included plotting relative read abundance (top 20 taxa) for eDNA samples by taxonomic group. Next, patterns of Shannon's alpha diversity were examined by calculating taxonomic richness for all ASVs per location. Rarefication to sample sizes of 1,000 was applied and multivariate analysis (PCoA/MDS, PERMANOVA) based on ASV abundance was conducted using the ordinate function in pyloseq and the vegan v.2.6-8 package (Oksanen et al. 2018). Statistical significance of the MDS clustering pattern was tested using the adonis2 function, with Brays distance, 999 permutations, and a confidence level of 0.05. Additionally, beta dispersion for the MDS was calculated from the Bray's distance matrix using the betadisper function from vegan. All plots were generated using base R or ggplot2 v3.5.1 (Wickham 2016).

Water and tissue comparisons

Patterns of ASV distribution across populations were compared between tissue and water eDNA samples taken across all eight sites to determine whether water-based eDNA samples were capturing representative patterns. Descriptive statistics (see Adams et al. 2022) were used to calculate proportional mean ASV read counts per sample method (i.e., water vs tissue) by calculating the read count of each recorded ASV as a proportion of the total read count per sample method.

Phylogentic analysis

A total of 61 G. *affinis* COI sequences were downloaded from National Center for Biotechnology Information (NCBI) on 06 January 2026 and stored in a FASTA file with the DNA sequence for ASV_1 from this study. All 62 sequences were then aligned using default parameters in MAFFT v7.453 (Katoh and Standley 2013). The aligned FASTA file was then loaded into Phylogeny.fr (https://www.phylogeny.fr/) to generate a phylogeny under default settings (HKY85 substitution model, gamma shape parameter: 8.544, transition/transversion ration 24.261, four categories, proportion of invariant sites: 0.712).

3.4 Results

General dataset characteristics

Two of the 96 samples did not pass filtering thresholds, resulting in a final total of 94 samples (48 eDNA, 46 tissue) *(Table A2.1)*. After filtering, the eDNA COI dataset comprised 2,534 taxa (908 taxa when rarefied to a sample size of 1,000).

Biodiversity assessment

Seven phyla (Annelida, Arthropoda, Chordata, Cnidaria, Platyhelminthes, Rhodophyta, and Rotifera) were detected overall across both catchment and habitat type samples, however relative read abundance plots for the top 20 taxa showed that biodiversity patterns varied across sites. At the catchment scale, Chordata dominated all samples in the Manawatū, while Rotifera dominated all samples at Waikato University and Rhodophyta dominated samples in the Riuwaka catchment (*Figure 3.3a*). The other catchments showed greater diversity within and across eDNA samples at the catchment scale and this did not broadly reflect geographic proximity (*Figure 3.3a*). Re-plotting this data by habitat determined that catchment generally impacted biodiversity patterns more than habitat type for creek, lake, pond, and stream habitats, while catchment and habitat were confounded for Moutere/river and Waitara/pond habitats (*Figure 3.3b*).

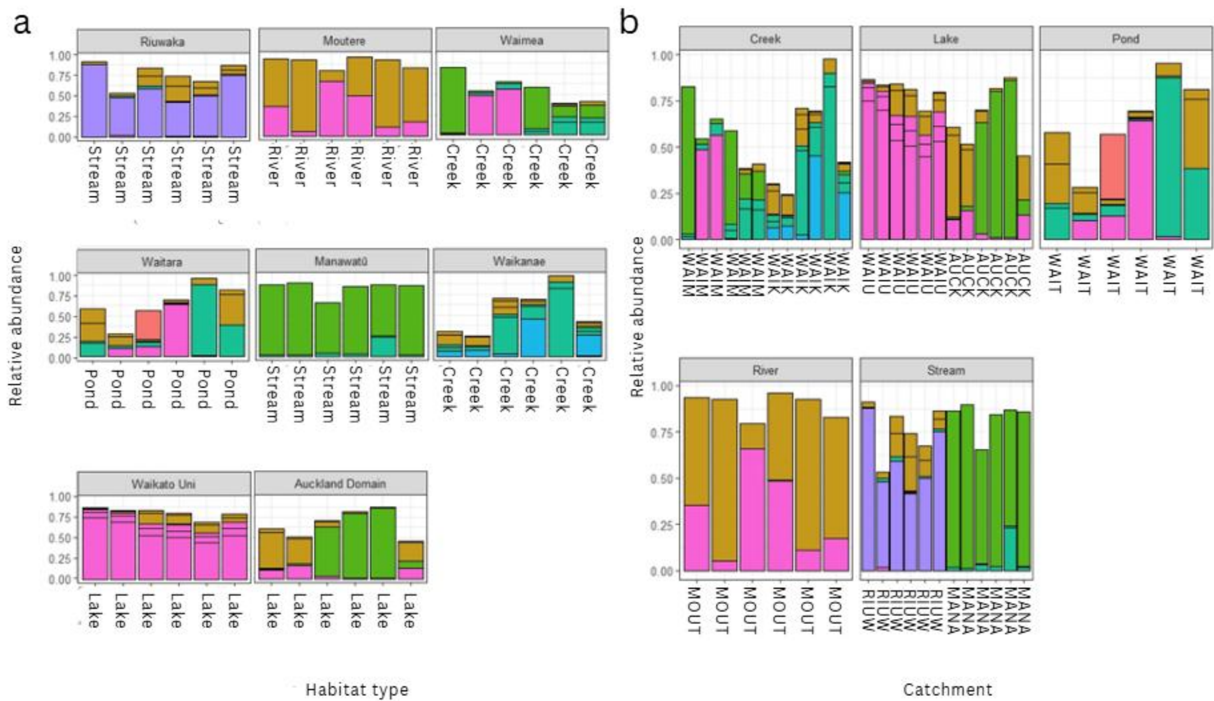

Figure 3. 3. Read count relative abundance plots per: (a) Catchment, with habitat type on the *x*-axis; and (b) Habitat type, with catchment on the *x*-axis. Phyla are represented by colour: Annelida (red), Arthropoda (gold), Chordata (dark green), Cnidaria (light green), Playhelminthes (blue), Rhodophyta (purple), and Rotifera (pink). In (a), catchments are plotted in approximate geographical order from North to South; abbreviations: AUCK=Auckland Domain; MANA=Manawatū; RIUW=Riuwaka; WAIK=Waikanae; WAIU=Waikato University; WAM=Waimea; WAIT=Waitara.

Patterns of alpha diversity varied by catchment and habitat type. At the catchment scale, alpha diversity was highest for Waikanae and Waitara and Moutere, showing a tight distribution of lower diversity values when compared to all other catchments (*Figure 3.4a*). At the habitat scale, alpha diversity observed in creek habitats and the pond site (Waitara) was higher than that observed for lakes, streams, and the river site (Moutere) (*Figure 3.4b*).

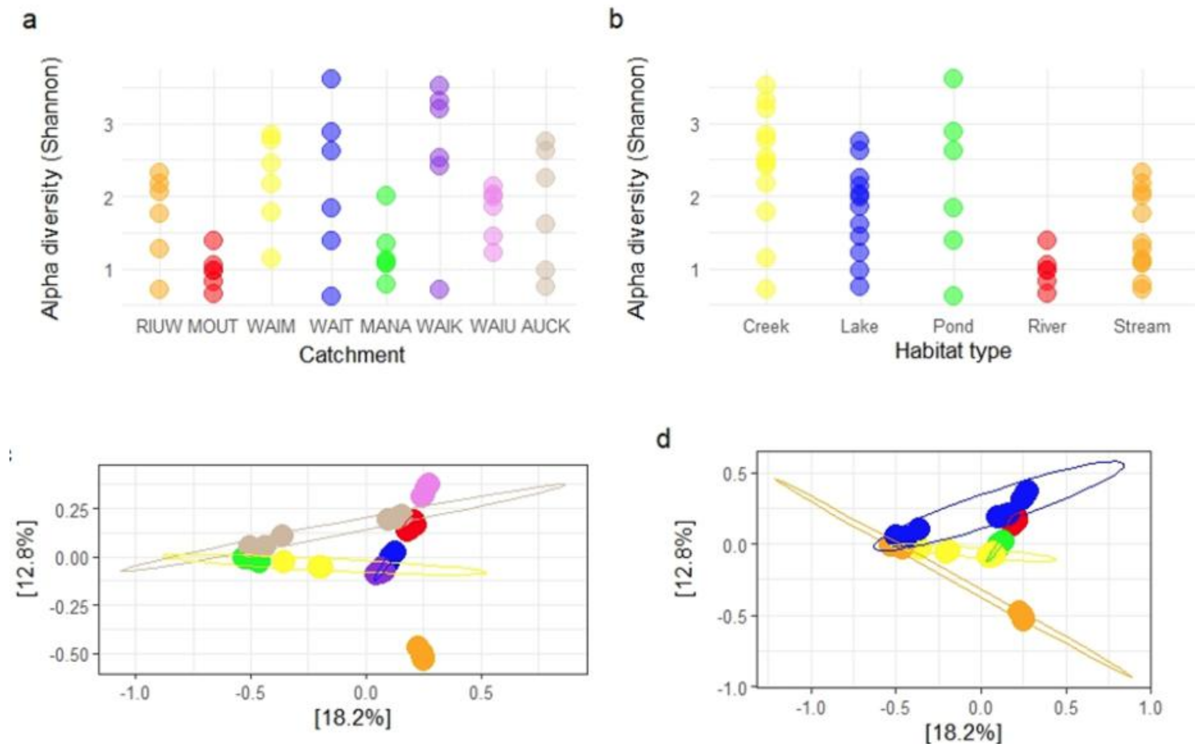

Figure 3.4. Plots of Shannon's alpha diversity for eDNA samples per: (a) Catchment; and (b) Habitat type. Ordination plots for eDNA samples per: (c) Catchment; and (d); Habitat type. In (a), catchments are plotted in approximate geographical order from South to North; abbreviations: RIUW=Riuwaka; MOUT=Moutere; WAIM=Waimea; WAIT=Waitara; MANA=Manwatū; WAIK=Waikanae; WAIU=Waikato University; AUCK=Auckland. In (c), gray=Auckland Domain; green=Manawatū; red=Moutere; orange=Riuwaka; purple=Waikanae; pink=Waikato University; yellow=Waimea; blue=Waitara. In (d), yellow=creek; blue=lake; green=pond; red=river; orange=stream.

Ordination plots showed significant differences in samples across catchments/habitat types (PERMANOVA: F=7.9023, p=0.001; BETADISPER: F=4.9213, p=0.003), with three general clusters. At the catchment scale, Riuwaka formed a discrete cluster, while the other sites formed two main overlapping sub-clusters (Waimea and Manawatū, and Waitara; and Moutere, Waikato University, and Waikanae) that themselves overlapped with Auckland Domain (*Figure 3.4c*). When re-plotted by habitat (PERMANOVA: F=4.8525, p=0.001; BETADISPERS: F=2.0707, p=0.109), stream samples overlapped with all other habitat types, while the lake cluster was slightly more separated from the river, pond, and creek habitats (*Figure 3.4d*). Pairwise analysis indicated that significant patterns in the MDS plots were driven by differences between populations including Auckland Domain and both Riuwaka and Manawatū (p_{perm}=0.035 for both), Manawatū and Waitara (p_{perm}=0.002), Manawatū and Waimea (p_{perm}=0.003); Riuwaka and Waitara (p_{perm}=0.001), and Riuwaka and Waimea (p_{perm}=0.006) (*Table 3.2*). At the habitat scale, signficant differences were only observed between river and creek sites (p_{perm}=0.027; *Table 3.2*).

Table 3. 2. Permuted p-values for pairwise comparisons betwee ncatchment (upper diagonal) and habitat type (lower diagonal). P-values <0.100 are indicated in bold, and those <0.050 are indicated by an asterix (*). Abbreviations: AUCK=Auckland Domain; MANA=Manawatū; RIUW=Riuwaka; WAIK=Waikanae; WAIU=Waikato University; WAM=Waimea; WAIT=Waitara.

	MANA	**MOUT**	**RIUW**	**WAIK**	**WAIU**	**WAIM**	**WAIT**	
	0.035*	0.803	**0.035***	0.846	**0.100**	0.408	0.218	**AUCK**
		0.090	0.796	0.011	0.666	**0.003***	**0.002***	**MANA**
River	**0.095**		0.104	0.652	0.178	0.320	0.171	**MOUT**
Pond	0.272	0.198		0.006	0.768	**0.006***	**0.001***	**RIUW**
Lake	0.597	0.137	0.911		**0.039***	0.456	0.165	**WAIK**
Creek	**0.052**	**0.027***	0.632	0.557		0.016	**0.009***	**WAIU**
	Stream	**River**	**Pond**	**Lake**			0.581	**WAIM**

Mitochondrial variation

Water and tissue comparison

Samples from all locations yielded a total of 88 unique ASVs characterised as *G. affinis DNA*, 50 of which were detected in the 46 tissue DNA samples while water samples (n=48) yielded 39 ASVs. One ASV (ASV_1) was common between the water and tissue sample types and this accounted for large proportions of the total *G. affinis* ASV reads in both tissue and water samples (99.2%, and 99.9% respectively; *Figure 3.5*). Of the remaining 87 ASVs that were attributed to *G. affinis* and only detected in one sample type, very low read count proportions were observed, the greatest of which was attributed to ASV_33 in the tissue samples (0.298% of all ASV reads detected) and ASV_2147 in the water samples (0.009%) (*Table A2.2*).

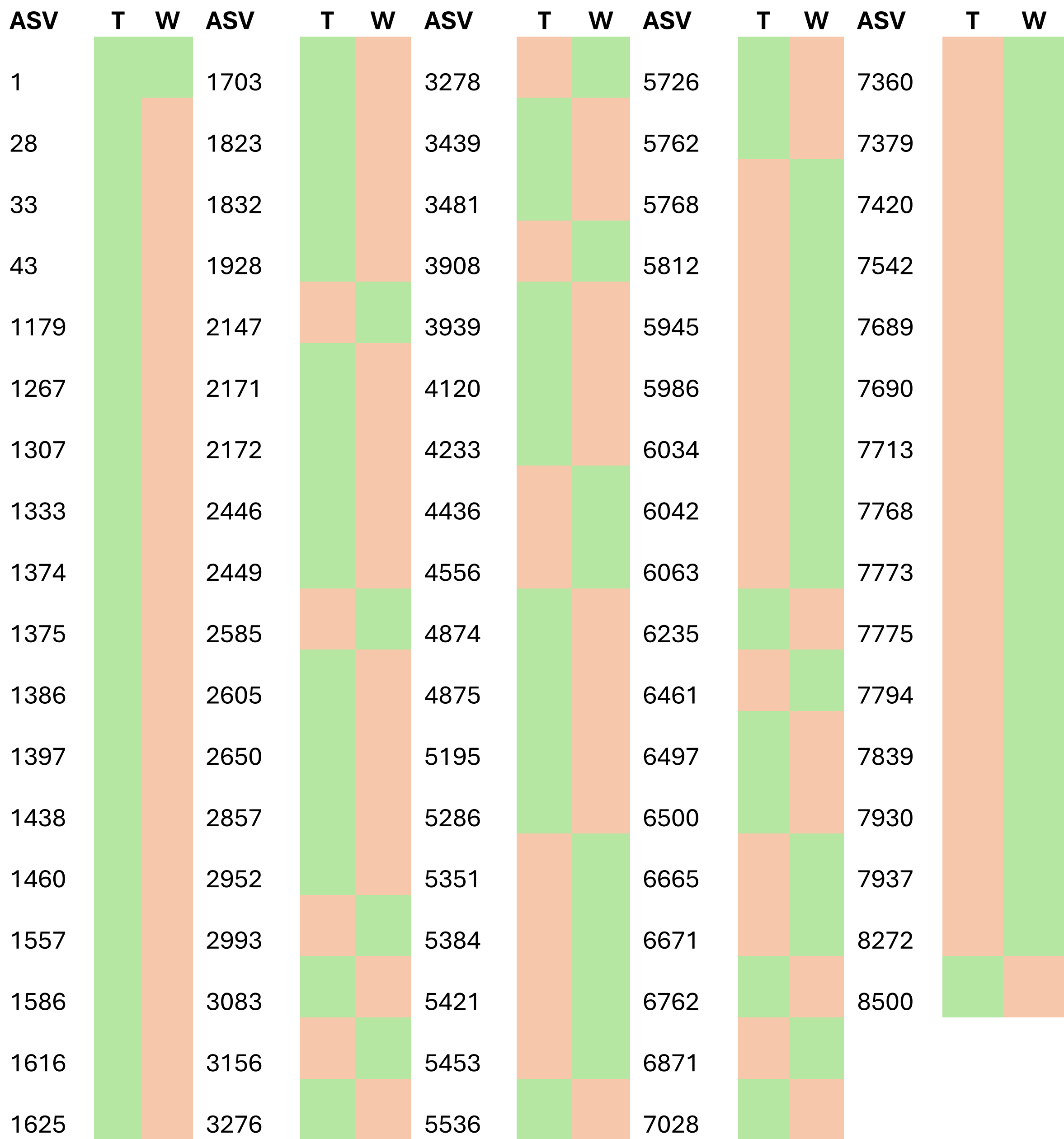

Figure 3. 5. Comparative visual schematic demonstrating which ASVs were detected per sampling method. Green=detected in tissue (T) or water (W); orange=not detected.

Geographical comparison

Of the 88 unique ASVs characterised as *G. affinis* DNA in this study, the greatest diversity within a population was found in the Waimea catchment (n=26 ASVs), with diversity detected in the remaining South Island populations (Moutere, n=8; Riuwaka, n=14). In the North Island, the greatest ASV diversity was detected in the Manawatū catchment (n=23), followed by the Waikanae (n=13). An ASV diversity of >10 was observed in the remaining populations in the North Island (Waitara, n=5; Waikato University, n=6; Auckland Domain, n=4).

A total of four ASVs were detected in more than one population. ASV_1 was detected in all eight populations, accounting for >97.7% of total ASV diversity detected (*Figure A2.2)*, while ASV_28 was found in Riuwaka, Moutere, and Waimea in the South Island as well as in Waitara and Waikanae in the North Island (0.570%, 0.190%, 0.509%, 0.227%, 0.308%, respectively; *Table A2.3*). All three South Island populations (Riuwaka, Moutere, and Waimea) also shared ASV_43 with Waitara in the North Island, and Riuwaka and Manawatū were the only populations that shared (low) read counts of ASV_3276 (10, and 2 reads, respectively); *Table A2.3*).

Phylogenetic analysis

Assessment of public records of COI data for *G. affinis* confirmed two main clusters of identical haplotypes and a third distinct haplotype exist throughout the invaded (excluding Australia and New Zealand) and native ranges of *G. affinis* (*Figure A2.3*). The first cluster included ASV_1 from this study and samples from Japan, Indonesia,

Bangladesh, China, Malaysia, Argentina, and Thailand (invaded range), as well as native range samples from Texas and Arkansas (United States) and Nuevo Leon (Mexico) (*Figure A2.3*). The second cluster included invaded ranges samples from Panama and Israel, and native range samples from Florida (United States) and Veracruz (Mexico) (*Figure A2.3*). Finally, the third, distinct haplotype pertained to a sample collected in the native range of *G. affinis* in the state of Veracruz (*Figure A2.*3).

3.5 Discussion

We undertook the first comparative analysis of tissue and water-based eDNA metabarcoding of mitochondrial DNA for *G. affinis* in New Zealand to explore biodiversity patterns across catchments and habits, alongside the utility of eDNA tools for genetic inference at the population level. We found that community-level biodiversity varied most strongly by catchment, while both water and tissue metabarcoding returned a single shared ASV at very high frequency.

eDNA for spatial biodiversity assessments

eDNA metabarcoding is a tool of increasing utility for biomonitoring applications - from the detection of a single species (Ficotela et al. 2008) to entire communities (Andres et al. 2023) - improving biodiversity estimates (Adams et al. 2019; Banks et al. 2021), and, in some cases, quantify population abundance (Adams et al. 2019). Here, we used eDNA to explore variation in overall biodiversity within and between sample sites to understand changes in the biotic community across catchments and habitat types. We expected to see catchment-scale differences that corresponded with site proximity; in particular, we expected the three proximate sites in the South Island (Riuwaka, Waimea, Moutere) to be similar, especially given uncertainties around the interconnectedness of these three catchments (H. McCaughan, pers. comms 2025). However, while we found evidence for catchment-scale differentiation, this did not generally correspond to geographic proximity. For example, the South Island Riuwaka catchment was quite different from all other catchments in our MDS plot, including the proximate Waimea and Moutere catchments. Meanwhile, the Manawatū site was

significantly different to two catchments in the South Island, but also to the North Island Waitara catchment.

We also hypothesised that sites with similar physical habitat parameters would be biotically similar, as flow variability is a principal mechanism determining habitat variability in riverine ecosystems (White 2019). However, we found that habitat type was generally not a significant driver of differentiation in our MDS plot, except between lotic systems (i.e., rivers and creeks). This finding could reflect increasing hydrological variation (i.e., fluctuations in discharge or flow velocity) across sites and suggests that flow rates should be assessed alongside alpha and beta diversity to determine biodiversity drivers in different habitats.

Our overall findings suggest that eDNA-derived biodiversity differences among sites are present but not clearly driven by geographic proximity or habitat type. However, disparities between the starting hypotheses and results of our study could be attributed to a number of factors. This includes misalignment in diversity classifications among sites - in particular, the assumptions of localisiation or regionality required to meet the definitions of alpha or beta diversity, respectively (see Heino 2011), would have required a greater number of samples taken at both site and catchment levels. Indeed, we sampled eight catchments and five habitat types, with confounding catchment/habitat for the Moutere River and Waitara Pond in particular. Thus, while our study confirms the value of eDNA sampling for detecting biodiversity, across spatial scales, it shows that relating biodiversity patterns to biotic drivers requires finer sampling and greater replication of those potential drivers - especially those that may be difficult to tease apart (e.g., habitat type and flow rate).

Mitochondrial variation

Recent developments in eDNA metabarcoding show its promise as a tool for population genetic analyses, particularly for both freshwater (Uchii et al. 2016; Gorički et al. 2017; Stepien et al. 2019; Marshall and Stepien 2019) and marine species (Eva Egelyng Sigsgaard et al. 2016; Stat et al. 2017; Baker et al. 2018; Turon et al. 2020; Adams et al. 2023). In our study, ASV_1 matched to *G. affinis* DNA and accounted for >99% of all COI diversity in both eDNA and tissue samples, suggesting similar rates of detection between sample methods for the same genetic variant across all sampled populations. However, none of the remaining 87 ASVs were shared across sample types, demonstrating variation in the detection of less abundant ASVs.

When accounting for the geographical spread of ASVs, low rates of recurrent ASV detection between populations was also observed, with three of the four common ASVs found in all three South Island populations, and varying configurations of North Island populations. The final shared ASV was detected in both Riuwaka and Manawatū - the two stream sites. Interestingly, the two more established populations - both lake sites - displayed among the lowest rates of ASV diversity (Auckland Domain, n=4; Waikato University, n=6) (see *Table 3.1* for date of first recorded population).

Although *G. affinis* was first detected in New Zealand in the 1930s, more recent introductions to the South Island took place in 2000 (Shaw and Studholme 2001). Thus, a single high-frequency haplotype may reflect the recent invasion history of the species. Consistent with this, our analysis of public records of *G. affinis* COI sequences showed that COI diversity for *G. affinis* is low across a range of global sites (only three

haplotypes in total), with our high frequency haplotype (ASV_1) corresponding to a global haplotype found in both native and invaded ranges. Population genetic analysis of *G. affinis* in New Zealand to date is based on microsatellite analysis and shows variation in the upper North Island that corresponds to three distinct clusters (Kevin M. Purcell and Stockwell 2015), but 97% of the New Zealand variation is shared with populations from the native range in Texas (K. M. Purcell et al. 2012). Collectively, these results suggest that the detected high-frequency ASV in our study most likely reflects the low global diversity of the species more generally, with the less abundant ASVs we detected likely reflecting technical considerations (e.g., poorly aligned shorter DNA fragments) as opposed to novel genetic diversity. This is supported by the patterns observed regarding ASV diversity between populations, which suggests physical habitat parameters may have an impact on the condition and presentation of DNA collected, irrespective of sampling method (i.e., tissue vs water). To explore this further, future studies should collect physical habitat parameters (e.g., temperature, salinity, pH, and turbidity) at each collection site and assess the DNA fragment length, in base pairs, as a proxy for condition of DNA fragments.

Multiple reviews assessing the utility of eDNA as a tool for population genetic inference mention the need for sequencing shorter, species-specific DNA fragments to obtain greater genetic resolution, or for size-based filtering to remove overly decayed DNA from the analysis (Rees et al. 2014; Adams et al. 2019; Andres et al. 2023)). Consistent with this, our eDNA data included two additional species in the Family Poeciliidae: *Xiphophorous helleri* (detected in a single sample) and *Poecilia reticulata* (detected in 53 samples). These species are not known to exist at the sample sites, and are only

present in localised, isolated populations in the wild in New Zealand (see McDowell 2000; Stoffels 2022; iNaturalist: available from https://www.inaturalist.org, accessed 3rd of December 2025), thus may represent misidentifications based on low-resolution fragments or errors in the reference database. However, both species can be purchased in the domestic aquarium trade (for example, https://www.hollywoodfishfarm.co.nz; https://bayaquatics.co.nz), thus further exploration of their potential presence in the wild should be explored.

Concluding remarks

We found that eDNA holds great promise for biomonitoring in freshwater environments but requires careful consideration in study design for use in assessing the drivers of biodiversity differentiation. A key component we were unable to achieve in the current study was the use of field controls alongside higher replication of biotic parameters among sampling sites. While every effort was made to eliminate cross-contamination between sites and PCR and sequencing controls were used to confirm an absence of laboratory contamination, field controls would have enabled us to remove potential field contaminants from the dataset. Finally, our assessment of the potential of eDNA for population genetic inference suggests that technical issues still limit this application, at least for recent biological invasions in which only a single high-frequency haplotype dominates the sequencing pool.

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4.1 General summary

My thesis used traditional (netting) and DNA metabarcoding approaches to investigate source-sink population dynamics of the invasive mosquitofish, *Gambusia affinis*. By combining both approaches, my studies provide a greater understanding of occupancy patterns at multiple scales, demonstrating how distribution and abundance of a species can vary drastically over both spatial and temporal scales, and the importance of critical analysis of scale when undertaking ecological studies on aquatic organisms.

At small spatial scales (**Chapter 2**), traditional netting surveys revealed presence and abundance to be a result of complex interactions with physical habitat parameters, demonstrating microhabitat selection in *G. affinis*. As spatial scales increased, presence was more highly attributed to variation in physical habitat parameters, such as the impact of less than average rainfall over the course of a year. These observations point to a need for incorporating greater consideration of wider temporal scales when assessing changes to the occupancy range of a species.

In **Chapter 3**, metabarcoding of both eDNA (water) and tissue samples produced insights into of how biodiversity differed across space and habitat type, while suggesting that eDNA for population genetic inference of recent biological invasions or low diversity species may be less useful. Thus, improvements in metabarcoding systems and techniques are needed to successfully understand occupancy patterns for such species.

4.2 Considerations and caveats

Both case studies sought to determine the ability of particular methods to detect occupancy patterns at different spatial and temporal scales, however the ecological and physical processes underpinning each method varied greatly. While many caveats relative to the studies have been considered and discussed in their respective chapters, interpretations of these findings must consider broader ecological and geophysical contexts.

The success of different sampling methods could be implicated by behavioural traits or personality of individuals, creating sampling bias in catch data (Pessanha Pais and Cabral n.d.; Álvarez-Quintero et al. 2021; Lamb et al. 2022). Additionally, exposure to tolerance limits of physical habitat parameters may have further extrapolated alterations in life-history patterns (Beaudouin et al. 2008). While careful consideration of the life-history and tolerance thresholds of a species can mitigate these effects, they do ultimately have implications on the accuracy of results. For example, in **Chapter 2**, detection rates did not reflect surveillance data supplied by the New Zealand Department of Conservation Motueka Operations District, despite dipnet sampling being attributed to the greatest catch levels for *G. affinis* (Howell et al. 2013; Cheng et al. 2018).

Similarly, DNA recovery and allelic attribution of eDNA can be complicated by responses to physical habitat parameters, such as temperature, pH, and salinity, as well as biotic factors, such as microbial loads (Lamb et al. 2022). While mitochondrial DNA is thought to provide the best opportunity for population genetic inference, as a

result of its high concentration (Andres et al. 2023) and slower decay rate compared to other gene regions, such as nuclear DNA (Eva E. Sigsgaard et al. 2020), there does not exist one preferred gene or region to undertake such studies with eDNA. As such, sampling bias may be implicated based on the gene region chosen. Adams et al. (2019) further states the use of eDNA for population genetics may still be in its infancy and requires further exploration. Indeed, in **Chapter 3** a single ASV accounted for >99% of all COI diversity in both eDNA and tissue samples, suggesting similar rates of detection between sample methods for the same genetic variant across all sampled populations. An additional set of >80 rare ASVs were also detected, but these reflected technical considerations versus additional genetic diversity, highlighting the current limitations of the field for some applications/species.

Overall, my thesis demonstrates that successful and accurate biomonitoring, particularly of source-sink dynamics across various spatial and temporal scales, requires a complex spectrum of sampling approaches to account for the dynamic network of interacting biotic and abiotic conditions, anthropogenic influences, and both fine and coarse-scale environmental variability.

4.3 Recommendations

My thesis coupled genetic techniques with systematic environmental monitoring, i.e., traditional survey methods, to improve understanding of source-sink dynamics of *G. affinis*. While specific recommendations have been considered for each in their respective chapter, several broader recommendations can be considered for future work.

Genetic and traditional methods have demonstrated strong potential to detect changes in occupancy patterns across dynamic spatiotemporal scales when combined. As shown in **Chapter 2**, traditional survey methods can influence understanding of changes to occupancy patterns as a response to environmental stochasticity over a defined period of time, while genetic techniques (**Chapter 3**) offer a more resource-sensitive approach that can enhance the spatial and temporal scales sampled. Expanding sampling indices across a greater number of invaded estuarine catchments, seasons, and years would facilitate the detection of long-term occupancy patterns. Additionally, incorporating comparative studies of related species occupying different niches, as demonstrated by Rodriguez-Silva et al. (2022) for four poeciliids, could further detect patterns in species behaviour in response to environmental stochasticity.

Methodological refinements could further enhance the information produced from occupancy patterns at various scales. For traditional netting, the incorporation of visible implant fluorescent elastomer (VIE; Northwest Marine Technology, Shaw Island, Washington, USA) has successfully shown that individuals can be tagged and

monitored over small distances with high rates of tag retention and minimal tagging-associated mortality (Chapman and Waburton 2006). Meanwhile, Adams et al. (2019) recommended the incorporation of multiple markers into sequencing of eDNA to improve success of population genetic studies. For example, the cytochrome b fragment has been used for freshwater fish species composition detection studies in Asia (Gillet 2008) and has been successfully used to understand population genetics of silver carp (*Hypophthalmichthys molitrix*) in the Mississippi River basin (Stepien et al. 2019).

Finally, future studies should incorporate samples sites within the invaded range but without known populations of *G. affinis* and undertake a more critical analysis of beta diversity to determine biotic dispersal drivers of individuals between sites. Particularly in conjunction with the aforementioned recommendations, this would build on the present study, which focused solely on abiotic dispersal drivers - incorporating mechanisms for active dispersal and providing a fuller picture of source-sink dynamics in *G. affinis*.

4.4 Concluding remarks

To conclude, my thesis highlights how advances in genetic techniques, particularly eDNA, can be incorporated into a multi-method sampling approach to understand complex spatiotemporal interactions in coastal aquatic systems in New Zealand. With some further refinement, such an approach could be incorporated into national management regimes to enhance long-term, coarse-scale biomonitoring, increasing capabilities for rapid management responses of already established and potentially new invasive species. This, ultimately, will facilitate greater protection of our native ecosystems and species, and enhancement of the human-environment system more in favour of naturogenic benefits.

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Appendix 1: Supplementary information: Chapter 2

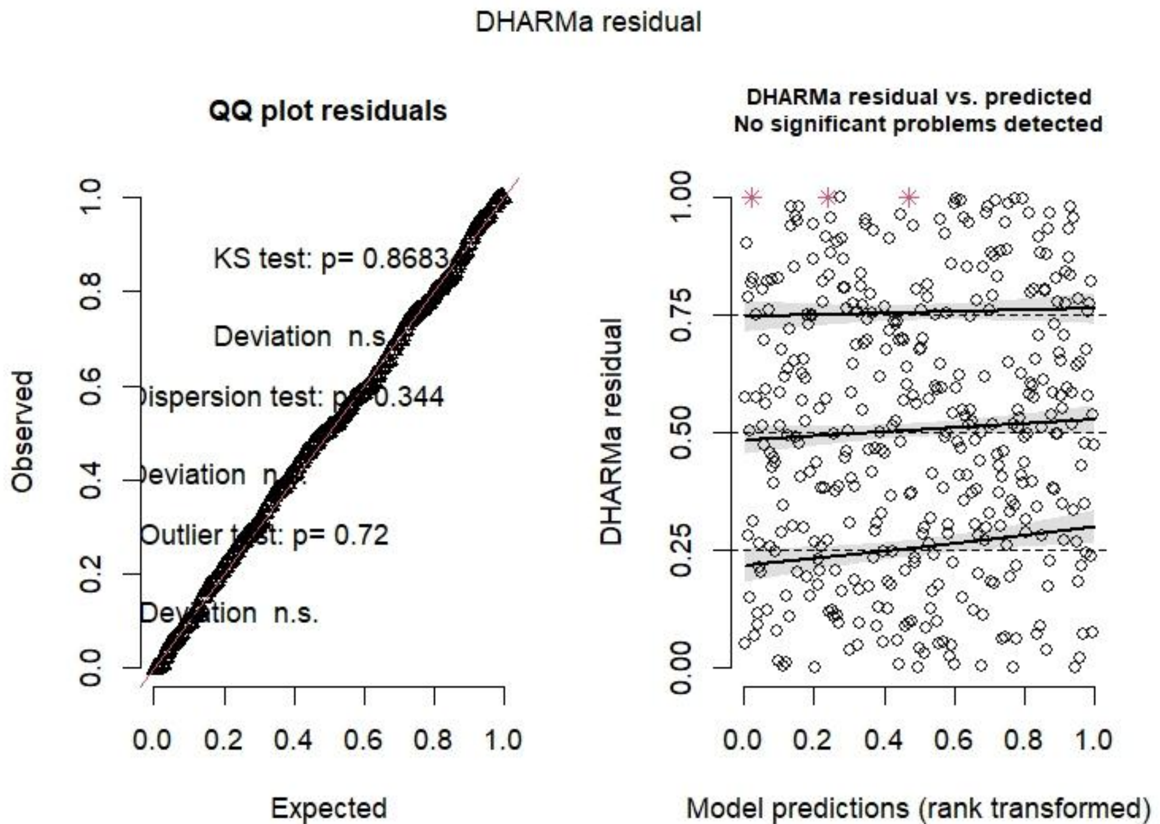

Figure A1. 1. Diagnostic plots of scaled residuals to assess fit of Negative Binomial Generalised Linear Mixed-effects Hurdle Model. The left panel shows QQ plot, while the right panel shows DHARMa residuals plotted against rank-transformed model predictions.

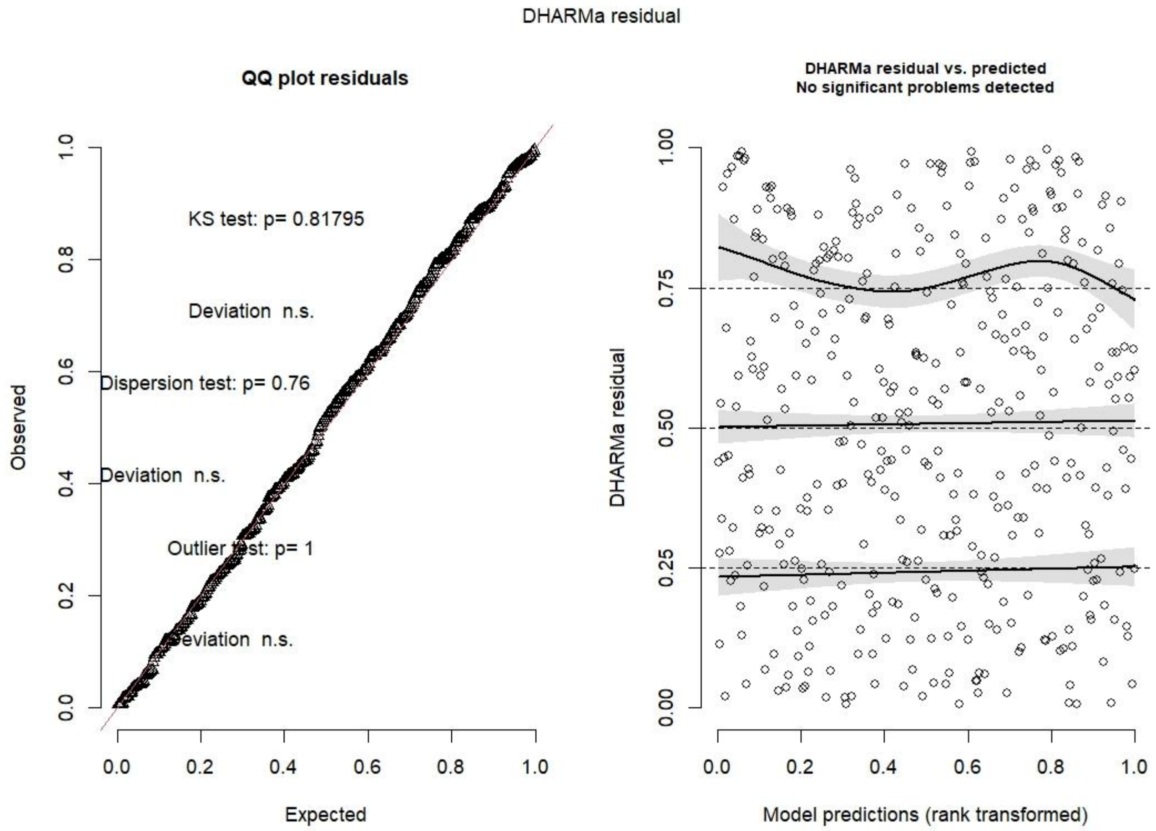

Figure A1. 2. Diagnostic plots of scaled residuals to assess fit of Negative Binomlial Generalised Linear Mixed-effects Model. The left panel shows QQ plot, while the right panel shows DHARMa residuals plotted against rank-transformed model predictions.

Appendix 2: Supplementary Information: Chapter 3

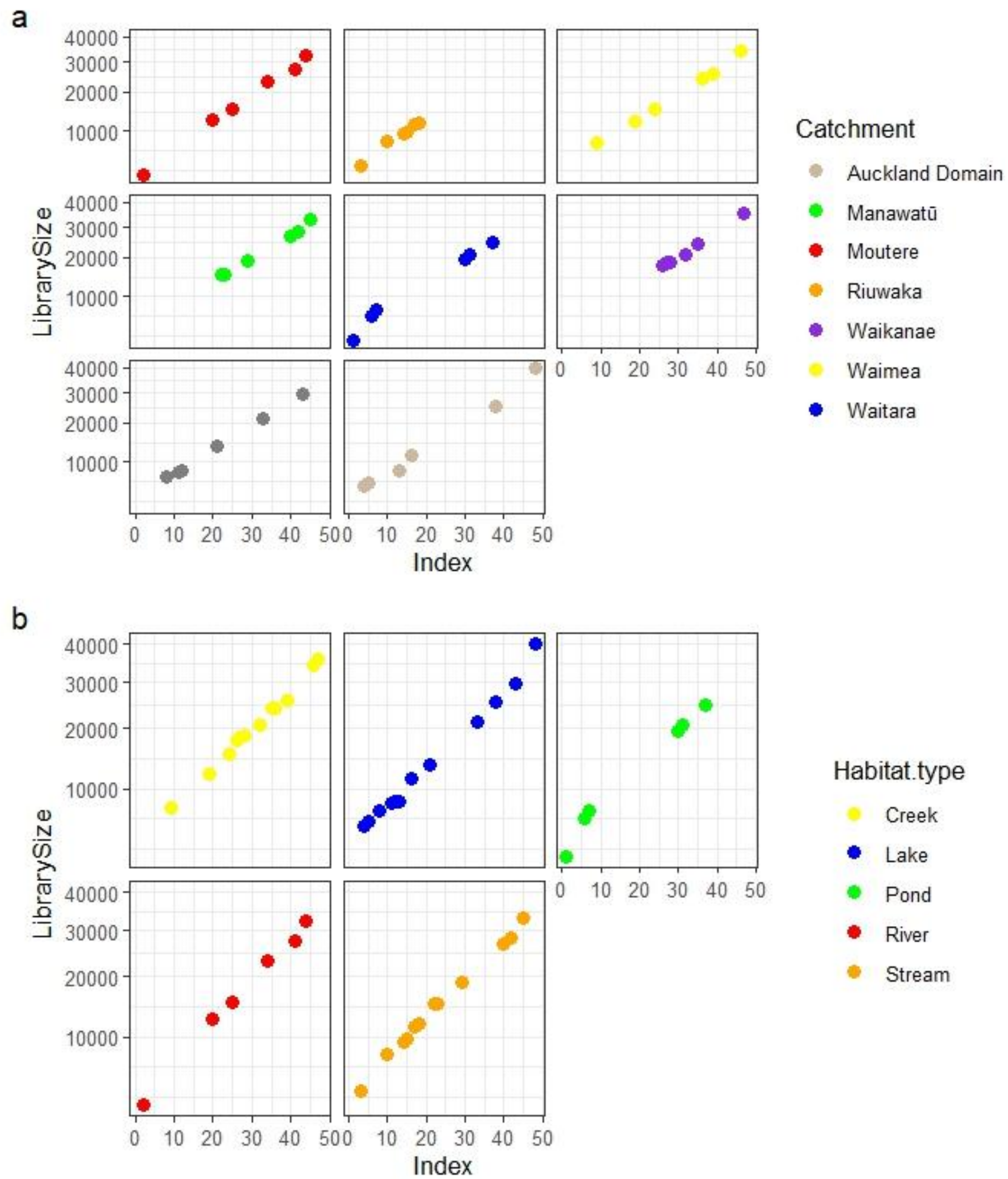

Figure A2.1. Library size plots (i.e., sequence output per sample) for the eDNA sample collection by: (a) Catchment; and (b) Habitat type.

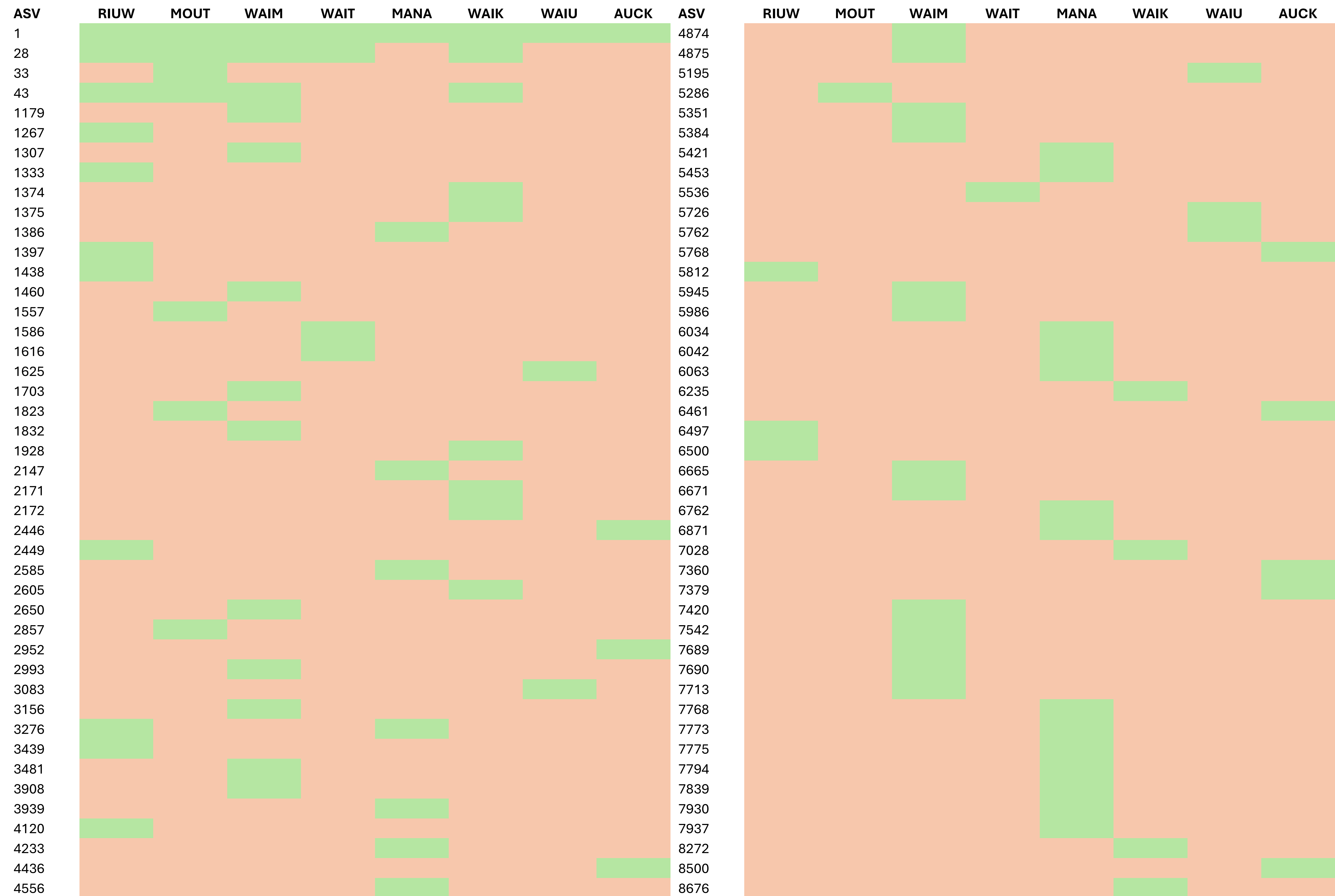

Figure A2. 2. Comparative visual schematic demonstrating which ASVs were detected per population. Green=detected in the populations, orange=not detected. Populations are plotted in approximate geographical order from South to North; abbreviations: RIUW=Riuwaka; MOUT=Moutere; WAIM=Waimea; WAIT=Waitara; MANA=Manawatū; WAIK=Waikanae; WAIU=Waikato University; AUCK=Auckland Domain.

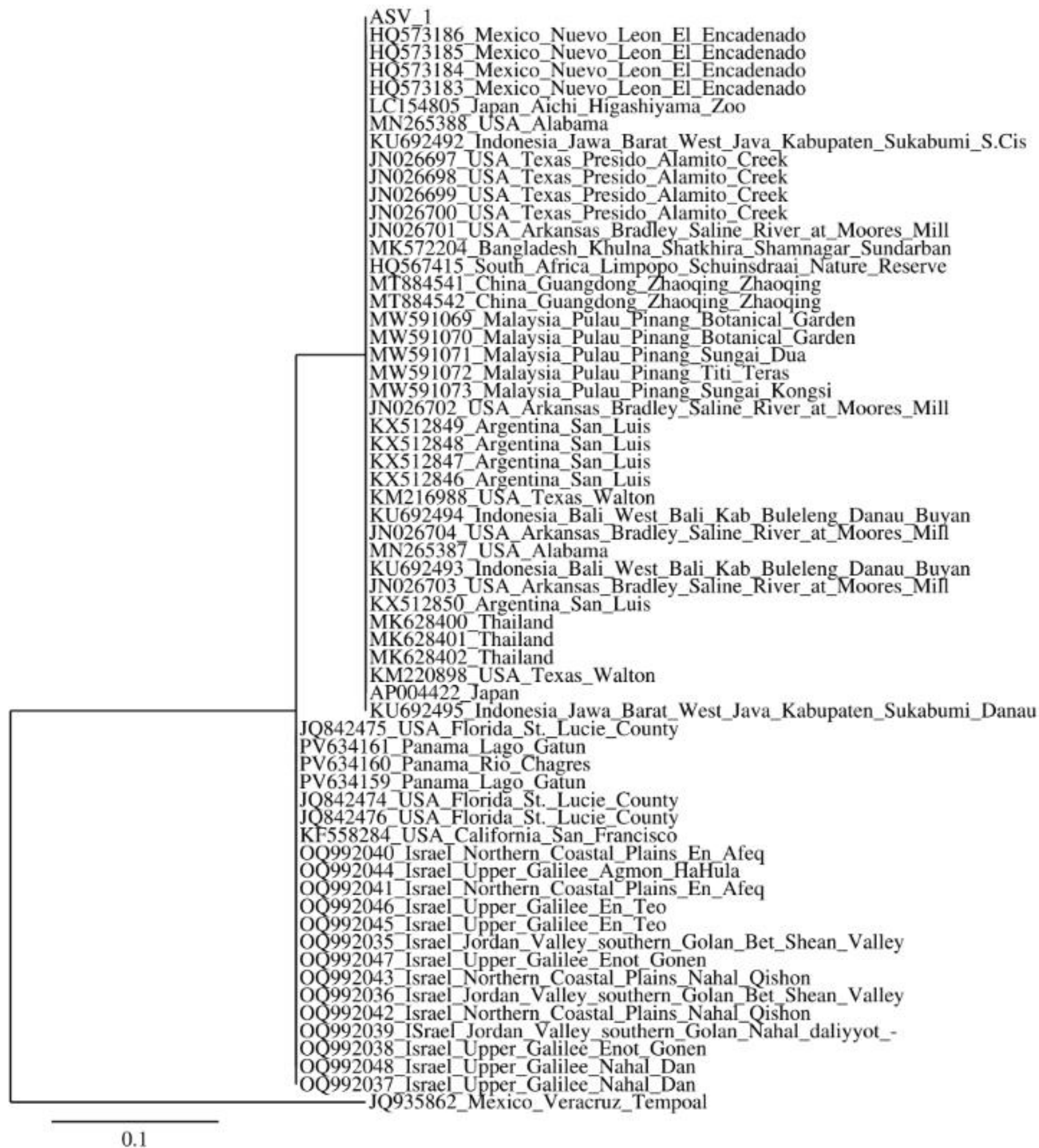

Figure A2.3. Phylogeny tree of COI sequences downloaded from the National Center for Biotechnology Information, including ASV_1 from this study.

Table A2.1. Per-amplicon sequencing statistics for each sample, including the number of raw (R), filtered (F), denoised (DF, DR for forward reverse reas, respectively), merged (M), and non-chimera (N) reads. Sample cose, location, latitude, longitude, sample method (SM), amount of water filtered per eDNA samples (mL), and collection date information are also included.

Sample	Location	Latitude	Longitude	SM	mL	Date	R	F	DF	DR	M	N
DOC-25-01-001	Moutere	-41.1491	172.9933	Tissue	0	19/12/2024	193863	115864	115746	115766	110621	110548
DOC-25-01-002	Moutere	-41.1491	172.9933	Tissue	0	19/12/2024	91616	54136	53882	54007	51322	51322
DOC-25-01-003	Moutere	-41.1491	172.9933	Tissue	0	19/12/2024	146436	89630	88465	89468	84621	84621
DOC-25-01-004	Moutere	-41.1491	172.9933	Tissue	0	19/12/2024	147949	87780	87068	87623	83027	83027
DOC-25-01-005	Moutere	-41.1491	172.9933	Tissue	0	19/12/2024	198768	117699	116848	117552	111793	111793
DOC-25-01-006	Moutere	-41.1491	172.9933	Tissue	0	19/12/2024	91421	54231	54011	54091	51526	51526
DOC-25-01-007	Moutere	-41.1491	172.9933	eDNA	220	19/12/2024	75234	43916	42901	43074	41239	41139
DOC-25-01-008	Moutere	-41.1491	172.9933	eDNA	240	19/12/2024	79615	47300	46961	46976	45248	45248
DOC-25-01-009	Moutere	-41.1491	172.9933	eDNA	300	19/12/2024	39886	23496	22534	23288	19825	19825
DOC-25-01-010	Moutere	-41.1491	172.9933	eDNA	180	19/12/2024	94692	55330	54110	54751	52643	52620
DOC-25-01-011	Moutere	-41.1491	172.9933	eDNA	300	19/12/2024	39985	24055	23314	23506	22596	22589
DOC-25-01-012	Moutere	-41.1491	172.9933	eDNA	240	19/12/2024	16797	9834	9121	9419	8583	8572
DOC-25-01-013	Riuwaka	-41.0780	172.9973	Tissue	0	19/12/2024	140030	82574	82421	82457	78977	78977
DOC-25-01-014	Riuwaka	-41.0780	172.9973	Tissue	0	19/12/2024	140056	84117	83888	84054	80849	80846
DOC-25-01-015	Riuwaka	-41.0780	172.9973	Tissue	0	19/12/2024	82784	49217	48971	49103	47307	47304
DOC-25-01-016	Riuwaka	-41.0780	172.9973	Tissue	0	19/12/2024	98155	57388	56749	57283	54473	54473

DOC-25-01-017	Riuwaka	-41.0780	172.9973	Tissue	0	19/12/2024	82077	49045	48808	48948	46985	46985
DOC-25-01-018	Riuwaka	-41.0780	172.9973	Tissue	0	19/12/2024	78704	47141	46757	47025	44653	44653
DOC-25-01-019	Riuwaka	-41.0780	172.9973	eDNA	60	19/12/2024	29948	17642	16843	17118	15911	15909
DOC-25-01-020	Riuwaka	-41.0780	172.9973	eDNA	60	19/12/2024	66736	39469	38796	39125	36342	36342
DOC-25-01-021	Riuwaka	-41.0780	172.9973	eDNA	70	19/12/2024	52382	30583	29763	29878	28425	28422
DOC-25-01-022	Riuwaka	-41.0780	172.9973	eDNA	120	19/12/2024	60719	35790	34563	35120	33059	33017
DOC-25-01-023	Riuwaka	-41.0780	172.9973	eDNA	90	19/12/2024	39681	23908	22964	23138	22142	22057
DOC-25-01-024	Riuwaka	-41.0780	172.9973	eDNA	60	19/12/2024	56039	33165	32528	32532	31364	31326
DOC-25-01-025	Waimea	-41.3289	173.1698	Tissue	0	18/12/2024	124133	73257	72927	73139	70362	70362
DOC-25-01-026	Waimea	-41.3290	173.1698	Tissue	0	18/12/2024	128648	76919	76367	76769	73381	73381
DOC-25-01-027	Waimea	-41.3291	173.1698	Tissue	0	18/12/2024	104203	61627	61159	61464	58456	58456
DOC-25-01-028	Waimea	-41.3292	173.1698	Tissue	0	18/12/2024	99861	58961	58384	58825	55724	55724
DOC-25-01-029	Waimea	-41.3293	173.1698	Tissue	0	18/12/2024	83167	48933	48492	48571	46555	46555
DOC-25-01-030	Waimea	-41.3294	173.1698	Tissue	0	18/12/2024	97650	58342	57912	58005	55428	55428
DOC-25-01-031	Waimea	-41.3295	173.1698	eDNA	100	18/12/2024	60425	35466	33210	34008	31334	30654
DOC-25-01-032	Waimea	-41.3296	173.1698	eDNA	180	18/12/2024	58613	34077	31441	32688	29883	29441
DOC-25-01-033	Waimea	-41.3297	173.1698	eDNA	120	18/12/2024	63917	36328	34228	34836	32148	31311
DOC-25-01-034	Waimea	-41.3298	173.1698	eDNA	130	18/12/2024	49707	29134	26207	27134	24690	24459
DOC-25-01-035	Waimea	-41.3299	173.1698	eDNA	450	18/12/2024	84565	50261	45788	46938	42049	39951
DOC-25-01-036	Waimea	-41.3300	173.1698	eDNA	400	18/12/2024	55881	33434	31223	31562	29447	29072

DOC-25-01-037	Manawatū	-40.2521	175.5950	Tissue	0	16/12/2024	122442	73015	72759	72936	69718	69718
DOC-25-01-038	Manawatū	-40.2521	175.5950	Tissue	0	16/12/2024	58849	34949	34742	34847	33389	33389
DOC-25-01-039	Manawatū	-40.2521	175.5950	Tissue	0	16/12/2024	60945	35912	35691	35761	33956	33956
DOC-25-01-040	Manawatū	-40.2521	175.5950	Tissue	0	16/12/2024	66846	39416	39222	39328	37657	37657
DOC-25-01-041	Manawatū	-40.2521	175.5950	Tissue	0	16/12/2024	48712	28732	28499	28629	27083	27083
DOC-25-01-042	Manawatū	-40.2521	175.5950	Tissue	0	16/12/2024	96422	57326	56829	56953	54533	54533
DOC-25-01-043	Manawatū	-40.2521	175.5950	eDNA	300	16/12/2024	61030	36258	34600	34984	32644	32599
DOC-25-01-044	Manawatū	-40.2521	175.5950	eDNA	450	16/12/2024	73618	43408	39770	41712	37579	37304
DOC-25-01-045	Manawatū	-40.2521	175.5950	eDNA	450	16/12/2024	61684	36611	33835	34291	31529	31246
DOC-25-01-046	Manawatū	-40.2521	175.5950	eDNA	450	16/12/2024	39133	23476	21604	21854	19684	19654
DOC-25-01-047	Manawatū	-40.2521	175.5950	eDNA	350	16/12/2024	32422	19104	17773	17970	17066	17044
DOC-25-01-048	Manawatū	-40.2521	175.5950	eDNA	350	16/12/2024	67950	40394	38339	38843	36492	36454
DOC-25-01-049	Waitara	-40.2521	175.5950	Tissue	0	16/01/2025	60594	35862	35712	35807	34245	34215
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DOC-25-01-054	Waitara	-40.2521	175.5950	Tissue	0	16/01/2025	75541	44468	44016	44292	42245	42192
DOC-25-01-055	Waitara	-40.2521	175.5950	Tissue	850	16/01/2025	55776	33121	30281	31149	28529	28248
DOC-25-01-056	Waitara	-40.2521	175.5950	Tissue	1000	16/01/2025	62237	35796	33170	34268	30073	29707

DOC-25-01-057	Waitara	-40.2521	175.5950	Tissue	750	16/01/2025	28331	14266	11413	12281	10710	10703
DOC-25-01-058	Waitara	-40.2521	175.5950	Tissue	600	16/01/2025	24448	14714	13714	13800	12639	12490
DOC-25-01-059	Waitara	-40.2521	175.5950	Tissue	200	16/01/2025	6002	3532	3167	3210	3080	3080
DOC-25-01-060	Waitara	-40.2521	175.5950	Tissue	1000	16/01/2025	56903	33635	32715	32846	31378	31331
DOC-25-01-061	Waikanae	-38.6623	177.9843	Tissue	0	14/12/2024	141807	84832	84320	84754	81272	81272
DOC-25-01-062	Waikanae	-38.6623	177.9843	Tissue	0	14/12/2024	112615	67122	66485	67017	63529	63529
DOC-25-01-063	Waikanae	-38.6623	177.9843	Tissue	0	14/12/2024	99301	58571	58129	58397	55557	55557
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DOC-25-01-066	Waikanae	-38.6623	177.9843	Tissue	0	14/12/2024	66270	39071	38850	38972	37402	37402
DOC-25-01-067	Waikanae	-38.6623	177.9843	eDNA	1000	14/12/2024	80514	47403	42388	43529	38702	37772
DOC-25-01-068	Waikanae	-38.6623	177.9843	eDNA	1000	14/12/2024	79703	46971	42347	43439	38896	37802
DOC-25-01-069	Waikanae	-38.6623	177.9843	eDNA	1000	14/12/2024	65076	38378	34604	35359	32296	31699
DOC-25-01-070	Waikanae	-38.6623	177.9843	eDNA	500	14/12/2024	58150	34556	31648	32148	28987	28682
DOC-25-01-071	Waikanae	-38.6623	177.9843	eDNA	500	14/12/2024	70569	41970	40705	40853	38725	37385
DOC-25-01-072	Waikanae	-38.6623	177.9843	eDNA	650	14/12/2024	56281	32222	29673	30370	27972	27957
DOC-25-01-073	Waikato University	-37.7865	175.3161	Tissue	0	20/01/2025	9489	5170	3840	5025	1748	1748
DOC-25-01-074	Waikato University	-37.7865	175.3161	Tissue	0	20/01/2025	32080	18971	18707	18876	17734	17734

DOC-25-01-075	Waikato University	-37.7865	175.3161	Tissue	0	20/01/2025	30959	18190	17905	18041	16217	16056
DOC-25-01-076	Waikato University	-37.7865	175.3161	Tissue	0	20/01/2025	23883	13934	13512	13771	11991	11956
DOC-25-01-077	Waikato University	-37.7865	175.3161	Tissue	0	20/01/2025	88227	52230	51853	52034	49090	48947
DOC-25-01-078	Waikato University	-37.7865	175.3161	Tissue	0	20/01/2025	49233	29564	28942	29446	28012	28012
DOC-25-01-079	Waikato University	-37.7865	175.3161	eDNA	150	20/01/2025	61827	34862	33500	33711	30774	30317
DOC-25-01-080	Waikato University	-37.7865	175.3161	eDNA	150	20/01/2025	88396	49971	47906	48386	44090	43362
DOC-25-01-081	Waikato University	-37.7865	175.3161	eDNA	150	20/01/2025	53272	31158	28575	29303	27159	26851
DOC-25-01-082	Waikato University	-37.7865	175.3161	eDNA	150	20/01/2025	80435	47302	43594	44789	40728	39995
DOC-25-01-083	Waikato University	-37.7865	175.3161	eDNA	150	20/01/2025	51475	30001	27707	28383	26265	25988
DOC-25-01-084	Waikato University	-37.7865	175.3161	eDNA	150	20/01/2025	47307	25553	23109	24064	22044	21896
DOC-25-01-085	Auckland Domain	-38.8596	174.7727	Tissue	0	19/01/2025	58614	34721	33765	34645	32636	32636
DOC-25-01-086	Auckland Domain	-38.8596	174.7727	Tissue	0	19/01/2025	87535	51709	51215	51569	48850	48850
DOC-25-01-087	Auckland Domain	-38.8596	174.7727	Tissue	0	19/01/2025	N/A	N/A	N/A	N/A	N/A	N/A

DOC-25-01-088	Auckland Domain	-38.8596	174.7727	Tissue	0	19/01/2025	47999	28231	28010	28141	27067	26836
DOC-25-01-089	Auckland Domain	-38.8596	174.7727	Tissue	0	19/01/2025	176424	104277	103809	103952	98990	98623
DOC-25-01-090	Auckland Domain	-38.8596	174.7727	Tissue	0	19/01/2025	N/A	N/A	N/A	N/A	N/A	N/A
DOC-25-01-091	Auckland Domain	-38.8596	174.7727	eDNA	150	19/01/2025	106438	62976	59866	60673	55842	52601
DOC-25-01-092	Auckland Domain	-38.8596	174.7727	eDNA	150	19/01/2025	122556	71208	68129	69064	65004	62225
DOC-25-01-093	Auckland Domain	-38.8596	174.7727	eDNA	150	19/01/2025	77477	46066	43467	43964	40920	39038
DOC-25-01-094	Auckland Domain	-38.8596	174.7727	eDNA	150	19/01/2025	105612	62474	60128	60912	57277	55252
DOC-25-01-095	Auckland Domain	-38.8596	174.7727	eDNA	150	19/01/2025	132124	77590	76502	76860	73998	73477
DOC-25-01-096	Auckland Domain	-38.8596	174.7727	eDNA	150	19/01/2025	126658	75495	73454	73814	70072	69327

Table A2.2. Total ASV counts per sample method expressed in both total count and proportion of total counts.

ASV	Tissue samples		eDNA samples	
	Total count	**Proportion**	**Total count**	**Proportion**
1	2347189	0.9920000	254178	0.999
28	7061	0.0029800	0	0
33	6091	0.0025700	0	0
43	4763	0.0020100	0	0
1179	65	0.0000275	0	0
1267	58	0.0000245	0	0
1307	55	0.0000232	0	0
1333	53	0.0000224	0	0
1374	51	0.0000216	0	0
1375	51	0.0000216	0	0
1386	50	0.0000211	0	0
1397	49	0.0000207	0	0
1438	47	0.0000199	0	0
1460	46	0.0000194	0	0
1557	40	0.0000169	0	0
1586	39	0.0000165	0	0
1616	38	0.0000161	0	0
1625	38	0.0000161	0	0
1703	35	0.0000148	0	0
1823	31	0.0000131	0	0
1832	31	0.0000131	0	0
1928	29	0.0000123	0	0
2171	24	0.0000101	0	0
2172	24	0.0000101	0	0
2446	20	0.0000085	0	0
2449	19	0.0000080	0	0
2605	18	0.0000076	0	0
2650	17	0.0000072	0	0
2857	15	0.0000063	0	0
2952	15	0.0000063	0	0
3083	14	0.0000059	0	0
3439	11	0.0000047	0	0
3481	11	0.0000047	0	0
3276	10	0.0000042	0	0
3939	9	0.0000038	0	0
4120	8	0.0000034	0	0
4233	8	0.0000034	0	0
4874	6	0.0000025	0	0

4875	6	0.0000025	0	0
5195	6	0.0000025	0	0
5286	5	0.0000021	0	0
5536	5	0.0000021	0	0
5726	5	0.0000021	0	0
5768	5	0.0000021	0	0
5812	4	0.0000017	0	0
6235	4	0.0000017	0	0
6497	3	0.0000013	0	0
6500	3	0.0000013	0	0
6762	3	0.0000013	0	0
7028	3	0.0000013	0	0
8500	2	0.0000009	0	0
2147	0	0	24	0.0000943
2585	0	0	18	0.0000708
4436	0	0	16	0.0000629
2993	0	0	14	0.000055
3156	0	0	13	0.0000511
3908	0	0	9	0.0000354
6461	0	0	8	0.0000314
4556	0	0	7	0.0000275
7379	0	0	6	0.0000236
7420	0	0	6	0.0000236
5351	0	0	5	0.0000197
5384	0	0	5	0.0000197
5421	0	0	5	0.0000197
5453	0	0	5	0.0000197
5762	0	0	5	0.0000197
5945	0	0	4	0.0000157
5986	0	0	4	0.0000157
6042	0	0	4	0.0000157
6034	0	0	4	0.0000157
6063	0	0	4	0.0000157
6665	0	0	3	0.0000118
6671	0	0	3	0.0000118
6871	0	0	3	0.0000118
3278	0	0	2	0.0000079
7542	0	0	2	0.0000079
7360	0	0	2	0.0000079
7689	0	0	2	0.0000079
7690	0	0	2	0.0000079
7713	0	0	2	0.0000079
7768	0	0	2	0.0000079
7773	0	0	2	0.0000079
7775	0	0	2	0.0000079

7794	0	0	2	0.0000079
7839	0	0	2	0.0000079
7930	0	0	2	0.0000079
7937	0	0	2	0.0000079
8272	0	0	2	0.0000079

Table A2. 3 Total ASV counts per population expressed in both total count and proportion of total count

ASV	Riuwaka		Moutere		Waimea		Manawatū		Waitara		Waikanae		Waikato University		Auckland Domain	
	Total count	**Proportion**	**Total count**	**Proportion**	**Total count**	**Proportion**	**Total count**	**Proportion**	**Total count**	**Proportion**	**Total count**	**Proportion**	**Total count**	**Proportion**	**Total count**	**Proportion**
1	486194	0.98993157	344300	0.97790275	386148	0.99278578	357578	0.99955275	227018	0.997364	350825	0.99216337	121903	0.99944249	266488	0.9997749
28	2800	0.00570103	671	0.00190582	1981	0.00509315	0	0	518	0.00227574	1091	0.00308544	0	0	0	0
33	0	0	6091	0.01730005	0	0	0	0	0	0	0	0	0	0	0	0
43	1880	0.00382784	927	0.00263292	483	0.00124179	0	0	0	0	1473	0.00416577	0	0	0	0
1179	0	0	0	0	65	0.00016711	0	0	0	0	0	0	0	0	0	0
1267	58	0.00011809	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1307	0	0	0	0	55	0.0001414	0	0	0	0	0	0	0	0	0	0
1333	53	0.00010791	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1374	0	0	0	0	0	0	0	0	0	0	51	0.00014423	0	0	0	0
1375	0	0	0	0	0	0	0	0	0	0	51	0.00014423	0	0	0	0
1386	0	0	0	0	0	0	50	0.00013977	0	0	0	0	0	0	0	0
1397	49	0.0000998	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1438	47	0.0000957	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1460	0	0	0	0	46	0.00011827	0	0	0	0	0	0	0	0	0	0
1557	0	0	40	0.00011361	0	0	0	0	0	0	0	0	0	0	0	0
1586	0	0	0	0	0	0	0	0	39	0.00017134	0	0	0	0	0	0
1616	0	0	0	0	0	0	0	0	38	0.00016695	0	0	0	0	0	0
1625	0	0	0	0	0	0	0	0	0	0	0	0	38	0.00031155	0	0
1703	0	0	0	0	35	0.0000900	0	0	0	0	0	0	0	0	0	0
1823	0	0	31	0.0000880	0	0	0	0	0	0	0	0	0	0	0	0
1832	0	0	0	0	31	0.0000797	0	0	0	0	0	0	0	0	0	0
1928	0	0	0	0	0	0	0	0	0	0	29	0.0000820	0	0	0	0
2171	0	0	0	0	0	0	24	0.0000671	0	0	0	0	0	0	0	0
2172	0	0	0	0	0	0	0	0	0	0	24	0.0000679	0	0	0	0
2446	0	0	0	0	0	0	0	0	0	0	24	0.0000679	0	0	0	0
2449	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0.0000750
2605	19	0.0000387	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2650	0	0	0	0	0	0	18	0.0000503	0	0	0	0	0	0	0	0
2857	0	0	0	0	0	0	0	0	0	0	18	0.0000509	0	0	0	0
2952	0	0	0	0	17	0.0000437	0	0	0	0	0	0	0	0	0	0
3083	0	0	15	0.0000426	0	0	0	0	0	0	0	0	0	0	0	0
3439	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0.0000563
3481	0	0	0	0	14	0.0000360	0	0	0	0	0	0	0	0	0	0
3276	0	0	0	0	0	0	0	0	0	0	0	0	14	0.00011478	0	0
3939	0	0	0	0	13	0.0000334	0	0	0	0	0	0	0	0	0	0
4120	10	0.0000204	0	0	0	0	2	0.0000056	0	0	0	0	0	0	0	0
4233	11	0.0000224	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4874	0	0	0	0	11	0.0000283	0	0	0	0	0	0	0	0	0	0
4875	0	0	0	0	9	0.0000231	0	0	0	0	0	0	0	0	0	0
5195	0	0	0	0	0	0	9	0.0000252	0	0	0	0	0	0	0	0

5286	8	0.0000163	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5536	0	0	0	0	0	0	8	0.0000224	0	0	0	0	0	0	0	0
5726	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0.0000300
5768	0	0	0	0	0	0	7	0.0000196	0	0	0	0	0	0	0	0
5812	0	0	0	0	6	0.0000154	0	0	0	0	0	0	0	0	0	0
6235	0	0	0	0	6	0.0000154	0	0	0	0	0	0	0	0	0	0
6497	0	0	0	0	0	0	0	0	0	0	0	0	6	0.0000492	0	0
6500	0	0	5	0.0000142	0	0	0	0	0	0	0	0	0	0	0	0
6762	0	0	0	0	5	0.0000129	0	0	0	0	0	0	0	0	0	0
7028	0	0	0	0	5	0.0000129	0	0	0	0	0	0	0	0	0	0
8500	0	0	0	0	0	0	5	0.0000140	0	0	0	0	0	0	0	0
2147	0	0	0	0	0	0	5	0.0000140	0	0	0	0	0	0	0	0
2585	0	0	0	0	0	0	0	0	5	0.0000220	0	0	0	0	0	0
4436	0	0	0	0	0	0	0	0	0	0	0	0	5	0.0000410	0	0
2993	0	0	0	0	0	0	0	0	0	0	0	0	5	0.0000410	0	0
3156	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0.0000188
3908	4	0.0000081	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6461	0	0	0	0	4	0.0000103	0	0	0	0	0	0	0	0	0	0
4556	0	0	0	0	4	0.0000103	0	0	0	0	0	0	0	0	0	0
7379	0	0	0	0	0	0	4	0.0000112	0	0	0	0	0	0	0	0
7420	0	0	0	0	0	0	4	0.0000112	0	0	0	0	0	0	0	0
5351	0	0	0	0	0	0	4	0.0000112	0	0	0	0	0	0	0	0
5384	0	0	0	0	0	0	0	0	0	0	4	0.0000113	0	0	0	0
5421	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0.0000150
5453	3	0.0000061	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5762	3	0.0000061	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5945	0	0	0	0	3	0.0000077	0	0	0	0	0	0	0	0	0	0
5986	0	0	0	0	3	0.0000077	0	0	0	0	0	0	0	0	0	0
6042	0	0	0	0	0	0	3	0.0000084	0	0	0	0	0	0	0	0
6034	0	0	0	0	0	0	3	0.0000084	0	0	0	0	0	0	0	0
6063	0	0	0	0	0	0	0	0	0	0	3	0.0000085	0	0	0	0
6665	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0.0000113
6671	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0.0000113
6871	0	0	0	0	2	0.0000051	0	0	0	0	0	0	0	0	0	0
3278	0	0	0	0	2	0.0000051	0	0	0	0	0	0	0	0	0	0
7542	0	0	0	0	2	0.0000051	0	0	0	0	0	0	0	0	0	0
7360	0	0	0	0	2	0.0000051	0	0	0	0	0	0	0	0	0	0
7689	0	0	0	0	2	0.0000051	0	0	0	0	0	0	0	0	0	0
7690	0	0	0	0	0	0	2	0.0000056	0	0	0	0	0	0	0	0
7713	0	0	0	0	0	0	2	0.0000056	0	0	0	0	0	0	0	0
7768	0	0	0	0	0	0	2	0.0000056	0	0	0	0	0	0	0	0
7773	0	0	0	0	0	0	2	0.0000056	0	0	0	0	0	0	0	0
7775	0	0	0	0	0	0	2	0.0000056	0	0	0	0	0	0	0	0
7794	0	0	0	0	0	0	2	0.0000056	0	0	0	0	0	0	0	0
7839	0	0	0	0	0	0	2	0.0000056	0	0	0	0	0	0	0	0

7930	0	0	0	0	0	0	0	0	0	0	2	0.0000057	0	0	0	0
7937	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0.0000075
8272	0	0	0	0	0	0	0	0	0	0	1	0.0000028	0	0	0	0