He Piko, He Repo:

Environmental DNA as a biomonitoring tool for wetland restoration

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Abstract

Wetlands are biodiversity hotspots, harbouring distinctive species while providing numerous ecosystem services. Unfortunately, throughout human civilisation, they have been systematically degraded, resulting in a dramatic global loss in wetland extent, the establishment of invasive species and the extinction of many natives and, in Aotearoa New Zealand, impediment of the special connection Māori share with their repo (wetlands). Monitoring wetland biodiversity to take appropriate restorative action and minimise further degradation is imperative, particularly in the face of climate change. Environmental DNA (eDNA; genetic material released by organisms into the environment) is an increasingly popular non-invasive biomonitoring method that provides information about the presence or potential absence of species inhabiting a given ecosystem, but has yet to be optimised for wetland environments. My thesis aimed to address the consequences of colonisation on the relationship between Māori and their wetlands, and to explore the value eDNA holds for wetland biomonitoring.

The first analysis (**Chapter 2**) delved into the past (post-European settlement; 1840s), the present (2023), and the future (2073) of Opuatia Wetland (Waikato, Aotearoa New Zealand) through a series of interviews with three individuals who whakapapa to (are connected with) Opuatia Wetland and its surroundings. I found that Opuatia Wetland once flourished with native biodiversity, and kai (food) was readily available. However, land confiscation by European settlers marked the downfall of this once productive and connected catchment such that it is presently in a fairly degraded state, dominated by exotic species, with Māori feeling disconnected from their whenua (land). Considering the future, the interviewees envisioned a revived abundance of native species and desire for restoration efforts to be facilitated by both mātauranga Māori (Māori knowledge) and science.

The second analysis (**Chapter 3**) examined a publicly available eDNA database to retrieve data from 26 wetland sites across Aotearoa New Zealand to understand taxonomic diversity patterns. Here, I showed that DNA sequence composition varied across the motu (country), illustrating that varying

environmental conditions among wetland types influences biodiversity. Notably, most sites were dominated by >50% exotic species. These findings highlight the value of publicly shared data for generating new insights, and the need for resource allocations to tackle the persistence of exotic species in wetland ecosystems.

The third analysis (**Chapter 4**) focused on analysing biodiversity dynamics at a single wetland and tested various eDNA sampling techniques. I sampled four spatially distant sites across Opuatia Wetland at three time points during an austral spring and simultaneously obtained data from conventional taxonomic surveys, tested three different filter sizes, and assessed DNA degradation rates using foreign DNA. I found significant differences in biodiversity across time and space, and when using different eDNA collection filters. Foreign DNA persisted for up to one week post-release within a 10 m radius, and combining conventional and eDNA methods provided a more comprehensive overview of biodiversity patterns. These findings collectively showcased key changes in biodiversity, even over short spatial and temporal scales, and identified parameters to consider for wetland biodiversity monitoring and associated data interpretation.

It is only a matter of time until the impacts of climate change and invasive species take firmer hold of Aotearoa New Zealand's distinctive and fragile environment. Undoubtedly, eDNA can be part of the solution to mitigate these challenges, owing to its versatility across ecosystem types, capacity to extend to various substrates, and accessibility for researchers and citizen scientists alike. As eDNA methods continue to improve and become integrated into ongoing biomonitoring schemes, building equitable and empowering relationships between Māori and researchers/government bodies will best ensure that Aotearoa New Zealand repo and other environments are maintained and conserved for future generations.

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My master's journey has come to an end, and what a privilege it has been. This achievement would not have been possible without the special people in this chapter of my life who have continuously and unconditionally encouraged and tautoko me through it all.

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Ko tooku awa koiora me oona pikonga, he kura tangihia o te maataamuri

The river of life, each curve more beautiful than the last

– Kiingi Taawhiao

Pai mārire

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Chapter 1.

General introduction

Design inspired by koru pounamu:
Represents new beginnings, harmony, hope for a positive future

1.1 Biodiversity

Biodiversity refers to biological variation that can span from the genetic diversity of individuals and populations to the richness of communities over entire ecosystems (Noss, 1990). Species richness – the number of different species in a community and the simplest measure of biodiversity (Mittelbach et al., 2001) – plays a pivotal role in sustaining the robustness and resilience of the environment (Hector & Bagchi, 2007; Schwartz et al., 2000). However, we are currently experiencing significant environmental change fueled by anthropogenic activities, which are posing a dire threat to biodiversity across the globe. The top five most widespread anthropogenic threats to biodiversity include over-exploitation, habitat changes, climate change, invasive species, and pollution (Fig. 1.1) (Pelletier & Coltman, 2018). The ability to monitor biodiversity dynamics is thus imperative to understand and minimise the effects of these threats on biodiversity loss.

Aotearoa New Zealand holds unique and distinctive biodiversity compared to its Gondwanan supercontinent counterparts. This distinctiveness arose from the biota evolving in isolation for 75-80 million years, including in the absence of mammalian predators (Atkinson, 2001; Gibbs, 2009), and has resulted in a high proportion of endemic taxa with unusual characteristics. For example, the kākāpō (*Strigops habroptilus*) is the world's only flightless parrot, and large insects like the Stephens Island giant wētā (*Deinacrida rugosa*) occupy niches that are typically inhabited by small rodents in other regions (Logan, 2001). However, Aotearoa New Zealand faced sudden and rapid changes following human settlement, including the introduction of invasive species, which have had detrimental impacts on native biodiversity.

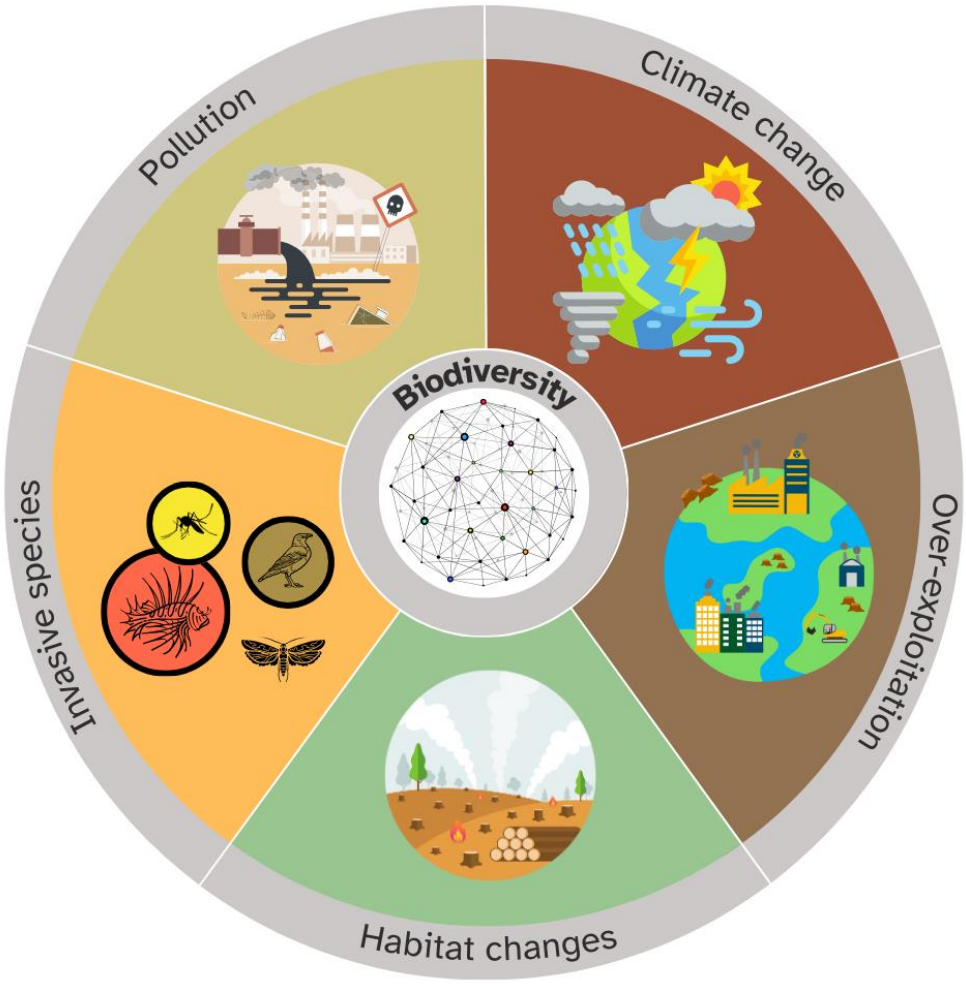

Figure 1.1 The five main anthropogenic threats on biodiversity globally, each of which also influences each other.

1.2 Invasive species

Invasive species – organisms that can expand demographically and spatially beyond their native range – are among the main drivers of biodiversity loss. Introduced both deliberately and inadvertently, invasive species can negatively impact their new range both ecologically and economically (Vitousek et al., 1997)(Fig. 1.1). In many cases, invasive species can have profound impacts on native ecosystems, affecting species richness, abundance, genetic composition, and behaviour. They also increase the risk of native species' extinction and can alter native species' phylogenetic diversity and trophic networks; in turn, these modifications can change ecosystem functions and services (Pyšek et al., 2020).

Fourteen percent of critically endangered terrestrial vertebrate species are at risk globally due to invasive species (Dueñas et al., 2021), which have an estimated annual global economic impact of US$423 billion (Roy et al., 2023). Aotearoa New Zealand is no exception, with 1,000 taxa threatened (and only 38% of native species described; Hare et al., 2019). Biological invasions in Aotearoa New Zealand from 1968 to 2020 incurred an estimated total cost of US$69 billion in economic damage and management expenses (Bodey et al., 2022). Invasive mammalian predators are widespread across the country, and have negative impacts on native invertebrates, reptiles, bats, freshwater fish, and birds in forest, riverine, and coastal environments (O'Donnell et al., 2015). Consequently, significant conservation efforts are underway to reduce or eradicate mammalian predators (e.g., aerial application of toxins, trapping, predator exclusion fences; Peltzer et al., 2019) with the goal of making Aotearoa New Zealand predator-free by 2050 (Predator Free 2050, 2022). To achieve this, monitoring of invasive species alongside pest management is crucial, particularly as rates of biological invasion are projected to increase with climate change (Bellard et al., 2013).

1.3 Wetlands

Throughout human civilisation, wetlands have been misunderstood ecosystems, often perceived as unproductive land (Fluet-Chouinard et al., 2023). Yet, they are actually one of the most productive land types globally and are home to many unique species (Clarkson et al., 2013). In Aotearoa New Zealand, under the Resource Management Act 1991, wetlands are defined as "permanently or intermittently wet areas, shallow water, and land water margins that support a natural ecosystem of plants and animals that are adapted to wet conditions" (pt 1). The type of wetland is determined by various factors, including water regime, substrate, nutrient status, and pH. Johnson and Gerbeaux (2004) recognised nine distinct wetland classes in Aotearoa New Zealand: bog, fen, swamp, marsh, seepage, shallow water, ephemeral wetland, pakihi and gumland, and salt marsh.

Wetlands provide a myriad of ecosystem services, including regulating greenhouse gases, maintaining water quality, flood control, securing shorelines, carbon sequestration, sediment retention, and nutrient cycling (Clarkson et al., 2013; de Groot et al., 2016). From a cultural perspective, wetlands provide numerous resources central to people's daily lives and significantly contribute to human well-being. For example, for Māori – the Indigenous People of Aotearoa New Zealand – wetlands are food gathering sites and form part of their food basket (Papa, 2017). Similarly, for the Indigenous People of Australia, wetlands bear intrinsic values embodying a sense of place, carrying knowledge and stories passed down through generations (Verschuuren, 2007). Additionally, wetlands serve as archaeological sites due to their anaerobic nature, creating ideal conditions for preserving organic material (e.g., Māori are known to have buried artefacts and waka (canoes) for either preservation or concealment purposes; Gumbley et al., 2005).

Between 1700 and 2020, the global extent of inland wetlands diminished by 3.4 million km^2, as they were drained and converted for croplands, rice cultivation, urbanisation, forestry, pasture, and peat extraction (Fluet-Chouinard et al., 2023). In Aotearoa New Zealand, the historical extent of wetlands encompassed ~10% of the mainland area (2.4 million ha; Ausseil et al., 2011a). However, their current extent represents <10% of this original coverage (Ausseil et al., 2011a; Myers et al., 2013), with the South Island retaining 16% of its historic wetlands and the North Island retaining just 4.9% (Ausseil et al., 2011a). The most common wetland types here are swamps and pakihi/gumland; however, swamps have experienced the most significant losses, retaining only 6% of their original extent due to their locality in lowland areas where conversion to productive land has been most pronounced (Ausseil et al., 2011b). This represents the highest decline in wetland areas globally (Myers et al., 2013) and this land-use change has led to the extinction of fifteen wetland bird species; a further ten bird species are classified as threatened, and several native freshwater fish have also declined (Ausseil et al., 2011b). Thus, wetlands have become one of the most threatened and degraded ecosystems at the national level (Ausseil et al., 2008).

Conserving wetlands has been formally recognised as an international priority since the signing of the Ramsar Convention, an intergovernmental treaty, in 1971 (Myers et al., 2013). To date, 172 contracting parties are committed to the national implementation of the Convention, including Aotearoa New Zealand, which became a signatory in 1976 (Ramsar, n.d.). These parties are obligated to establish wetland policies and report on wetland extent and condition (Myers et al., 2013). In Aotearoa New Zealand, the National Policy Statement for Freshwater Management 2020 (amended 2023; Ministry for the Environment, 2023) established nationally consistent guidelines for local authorities to manage freshwater resources, as mandated by the Resource Management Act 1991 – legislation which stipulates the preservation of wetlands as a matter of national importance. However, despite this national legislation, many wetlands continue to degrade and decline (Clarkson et al., 2013; Denyer & Peters, 2020), with this expected to worsen with exposure to additional threats associated with climate change. In particular, hydrological regimes are expected to fluctuate with more variable precipitation rates, and wetlands will experience increases in temperature, evaporation, transpiration, and fire, resulting in reduced species richness and directly impacting ecosystem services (Jisha & Puther, 2021).

1.4 Monitoring tools

1.4.1 Cultural monitoring

Understanding ecosystem dynamics and processes between physical and biological elements is fundamental for society's capacity to respond and adapt to environmental shifts. This practice of observing and monitoring the environment is deeply entrenched in traditional knowledge. Indigenous People tend to consider humankind as part of, and in balance with, the natural world – integral to sustaining human life (Garnett et al., 2009; Harmsworth & Awatere, 2013). Historically, and in modern times, Māori have relied on environmental tohu (signs, cues) to determine the best times to perform certain activities. For example, the maramataka (Māori lunar calendar) serves as a predictive tool that

assists Māori in successfully scheduling activities, such as fishing, planting, and harvesting food, which are essential to survival and well-being (Hikuroa, 2017; Warbrick et al., 2023). In response to contemporary challenges, several cultural monitoring frameworks (e.g., the Cultural Health Index, Māori wetland indicator, and the Mauri Assessment model) have emerged as a means of assessing and monitoring environmental changes from a te ao Māori (Māori worldview) perspective (Harmsworth & Awatere, 2013). These approaches have revitalised traditional knowledge, generated new insights, and made contributions to environmental decision-making. However, their implementation has not been without challenges. For example, cultural monitoring has been subjected to institutionalisation within policy and planning, and conceptualised within Western science frameworks (Tadaki et al., 2022).

1.4.2 Environmental DNA

Biological monitoring tools are used to monitor species diversity, distributions, and abundances both spatially and temporally. However, conventional approaches – such as acoustic recording (Takahara et al., 2020), auditory callback (Neice & McRae, 2021), hand-capturing (Doi et al., 2017), active search (Plante et al., 2021), camera-trapping (Leempoel et al., 2020), vegetation plots (Ariza et al., 2023), electrofishing, and netting (Wang et al., 2021) – are often time-consuming and entail significant labour and cost commitments. Environmental DNA (eDNA) refers to the sampling of genetic material that is shed by organisms into the environment as they pass through it. This genetic material – such as skin, hair, and bodily fluids – can be extracted from environmental samples (e.g., water, soil, air), to provide information about the species inhabiting that ecosystem (Dickie et al., 2018). eDNA has received increased attention as a tool for monitoring biodiversity dynamics as it can overcome some of the challenges associated with conventional monitoring (Beng & Corlett, 2020).

eDNA analysis involves sample collection followed by DNA extraction, purification, sequencing, and analysis (Fig. 1.2). The particular method employed at each step varies (Tsuji et al., 2019). In brief, a common method employed to collect eDNA from water samples is the filtration method. This involves passing large volumes of water (six replicates of 1 L is the recommended

standard; Smith et al., 2023) through a filter that captures the DNA. The DNA is then extracted using specialised laboratory techniques and amplified using polymerase chain reaction (PCR). There are two primary eDNA sequencing methods currently used: barcoding and metabarcoding. Barcoding uses species-specific primers to amplify and detect short DNA fragments from a target species through PCR, while metabarcoding employs universal (i.e., non-specific) primers to simultaneously detect DNA fragments from an entire assemblage or community of interest. Analysis of eDNA data involves matching the short sequence fragments against a reference database to determine the taxonomic identity of species present in the eDNA sample (Beng & Corlett, 2020; Tsuji et al., 2019).

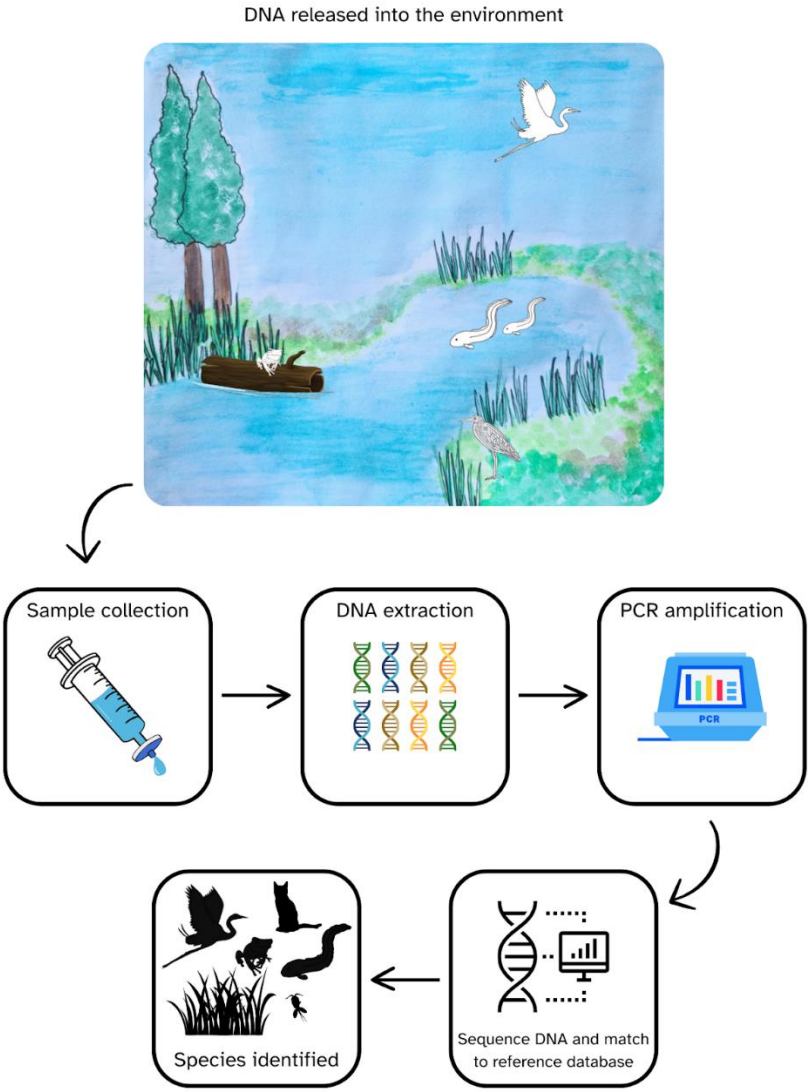

Figure 1.2 Schematic depicting how eDNA is processed from a wetland environment.

eDNA methods have been praised for their versatility and application across a range of environments to survey the tree of life. For example, eDNA can be used to detect and monitor cryptic and endangered species from snow, estuaries, and streams (Franklin et al., 2019; Shelton et al., 2019; Takahara et al., 2020) and can also reveal spatial and temporal biodiversity patterns (Bedwell & Goldberg, 2020; Hervé et al., 2022). However, as with any procedure, there are a number of challenges and limitations to using eDNA. For example, eDNA results can return false negatives (failures to detect species of interest when they are actually present) and false positives (detections of species when they are actually absent) (Larson et al., 2020), and incomplete reference sequence databases can limit the method's taxonomic resolution (Hotaling et al., 2021) – providing important considerations for experimental design and data interpretation.

Wetland environments present unique methodological challenges for eDNA sampling. For example, they pose accessibility challenges as they must be wadeable, considering that a shore sample may not accurately represent the entire wetland. Additionally, wetlands typically harbour high sediment volumes, which can clog eDNA filters (Goldberg et al., 2016). Consequently, a standardised eDNA methodology is essential for wetland environments, particularly in the context of global biodiversity decline and the threat of invasive species to indigenous species.

1.5 Thesis structure

My thesis aims to explore spatial and temporal biodiversity patterns in the context of Aotearoa New Zealand wetlands, with a particular focus on eDNA tools and invasive species distributions. Following this Introductory chapter, **Chapter 2** involves interviews conducted with mana whenua (Māori who have general authority over a particular area of land) to present historical and contemporary accounts of Opuatia Wetland (Waikato, Aotearoa New Zealand) from both environmental and cultural perspectives and to examine their aspirations for the wetland moving forward. This chapter's writing style differs from the subsequent chapters as it provides social context and sets the tone. **Chapter 3** investigates publicly available eDNA records collected from various wetland types across Aotearoa

New Zealand, illustrating how eDNA can be used to assess the biological condition of these environments with a special focus on invasive species patterns. In **Chapter 4**, various eDNA sampling techniques are tested to understand spatiotemporal biodiversity signals at Opuatia Wetland, with the goal of optimising their application in wetland environments. Finally, **Chapter 5** synthesises the key findings of my thesis, contextualising them within a broader framework and outlining recommended steps for future research.

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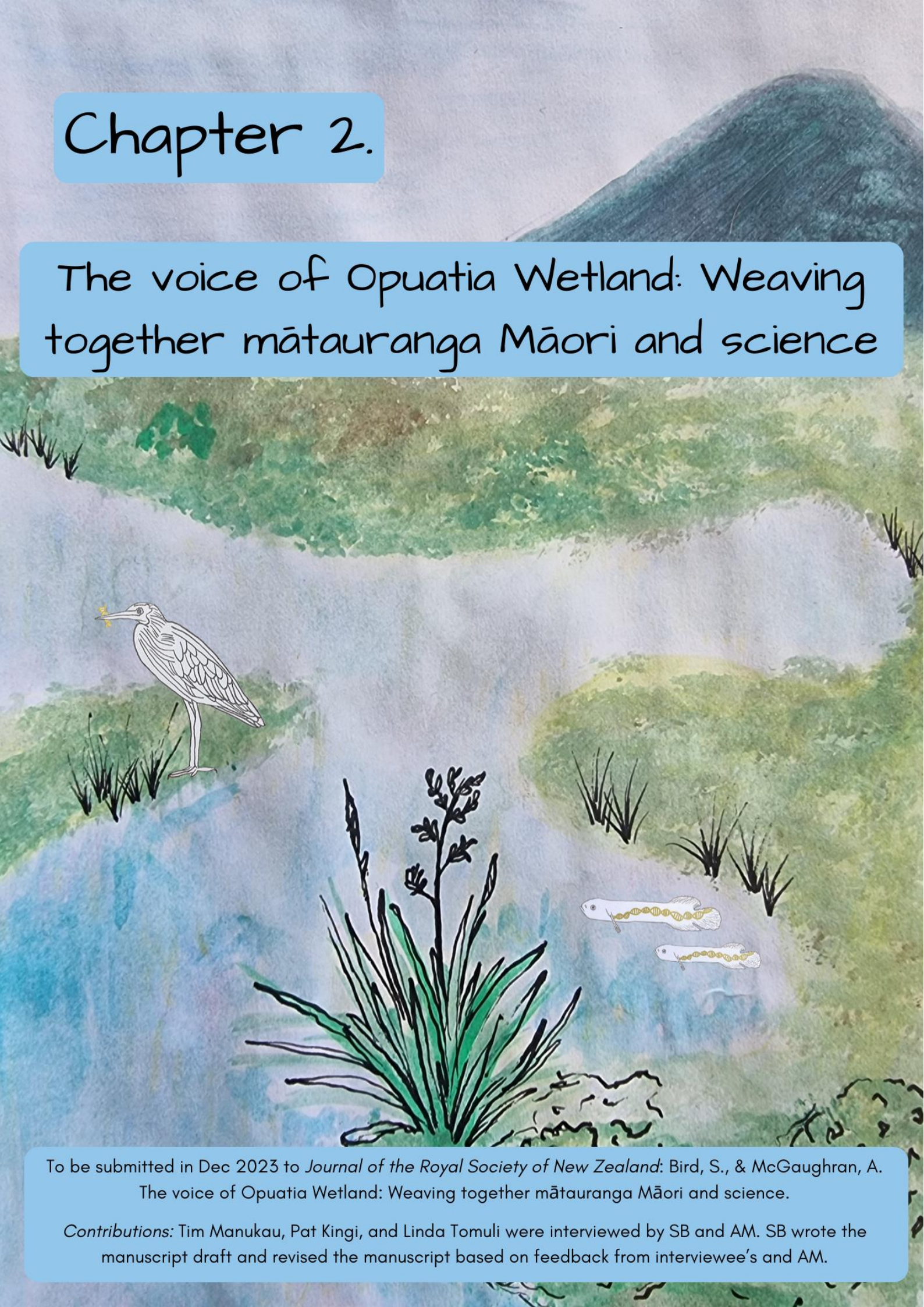
Chapter 2.

The voice of Opuatia Wetland: Weaving together mātauranga Māori and science



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Contributions: Tim Manukau, Pat Kingi, and Linda Tomuli were interviewed by SB and AM. SB wrote the manuscript draft and revised the manuscript based on feedback from interviewee's and AM.

2.1 Abstract

Wetlands hold cultural, spiritual, and historical significance for Māori and are integral to their tribal identity. For tangata whenua, wetlands serve as reservoirs of knowledge, habitats for taonga species, and food-gathering sites, and are themselves regarded as taonga. However, their relationship with wetlands and the surrounding environment changed following colonisation by European settlers, whereby wetlands were drained and converted for agricultural purposes and urban development and now encompass <10% of their original extent.

Here, we interviewed three mana whenua with a special relationship with Opuatia Wetland (Waikato, Aotearoa New Zealand). Using the Ake Ake Model as our interview framework, we delved into the past (post-European settlement; 1840s), the present (2023), and the future (2073) of Opuatia Wetland and its broader catchment from four perspectives – environmental, species composition, cultural, and social (including recreational) – to identify common themes.

We found a stark contrast in the state of Opuatia Wetland and its surrounding catchment between the past and present – transforming from a communal food basket abundant with native biodiversity into a landscape dominated by exotic species. Today, mana whenua aspire to see restoration efforts supported by mātauranga Māori and science, and to reignite the deep connection that tūpana once held with their whenua for future generations. Thus, our research revealed the sustained impact colonisation has had on Māori, the critical state of Aotearoa New Zealand wetlands, and the importance of cultivating an equitable and empowering relationship between researchers and indigenous communities moving forward.

Ake Ake
Forever and ever

Aotearoa
Māori name for New Zealand

Mana whenua
General authority exercised by an iwi, hapū or individual over a particular area of land

Māori
Indigenous people of Aotearoa

Mātauranga Māori
Māori knowledge systems

Tangata whenua
People of the land

Taonga
Things that are treasured by Māori

Taonga species
Native and endemic species that are precious and treasured by Māori

Tūpana
Ancestors

Whenua
Land

2.2 Introduction

Hauanga kai
Food gathering sites

Hauora
Health and wellness

Iwi
Tribes

Kai
Food

Mana
Spiritual authority, prestige

Mauri
Life force, spiritual energy

Pūrakau
Story, legend

Raranga
Weaving

Raupatu
Confiscation

Repo
Wetlands

Rongoā
Māori medicinal use

Te tiriti o Waitangi
The treaty of Waitangi

Tikanga
Māori practices/protocol

Tino rangatiratanga
Sovereignty, self-determination

Tuna
Freshwater eels

Tupana awa
Ancestral river

Waikato-Tainui
Tribal people of the central North Island

Wairua
Spirit

Waka ama
Outrigger canoe

Whakapapa
Genealogy, ancestral connections

Repo are highly valuable ecosystems, not only from an environmental and biodiversity standpoint (Clarkson et al., 2013) but also from a cultural perspective (Papa, 2017). Māori hold a special and intrinsic relationship with repo that spans many generations (*Her Majesty the Queen in Right of New Zealand and Waikato-Tainui: Deed of Settlement in Relation to the Waikato River*, 2009; hereafter referred to as The Deed, 2009). For Māori, repo hold whakapapa, pūrākau, hauanga kai and kai (e.g., tuna; Watene-Rawiri, 2021), and materials for cultural practices such as raranga and rongoā (Harmsworth, 2021). They also serve as the spawning grounds for taonga species (Papa, 2017) and provide space for cultural activities, such as waka ama. The mauri of repo is connected to the hauora of the ecosystem and tangata whenua (Harmsworth, 2021).

The Waikato River is the tupana awa of Waikato-Tainui and is a source of their tribal identity (The Deed, 2009). It has mana and mauri and thus represents the mana and mauri of Waikato-Tainui (The Deed, 2009). The surrounding repo acts as the River's kidneys – cleansing and filtering pollutants and sediment before entering their tupana awa (Papa, 2017). Ultimately, the Waikato River and surrounding repo have sustained, and continue to sustain, the people of Waikato both physically and spiritually. Equally, it is the responsibility of the people to safeguard the River and its wairua; if the wairua of the River is degraded and ignored, so too will the physical and spiritual wellbeing of the tangata whenua be diminished (The Deed, 2009).

The Deed (2009) emphasises the essence of the River claim. Under Article Two in Te Tiriti o Waitangi (signed 6 February 1840), the founding document between Māori and the British Crown (hereafter referred to as the Crown), Māori are guaranteed tino rangatiratanga over their lands and taonga. Thus, Waikato-Tainui and other Waikato River iwi have the right to follow their tikanga regarding

Mana whakahaere
Authority, rights of control

Marae
Social cultural centre/village

Waka
Canoe

the Waikato River (The Deed, 2009). However, the Crown's military forces invaded Waikato – resulting in a war between 1863-64 and causing many communities to be driven out of the region (The Deed, 2009). In 1865, confiscation of Waikato lands occurred, and the Crown assumed control over the Waikato River (The Deed, 2009). After the raupatu, settlers established farms and towns along the River, resulting in a shift in the utilisation of the waters for farming, coal mining, power generation schemes, waste disposal, and domestic and industrial water abstraction (The Deed, 2009). Although this supported economic growth, it contributed to the pollution and degradation of the River and led to declines in native taonga species which had been depended upon by Māori for many generations (The Deed, 2009). The Crown's assumed control and management of the Waikato River and their exclusion of Waikato-Tainui from decision-making severely compromised Waikato-Tainui mana whakahaere to protect the health and well-being of the Waikato River (The Deed, 2009).

Following colonisation, European settlers also drained and cleared wetlands across Aotearoa New Zealand, with the repo in the Waikato reduced from 350,000 ha to 28,000 ha over ~150 years (Myers et al., 2013). This hydrological modification accompanied land-use changes – particularly in agriculture – which together resulted in increased nutrient input that has facilitated opportunities for introduced plant species to establish in vulnerable wetland ecosystems (Singers, 2019). In addition, habitat changes have enabled introductions of mammalian predators and significantly impacted indigenous wetland bird populations (O'Donnell et al., 2015).

Opuatia Wetland, located in the lower Waikato River catchment north of Lake Whangape, in Te Ika-a-Māui, Aotearoa (Fig. 2.1), has been no stranger to anthropogenic pressures (Browne & Campbell, 2005). Opuatia Wetland holds special significance to Horahora Marae – descended from the great voyaging waka Tainui –

Kaumātua
Elder

Kōrero
Conversation

Ngāti Pou
Interview participants hapū

who have stood witness to change over generations. Here, we interviewed three mana whenua from Horahora Marae, using the Ake Ake Model as our framework (see below), to delve into the past, present, and future value of Opuatia Wetland and its wider catchment. We identified the common themes and values that emerged from this kōrero, with a particular focus on identifying culturally informed steps that would protect and enhance the hauora and mauri of Opuatia Wetland and its broader catchment moving forward.

Figure 2.1 Opuatia Wetland. Left: The wetland and surrounding rivers and lakes referred to in the text (inset: location of Opuatia in Aotearoa New Zealand's North Island). Satellite image obtained from Google Earth Pro 2023; Right: Illustration of Opuatia Wetland with Mount Taupiri set in the background (He Piko, He Repo – Wetland at every bend of the river). Illustration: Starsha Bird.

2.3 Interviews

2.3.1 Ake Ake model and common themes

Interviews were conducted with three Waikato-Tainui, Ngāti Pou members who whakapapa to Horahora Marae. Pat Kingi (kaumātua) and Linda Tomuli were

Hapū
Sub-tribe

interviewed together, and Tim Manukau was interviewed alone at a later date. The interviews loosely followed the Ake Ake Model described in the Taura et al. (2017) handbook, developed by Lorraine Dixon and John Te Maru, with Opuatia Wetland and the surrounding catchment investigated in the context of three time points: (i) Exploring the past; (ii) Describing the present situation; and (iii) Identifying the future (~50 years) aspirations of iwi/hapū/whānau (Appendix A2.1).

For each time point, we considered four perspectives: environmental, species composition, cultural, and social (including recreational), and identified common themes (Table 2.1), along with cultural indicators (Table 2.2). Interviews were conducted with ethics approval obtained from The University of Waikato Human Ethics Committee (HREC(HECS)2023#34). Participants were sent a copy of a Participant Information Sheet (Appendices A2.2, A2.3) and Consent Form (Appendix A2.4) and a copy of the transcript was emailed to participants to edit as required.

Table 2.1 Common themes for each of the four perspectives over past, present, and future time points.

Environmental		
Past (Post-European settlement; 1840s)	**Present (2023)**	**Future (2073)**
Opuatia Stream was big	Opuatia Stream smaller	Opuatia Stream becomes bigger
Catchment connected – Opuatia Wetland, Lake Whangape, Waikato River	Lake Whangape shallow and muddy	Connect Opuatia back to Lake Whangape
Water quality clearer and cleaner – able to drink from it	Fairly degraded state	Good water quality
Food basket for people from Kawhia, Huntly, Rangariri, Port Waikato	Stopped collecting tuna and other hauanga kai	Opuatia Stream to be full of tuna
Lived off the land	Progress has resulted in less food	See success from plantings; abundant bird life
Diverse range of species; pristine and productive	Flood control scheme reduced wetland habitat	All willow removed and replaced with natives

Abundance of huge tuna	Introduced species, intensive farming, housing developments caused a lot of changes	Continue to protect the rivers, lakes, and wetlands per Waikato River Vision and Strategy
No roads	Roadway where Opuatia stream used to be	
Confiscation the starting point of degradation of wetlands, lakes and rivers	Plantings to occur at Opuatia Wetland	
Willow widespread along the banks	Bird life coming back from plantings around Lake Whangape	

Species composition		
Past (Post-European settlement; 1840s)	**Present (2023)**	**Future (2073)**
Native species: watercress – leaves were big (naturalised), pūhā/sow thistle, kererū/wood pigeon, matuku/Australasian bittern, pūkeko/swamp hen, matamata/īnanga/white bait, mullet, puhi (male short-finned eel), tuna/freshwater eels – key food source	Native species: watercress no longer present, less matamata/īnanga/white bait, less tuna/freshwater eels, no flounder	Native fish are abundant – flounder, matamata/īnanga/white bait, tuna/freshwater eels
Invasive species: willow, cats, cows, deer, pigs, ducks, swans, carp, catfish, flounder, goldfish	Invasive species: willow, cats, cows, deer, pigs, ducks, geese, swans, carp, goldfish, gold clam	No pest plants and fish

Cultural		
Past (Post-European settlement; 1840s)	**Present (2023)**	**Future (2073)**
Tuna caught for koroneihana (coronation) held at Tūrangawaewae Marae	Feathers from ducks and swans for korowai (cloaks)	Rekindle Waikato-iwi relationship to Opuatia Wetland and wider surroundings
Weave baskets with flax or willow to carry kai	Plastic or reusable bags used to carry kai	

Social & Recreational		
Past (Post-European settlement; 1840s)	**Present (2023)**	**Future (2073)**
Whānau collected hauanga kai together	No longer interacting with Opuatia; disconnected people's relationship with the whenua (land)	Collect hauanga kai again
Wetlands and rivers used for transport via boats	Now use cars and roads	Effort on everyone's behalf to restore and protect Opuatia Wetland and wider surroundings

Table 2.2 Cultural indicators signalling the health and wellbeing of the environment.

Cultural indicators	
Watercress	Only grows in clean, flowing water
Flounder	Only present in healthy waterways

2.4 Narratives about the themes

2.4.1 Mapping the past

The description of Opuatia Wetland and the wider catchment in the past seems almost ethereal – teeming with native biodiversity and a way of life that operated at a slower pace. Opuatia Wetland was strongly connected to Lake Whangape and the Waikato River. Notably, Opuatia Stream, which runs through the Wetland, was wider, while the Waikato River was smaller. The significance of Opuatia Wetland is connected to the surrounding repo as “everything is all interconnected . . . all those wetlands whakapapa because they all connect through to the Waikato River” (Manukau).

Opuatia Wetland and the surrounding repo were quite productive and pristine. They were the food basket for whānau from Kawhia, Huntly, Rangariri, and Port Waikato. Tomuli stated that “The food was plentiful there, but we would only

Matuku
Australasian bittern

take enough for a meal". Kingi responded with, "You only take enough for the kai. That's all you need. You can always go back and get some later". The tuna were huge and formed a staple diet for the Waikato tribes, being high in protein and omega-3. The importance of tuna to the tribes of Waikato is rather understated:

> "Think about why tuna was important, you gotta think about if you're living in the Waikato in the middle of the basin, in the middle of winter, you better be getting your protein source from somewhere, and what better protein source there is, is the eels, is the tuna" (Manukau).

Another source of kai was matuku. It was plentiful, and the people would go matuku hunting, following the birds' booming call. Exotic species, including carp, goldfish, catfish, and duck and swan eggs, were also part of the cultural food basket. Cats would accompany their tūpana when they went fishing and would help to remove pest fish. Food was seasonal, illnesses were less apparent, and life expectancy was longer (~100 years), all because they lived off the land. The waterways were healthy and Kingi stated: "We were drinking it". Willow trees were widespread along the stream banks and were utilised for weaving baskets if flax was unavailable to bring the tuna and fish home. It was also used for firewood. However, Māori recognised that willow were pests, dominating the landscape and taking all of the water: "Those willows are no good in there" (Kingi), and Māori attempted to remove them.

Repo, the Waikato River, and connecting awa were a significant means of transport for Māori in the past; what Tomuli described as their "State Highway 1". However, the confiscations brought massive change within the Waikato River catchment and were the starting point of the degradation of the repo and the Waikato River:

Kīngitanga
King movement developed in the 1850s to stop inter-tribal war and loss of land to colonists

> "The confiscations caused the degradation of the lakes and wetlands. They became polluted and drained as a result of the confiscations. Confiscations by the colonial government and their desire to acquire tribal lands for settlement. The government viewed the Kīngitanga as a hindrance and a threat to settler occupation. The Kīngitanga was to unite the tribes and to prevent alienation of their lands and natural resources. The impacts from the confiscations is intertwined in the historic degradation of those wetlands" (Manukau).

Following the raupatu, the repo and awa began to deteriorate, and it was only a matter of time before they would inevitably collapse. "We should never forget that confiscation caused the degradation of our wetlands" (Manukau).

2.4.2 Mapping the present

The current situation within Opuatia Wetland and the wider catchment strongly contrasted with the past from all four perspectives. Opuatia and surrounding wetlands are in a fairly degraded state. The interviewees have seen a substantial increase in introduced species (e.g., the gold clam was recently discovered in the Waikato River; "Newly discovered invasive gold clam," 2023), and Tomuli noted that invasive species have "actually caused a lot of the changes". Coupled with intensive farming, housing, and the development of roads, these changes have caused Opuatia Stream to become smaller, and therefore, hauanga kai sources to diminish. The matuku "are all gone" (Kingi), and are classified as nationally critical conservation status due to habitat loss. Thus, in present times, Māori have ceased their collection of hauanga kai to allow Opuatia Wetland to heal and replenish: "Most of us have left that stream. We've tried to leave it so it grows. But if it's got nowhere to go, it's not going to grow" (Tomuli).

The hydrological dynamics have significantly shifted compared to the past. The interviewees have noticed that Opuatia Wetland dries up, particularly over the summer. The Waikato River has become bigger due to the instalment of eight dams. The Whangape Stream has been cut off from Opuatia Wetland (the awa connecting Opuatia with Lake Whangape): “It's the connection between the Opuatia Stream, the Whangape Stream, and the Whangape Lake. There was always a relationship there. To shut that stream off from all of that, I think therein lies our problem, of where there's hardly any eels or any white bait” (Tomuli).

On top of that, raupatu has caused *legacy effects* culturally and recreationally. In other words, raupatu have severed the relationship and connection mana whenua have with their land: “To see it how it was . . . to see it how it is now, it saddens me . . . it’s sad we don’t rush up there and get the tuna because there’s nothing out there” (Tomuli). That said, mana whenua are unwavering in their determination to restore and protect their repo:

> “We don’t walk away from something just because it’s too late or too hard to restore . . . when we did the Waikato River Settlement negotiation, which includes all of these wetlands . . . we talked about needing to focus on restoration and protection, and some people were saying, oh, look, it's too late, it’s too hard, let's throw the towel in. We always tell them, look, that's what our ancestors could have done after the land wars and after the confiscations, they could have just thrown the towel in, but they didn’t, and hence the reason why we are here today” (Manukau).

2.4.3 Mapping the future

From the interviews, it was evident that mana whenua have strong aspirations for Opuatia and the Waikato River. This overarching objective aligns with the

government's objectives: to "restore and protect the health and wellbeing of the Waikato River for future generations" and ensure the River is not subjected to further degradation from anthropogenic sources (The Deed, 2009; quote from p. 22). A key aspiration expressed by Kingi is for the awa "to be full of eels". More broadly, mana whenua aspire for an abundance of native fish, including flounder and īnanga, coupled with a profusion of native bird life thriving from intentional plantings guided and informed by mātauranga Māori and science. Equally, there was a wish for willow and pest fish to be absent from the landscape: "Get all that crack willow out" (Tomuli). This desired abundance of native species would be supported by the reconnection of Opuatia to Lake Whangape via Whangape Stream: "I'd love to see that stream opened on to Whangape, so that it flows and that the tuna can come back and all our food" (Tomuli). An additional aspiration of current restoration efforts (collective efforts on everyone's behalf) is to rekindle the deep connection that tūpana once shared with their whenua in future generations.

2.5 Conclusions

Using the Ake Ake Model, we shed light on the pressures the Waikato catchment has experienced and continues to endure. These pressures have jeopardised environmental processes, species composition, and cultural and recreational realms, transforming the repo from a communal food basket into a landscape dominated by exotic species. Mana whenua aspires for Opuatia Wetland and the surrounding catchment to be connected and abundant with native flora and fauna, with restoration efforts supported by mātauranga Māori and science.

One potential method to assist the aspirations of mana whenua is to monitor wetland health through environmental DNA (eDNA; DNA released by organisms into the environment). For example, the gold clam (*Corbicula fluminea*) mentioned above

was detectable in eDNA samples prior to its initial discovery in the Waikato River, and eDNA samples are currently being used to monitor the clam's spread (Wilderlab NZ, 2023). This highlights the value of environmental monitoring tools as early warning systems for potentially harmful species incursions. They can also be used before and after mitigative or restoration efforts to provide a measure of their associated impacts (Hogg et al., 2019).

Alongside science, there was a strong interest from the interviewees in utilising mātauranga to gain a better understanding of degradation and support restoration efforts in wetland environments. For example, intergenerational knowledge was used to identify the traditional distribution range and size of various taonga species in traditional coastal areas (Paul-Burke et al., 2020). This information then served as a foundation for field research surveys, ultimately aligning the shared interest of both Māori and government entities in preserving marine taonga species for future generations. Intergenerational knowledge is invaluable because it provides rich temporal and spatial perspectives that can revolutionise our understanding of the unique biodiversity and ecosystems in Aotearoa New Zealand (Collier-Robinson et al., 2019; McAllister et al., 2020). Moreover, mātauranga Māori unlocks perspectives from the past and also illuminates humanity's relationship with the environment and culturally appropriate management practices (McAllister et al., 2020).

Drawing upon two knowledge systems contextualises and enhances conservation outcomes while upholding Te Tiriti o Waitangi. We can avoid repeating the negative experiences of colonisation and dispossession by acknowledging and embodying indigenous perspectives and fostering self-determination. This, in turn, establishes an equitable and empowering relationship between researchers and indigenous communities. There are growing kōrero in this interspace, with guidelines

and resources frequently becoming available (e.g., Te Nohonga Kaitiaki; Hudson et al., 2021; Kapu tī 101; Van Schravendijk-Goodman, 2017) to support the relationship between Māori and non-Māori, science and mātauranga. These initiatives inspire hope that Opuatia Wetland and our other precious repo will be in safe hands moving forward.

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Chapter 3.

Wetland biodiversity in Aotearoa New Zealand: An eDNA perspective on exotic and non-exotic species

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Contributions: SB and AM conceived the research project. SB analysed the data provided by Wilderlab New Zealand, wrote the manuscript draft, and revised the manuscript based on feedback by AM and SW.

3.1 Abstract

Invasive species threaten biodiversity in Aotearoa New Zealand by out-competing native species, introducing parasites and disease, and causing immense environmental damage. In wetlands − significant sites that provide an array of ecosystem and cultural services but represent <10% of their original extent − invasive species can affect hydrological function, nutrient regimes, and overall ecological functionality. Environmental DNA (eDNA) has emerged as a valuable biomonitoring technique for cataloguing biodiversity and detecting biological incursions, but little is known about how species distributions vary in wetlands over fine and broad spatial scales.

Here, we examined a publicly available eDNA wetland database established by Wilderlab New Zealand − a commercial provider of eDNA services. Using eDNA samples collected at 26 sites representing various wetland types, we assessed taxonomic diversity patterns and assessed the presence of exotic and non-exotic (native and endemic) species across wetlands in Aotearoa New Zealand.

We found significant spatial variation in DNA sequence composition, even among neighbouring sites. Exotic species prevailed across all wetland locations, with only three sites harbouring exactly 50% non-exotic species and the rest having exotic species proportions exceeding 50%. Our results provide new information on the current state of wetland biodiversity in Aotearoa New Zealand and highlight the value of eDNA databases for generating new insights from publicly shared data. They also emphasise an urgent need for resource allocation to conservation and restoration initiatives in Aotearoa New Zealand that will ensure the persistence of treasured native and endemic wetland species.

3.2 Introduction

Concern regarding global change and alarming associated declines in biodiversity is escalating (Hohenlohe et al., 2021). Many species face numerous threats, including habitat loss and fragmentation, invasive species, disease, direct hunting, and climate change (Forsdick et al., 2022; Hohenlohe et al., 2021). Among these, invasive species – organisms that can expand demographically and spatially beyond their native range – pose a significant threat (Goldson et al., 2015), with a recent report from the United Nations Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services estimating a global economic impact of invasive species in 2019 of US$423 billion (Roy et al., 2023). This included management costs of just 8%, with the remaining 92% incurred from other factors, such as disease transmission and agricultural crop losses.

Aotearoa New Zealand separated from the Gondwanan continent approximately 75-80 Mya, thereby becoming isolated from the impacts of terrestrial mammals (Atkinson, 2001; Gibbs, 2009). This isolation also played a significant role in the formation of a distinctive and intricate flora and fauna with high levels of endemism (Wallis & Trewick, 2009). The initial absence of mammalian predators left native animals and plants ill-equipped for their later invasion (Atkinson, 2001; Gibbs, 2009), which was facilitated by the arrival of Polynesians in the 13th Century and Europeans in the 1800s (Duncan & Blackburn, 2004), and caused catastrophic effects on indigenous biodiversity (Atkinson, 2001). For example, many avian species were poor fliers or entirely flightless, with minimal avoidance behaviours, rendering them susceptible to predation by introduced mammals (O'Donnell et al., 2015).

Among Aotearoa New Zealand's unique ecosystems, wetlands are areas that encompass the interface between land and a water body and harbour specialised flora and fauna (Ausseil et al., 2011b; Sorrell & Gerbeaux, 2004). They provide numerous ecosystem services, such as maintaining water quality and flood control (Clarkson et al., 2013), and providing cultural and recreational resources (Harmsworth, 2021). Due to their shallow water bodies being located in depressions, wetlands are sensitive to shifts in nutrient and sediment inputs. Consequently, they are prone to rapid transformations in response to hydrological and nutrient disturbances (Sorrell & Gerbeaux, 2004),

which alter both species compositions (Ausseil et al., 2011b) and ecological processes (Ausseil et al., 2008). For example, floodplain habitats serve as spawning grounds for invasive fish, such as common carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*), which are notorious for disturbing and mobilising fine sediment and thereby altering freshwater ecosystems (Wu et al., 2013).

Invasive species are a pervasive issue in the wetlands of Aotearoa New Zealand. Coupled with a reduction of >90% in the original extent of wetland coverage (Ausseil et al., 2011a), invasives pose a significant threat to the persistence of indigenous species. For example, the grey willow (*Salix cinera*) – a deciduous shrub – was introduced to Aotearoa New Zealand from Europe. It has since formed dense monospecific stands across wetlands in the North and South Islands, impeding the regeneration of native and endemic flora and altering ecosystem composition, structure, and function (Griffiths et al., 2018). Meanwhile, feral cats (*Felis catus*), domestic dogs (*Canis familiaris*), mustelids (e.g., stoats, *Mustela erminea*; weasels, *Mustela nivalis*; ferrets, *Mustela furo*), and rats (Norway rat, *Rattus norvegicus*; ship rat, *Rattus rattus*) are introduced mammals frequently reported to predate on wetland birds, such as Australasian bittern (*Botaurus poiciloptilus*), brown teal (*Anas chlorotis*), marsh crake (*Porzana pusilla*), spotless crake (*Porzana tabuensis*), banded rail (*Gallirallus philippensis*), and fernbird (*Bowdleria punctata*) (O'Donnell et al., 2015). However, little is known about how species distributions – for both exotic and non-exotic (i.e., native and endemic) species – vary in wetlands over fine and broad spatial scales.

Understanding wetland biodiversity dynamics is crucial for identifying current conditions, tracking shifts, and anticipating future changes. Resources like the Wetland Restoration Handbook (Peters & Clarkson, 2010) and the Wetland Cultural Health Indicator (Robb, 2014) offer comprehensive guidelines that support monitoring and restoration efforts in Aotearoa New Zealand wetlands. However, environmental DNA (eDNA; genetic material shed by organisms into the environment) is a time-efficient biomonitoring technique that is gaining popularity for monitoring and surveilling biodiversity (Beng & Corlett, 2020). For example, eDNA can detect rare and endangered species – even when their populations are relatively sparse – and can out-perform traditional survey methods in

detection capability with less associated time and labour (Franklin et al., 2019; Shelton et al., 2019; Takahara et al., 2020).

eDNA has also been useful for detecting invasive species. For example, the gold clam (*Corbicula fluminea*), was found to have been detectable in eDNA samples prior to its recent discovery in the Waikato River, Aotearoa New Zealand (Wilderlab NZ, 2023). It has also been used to detect common carp (*C. carpio*) in coastal wetlands in the Great Lakes of North America (Turner et al., 2012). But its value in detecting invasives in wetlands is yet to be fully realised due to various challenges and limitations associated with working in turbid environments, where filter clogging is more prominent (Goldberg et al., 2018), and the reliance on reference databases to match unknown genetic sequences that often contain a considerable amount of missing data (Hotaling et al., 2021).

However, the relative ease of collecting eDNA samples also makes it amenable for use by citizen scientists, fostering community engagement with the environment (Goldson et al., 2015). For example, Miya et al. (2022) recruited six groups of parents and children to collect eDNA samples from a coastal marine environment in Japan. After attending an educational seminar and workshop on sampling protocols, the groups freely selected their sampling sites based on personal preference, with the resulting samples detecting 140 fish species. These findings highlight the potential of eDNA as an educational tool that fosters public awareness and empowers self-directed exploration of the environment.

Here, we examined a publicly available eDNA database established by Wilderlab New Zealand (https://www.wilderlab.co.nz/explore) − a commercial provider of eDNA services − and contributed to by academic and citizen scientists since 2019. We aimed to identify spatial biodiversity patterns across the country and placed a special focus on invasive species patterns, examining the proportion of exotic and non-exotic species across each wetland site.

3.3 Methods

3.3.1 Site selection and data generation

Multi-species DNA metabarcoding data from 18 wetland locations (as classified by the sample submitter) was downloaded from Wilderlab New Zealand's public database. Seven of these wetland locations included >1 sampling site, bringing the total number of individual sites to 28. The geographic location for all sites was cross-checked using Google Earth's aerial imagery and roadside view to ensure the eDNA samples were taken from a wetland environment. The wetland type (e.g., bog, fen, swamp, marsh, dune) was determined based on client self-reporting, Google Earth's aerial imagery and roadside view, or websites established by government bodies or trusts. The Wilderlab dataset included DNA matches from all taxonomic ranks, which were filtered to retain only those sequences for which species and/or genus level identification could be determined. After reviewing the locations against this criteria, two sites were removed, leaving a final total of 16 locations (26 sites; Table 3.1).

3.3.2 Determination of species status

To understand how native and non-native species are distributed across Aotearoa New Zealand wetlands, we first classified all species (n = 388) in our dataset. We used the classifiers 'exotic' or 'non-exotic' for consistency with public databases and to minimise the anomalies in invasion biology surrounding terminology (Blackburn et al., 2011). Here, we consider an exotic species to be an organism that is introduced to Aotearoa New Zealand from outside its native geographic range, and a non-exotic species to include both native and endemic species. The former classification encompasses both invasive species, which have economic (Diagne et al., 2021) and environmental impacts (Doherty et al., 2016), and non-native species, which have become naturalised with possible benign effects (Schlaepfer et al., 2011). We primarily used the New Zealand Organisms Register (NZOR; https://www.nzor.org.nz/search) and iNaturalistNZ (https://inaturalist.nz/) to make these classifications. Additional used databases included Biota of New Zealand (https://biotanz.landcareresearch.co.nz/), Plants of the World Online (https://powo.science.kew.org/), New Zealand Birds Online (https://www.nzbirdsonline.org.nz/), and

New Zealand Plant Conservation Network (https://www.nzpcn.org.nz/). In cases where the species could not initially be classified, broader search terms that included the genus name and "native range" were employed in Google's search engine to further attempt to define exotic/non-exotic status. We were ultimately able to classify the status of 336 species, leaving 52 with an unknown status, which we excluded from our analyses.

3.3.3 Data analysis

To visualise differences among DNA sequences across wetland sites, non-metric multidimensional scaling (nMDS) plots were generated using the metaMDS function from the vegan v.2.6–4 package (Oksanen et al., 2022) in R v.2023.06.1+524 (R Core Team, 2023). To understand variation in taxonomy across space, the proportion of phyla was calculated for each site. Finally, to investigate spatial patterns in non-exotic species persistence across Aotearoa New Zealand, the proportion of exotic and non-exotic species at each site was plotted and species were ranked according to their eDNA read count to identify the top ten exotic and non-exotic species at each site.

Table 3.1 A summary of wetland locations used in this study, presented in geographical order (from the top of the North Island to the bottom of the South Island), including location code, latitude and longitude, sample collection date, the number of sites within each location number of eDNA replicates filtered, volume of water filtered (mL), wetland type, a brief site description, and ecological integrity (EI) index values observed from Ausseil et al. (2008).

Location name	Location code	Latitude	Longitude	Collection date	No. sites	Replicates	Volume filtered (mL)
Te Ahu Ahu Rd	TAA	-35.307736	173.928037	26/10/2022	1	1	1000
Te Henga Wetland					2		
	HEN 1	-36.865878	174.489567	01/07/2022		6	540
	HEN 2	-36.865725	174.488586	29/03/2022		3	350 - 500
Matarangi Wetland					2		
	MAT 1	-36.735661	175.664797	22/06/2022		6	50 - 60
	MAT 2	-36.73729	175.68806	30/07/2022		1	Unknown
Opuatia Wetland					3		
	OPU 1	-37.434073	175.063982	22/12/2021		4	57 - 113
	OPU 2	-37.428165	175.069727	22/12/2021		1	20
	OPU 3	-37.410144	175.057712	10/02/2022		8	82 - 500
Rangiriri	RAN	-37.397774	175.113333	08/09/2021	1	5	1000
Pongakawa Wetland	PON	-37.838270	176.47816	16/06/2021	1	1	205
Waikahu Wetland	WKU	-39.562131	176.921211	20/01/2022	1	6	1000
Ngatotora Lagoon				18/10/2022	3		
	NTT 1	-40.733931	175.154688			6	100 - 120
	NTT 2	-40.733083	175.155938			6	47 - 54

	NTT 3	-40.732835	175.15664			6	250 - 300
Kawakahia Wetland/ Te Harakeke Swamp				16/09/2021	3		
	KAW 1	-40.845114	175.053323			6	Unknown - 600
	KAW 2	-40.844990	175.053611			6	Unknown - 600
	KAW 3	-40.844561	175.053706			6	Unknown - 360
Ngā Manu Nature Reserve	NGĀ	-40.859238	175.05954	21/04/2022	1	1	1000
Old State Hwy 1	OLD	-40.884564	175.053658	16/09/2021	1	6	Unknown - 720
Queen Elizabeth Park				12/10/2022	2		
	QEP 1	-40.954389	174.983597			6	70 - 125
	QEP 2	-40.94246	174.99017			6	55 - 75
Wetland inlet and outlet				10/05/2021	2		
	INL	-41.15976	175.391769			1	1000
	OUT	-41.160746	175.391392			1	1000
Hororata	HOR	-43.584889	172.033694	22/06/2022	1	6	420 - 1000
Redcliff Wetland Reserve	RED	-45.659051	167.673184	23/03/2022	1	1	320
Rakatu Wetland	RAK	-45.663374	167.653958	23/03/2022	1	1	660

Location name	Location code	Wetland type	Site description	Site EI index
Te Ahu Ahu Rd	TAA	Riparian wetland complex	Mānuka-kiokio-Machaerina wetland (0.06 ha) transitioning to an *Eleocharis-Schoenoplectus-Machaerina* (0.09 ha) wetland, surrounded by exotic and tōtara forest, degraded by stock access (Wong et al., 2020).	
Te Henga Wetland	HEN 1 HEN 2	Freshwater swamp	The 168 ha freshwater wetland contains a mosaic of cabbage trees, flax, mānuka, kuta, swamp millet, with patches of *Machaerina* and large areas of raupō reedland (Tiaki Tāmaki Makaurau Conservation Auckland, n.d.).	0.66
Matarangi Wetland	MAT 1 MAT 2	Coastal Coastal	Unmodified wetland reserve, fed by natural springs and run off (Rings Beach Wetland Group, n.d.).	
Opuatia Wetland	OPU 1 OPU 2 OPU 3	Marsh Fen Opuatia Stream (Wetland Outlet)	A mosaic of wetland types surrounded by farmland (Barnes et al., 2001; Browne & Campbell, 2005).	0.23
Rangiriri	RAN		No information available. Aerial imagery/roadside view suggests constructed or modified wetland.	
Pongakawa Wetland	PON		A 1.5 ha restored wetland (Western Bay of Plenty District Council, 2021).	
Waikahu Wetland	WKU		The 15 ha constructed wetland (Tuia Pito Ora New Zealand Institute of Landscape Architects, 2019).	
Ngatotora Lagoon	NTT 1	Dune lake-wetland Open water	The dune lake is surrounded by a nationally vulnerable wetland ecosystem exhibiting a transition from wet to dry vegetation zones. The wetland includes areas of raupō reedland and flaxland.	

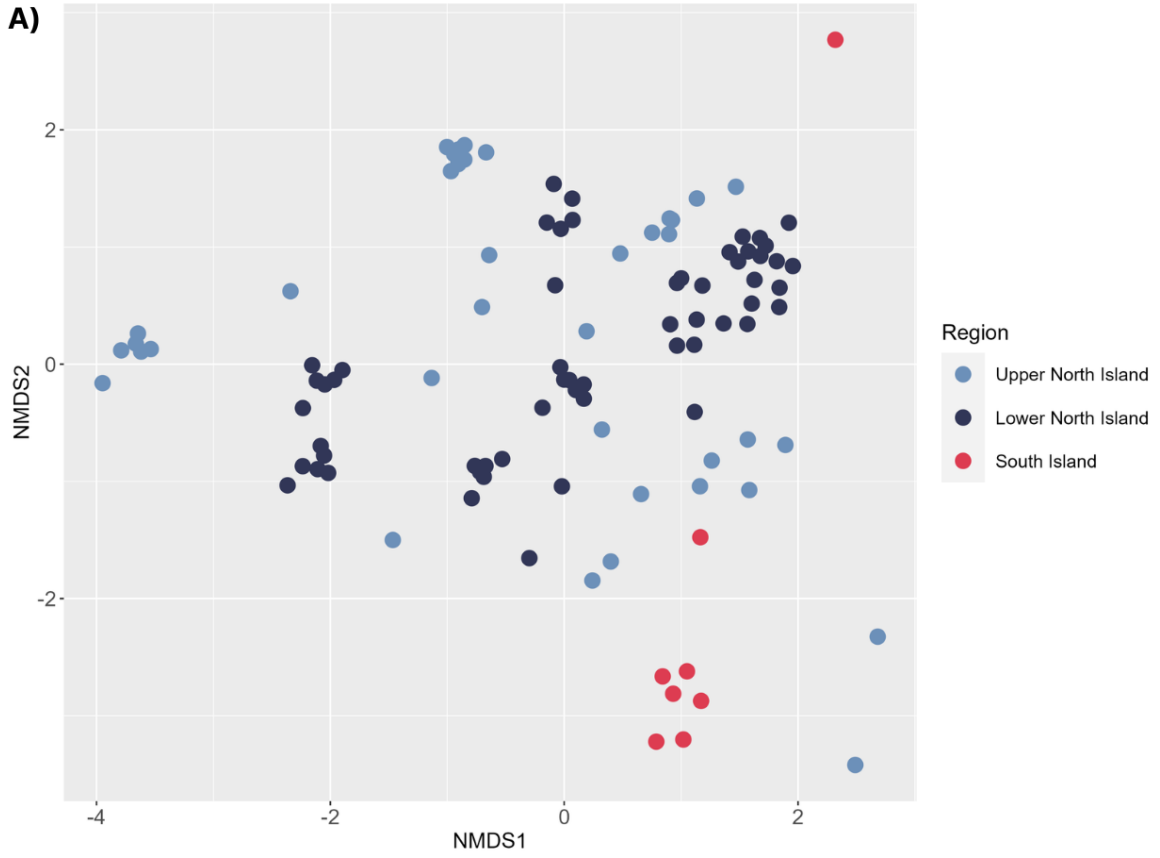
	NTT 2 NTT 3	Wetland Drain	Although it is small, fragmented, and unfenced, the area provides habitat for several vulnerable and at-risk species (Kapiti Coast District Council, 2022).	
Kawakahia Wetland/ Te Harakeke Swamp	KAW 1 KAW 2 KAW 3	Dune	Kawakahia Wetland (58.2 ha) stands as the largest surviving dune swale wetland in a relatively unaltered condition (Waka Kotahi NZ Transport Agency, 2012), comprising several vegetation communities, such as sedgeland, raupō reedland, flaxland (Smale & James, 2014), and providing a high-quality habitat for threatened and at-risk avian species (Waka Kotahi NZ Transport Agency, 2012).	0.32
Ngā Manu Nature Reserve	NGĀ	Lowland swamp forest	The 14 ha reserve comprises a diverse range of high-quality habitats for freshwater species, particularly threatened and at-risk avian species, with extensive raupō reedlands and flaxlands and contains 400 year old kahikatea (Ngā Manu Nature Reserve, n.d.; Waka Kotahi NZ Transport Agency, 2012). Samples were taken from the reserve's stream outflow.	
Old State Hwy 1	OLD		No information available. Aerial imagery suggests riparian area.	
Queen Elizabeth Park	QEP 1 QEP 2	Peat Drain outlet Drain	The area transitions from farmland into newly formed native forest, with regenerating native bush and newly established natural wetlands. Further peat restoration projects are planned (Greater Wellington Regional Council, 2022).	
Wetland inlet and outlet	INL OUT		No information available. Aerial imagery suggests area is a drain/channelised.	
Hororata	HOR		No information available. Aerial imagery suggests a riparian area.	
Redcliff Wetland Reserve	RED	Swamp and riverine wetland complex	Redcliff is part of a series of spring-fed wetlands, including the Rakatu Wetland system. The 50 ha of open water, native wetland vegetation and regenerating shrubland support a diverse range of bird and fish species (National Wetland Trust of New Zealand, n.d.).	

Rakatu Wetland	RAK	Swamp/constructed wetland	The 278 ha flood plain area was developed to enhance habitat for fish and wildlife in the region (Futter, 2008). A total of 90 wetland habitats have been interconnected (McCulloch, 2019).

3.4 Results

3.4.1 Spatial differences in wetland species composition

nMDS analysis identified spatial differences in DNA sequence composition among wetland sites of New Zealand, with the upper North Island showing more variation than the lower North Island, and the South Island wetlands clustering outside the main group (Fig. 3.1A). These differences were statistically significant ($F_{2,23}$ = 1.4104; p = 0.001; PERMANOVA; $F_{2,23}$ = 13.572; p = 0.001; PERMDISP), for all pairwise comparisons among the three regions (p = <0.001 for upper North vs. lower North Island, upper North vs. South Island, and lower North vs. South Island).



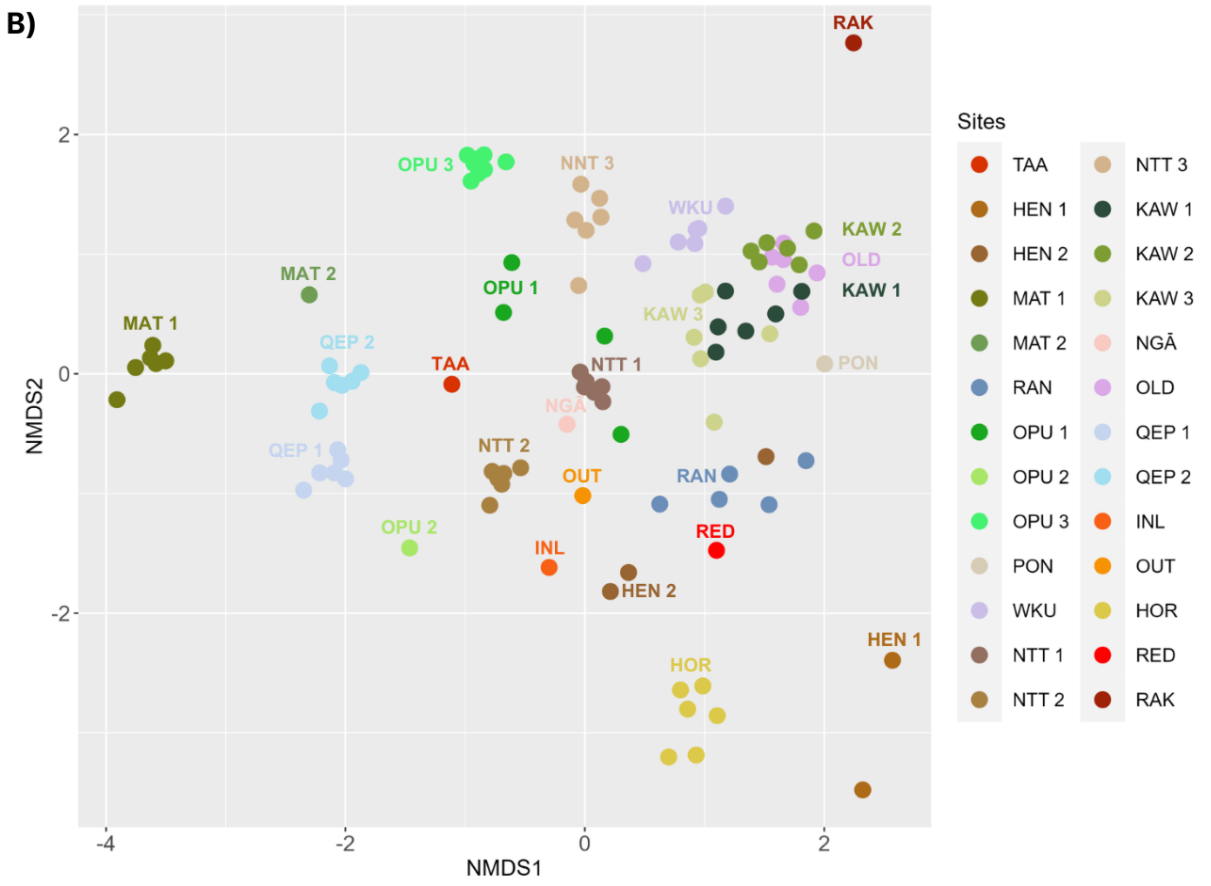


Figure 3.1 nMDS plot comparing: **A)** Wetland locations for broad Aotearoa regions – upper (locations TAA - PON) and lower North Island (WKU - OUT), and South Island (HOR - RAK); and **B)** All 26 individual sites. Locations are presented in the key in geographical order (from the top of the North Island to the bottom of the South Island).

For individual sites, the nMDS plot showed a general pattern in which samples from wetlands in close geographical proximity (e.g., KAW, OLD, NGĀ, and NTT) typically clustered together (Fig. 3.1B). Exceptions to this included RED and RAK wetlands (1.6 km apart), which clustered separately despite their neighbouring locations (Figs. 3.1B, A3.1). Multiple samples collected from the same wetland system exhibited high similarity for some sites (e.g., KAW 1 and KAW 2; QEP 1 and QEP 2), and significant dissimilarities for others (e.g., OPU 1-3) (Fig. 3.1B).

Analyses of the proportions of taxonomic phyla at each wetland showed overall variation in biodiversity among sites (Figs. 3.2, A3.2). The top five phyla across all sites were Annelida, Arthropoda,

Chordata, Cnidaria, and Streptophyta. However, a notable difference when comparing the North and South islands was that none of the South Island wetlands contained Streptophyta, while HOR (South Island) was the only wetland where the proportion of Bacillariophyta exceeded 1%. Moreover, MAT 1 and MAT 2 (upper North Island) showed the highest proportions of Annelids (84.4% at MAT 1) and Chordates (99.7% at MAT 2), while RAK (South Island) had the highest proportion of Arthropods (94.3%). A distinction between the North Island clusters was the greater variation of phyla in the lower North Island compared to the upper North Island when considering proportions >1%. For example, the primary phyla within the upper North Island wetlands were Annelida, Arthropoda, Chordata, and Streptophyta, while the lower North Island included these phyla but also harboured Mollusca and Cnidaria.

Even samples from wetlands in close geographical proximity demonstrated subtle differences when considering phyla proportions >1%. For instance, RAN and OPU 1-3 (6 km apart, upper North Island) displayed varying proportions of Chordata, Arthopoda, and Annelida but the OPU sites included Streptophyta, while RAN had Cnidaria and Mollusca. Similarly, NGĀ and OLD (2.9 km apart, lower North Island) had varying proportions of Annelida, Arthopoda, Chordata, and Cnidaria but only NGĀ contained Nemertea and Streptophyta. In the South Island, RAK was dominated by Arthropoda (as outlined above) and Chordata (5.7%), while the neighbouring RED wetland (1.6 km away) had a much higher proportion of Chordata (69.7%), only 6.2% Arthropoda, and low representation from Annelida (21.2%) and Nemertea (1.6%) (Fig. 3.1B).

3.4.2 Differences in exotic/non-exotic species ratios across wetlands

The majority of locations harboured >50% exotic species, while HEN 2, MAT 2, and KAW 2 each consisted of exactly 50% exotic species (Fig. 3.3A). MAT 1 and OPU 2 completely lacked any non-exotics, though the number of species differed between the two wetlands, with 8 and 17 exotic species at MAT 1 and OPU 2, respectively. Samples from spatially distant wetlands HEN 1 (n = 15), NGĀ (n = 66), HOR (n = 48), and RAK (n = 9) each exhibited 67% exotic species and 33% non-exotic species. Similarly, PON (n = 20) and NTT 1 (n = 40) were dominated by 75% exotic species (Fig. 3.3A).

The top ten exotic species across all sampled sites included fish (n = 6) and worms (n = 4) (Fig. 3.3B). Among these, *Acanthocyclops robustus* (copepod), *Gambusia affinis* (mosquito fish), and *Anas platyrhynchos* (mallard duck), were present at 21, 6, and 17 of the 26 sites, respectively. Interestingly, the highest eDNA read count for mosquito fish (n = 97,470 reads) was detected at OPU 3, while the remaining five sites that detected this species did so in much lower read counts (ranging from 2,356 to 11,906). The top ten list of non-exotic species (native and endemic) included six fish, two birds, a copepod, and a hydrozoan (Fig. 3.3B). *Anguilla australis* (tuna/short-finned eel), *Mesocyclops leuckarti* (copepod), and *Porphyrio melanotus* (pūkeko/swamphen) were observed at 22, 12, and 16 of the 26 sites, respectively; while *Porphyrio melanotus* was not detected in the South Island wetlands and *Gobiomorphus breviceps* (uplands bully) and *Neochanna apoda* (waikaka/hauhau/brown mudfish) were each only detected at a single site (HOR and NTT 2, respectively) (Fig. 3.3B).

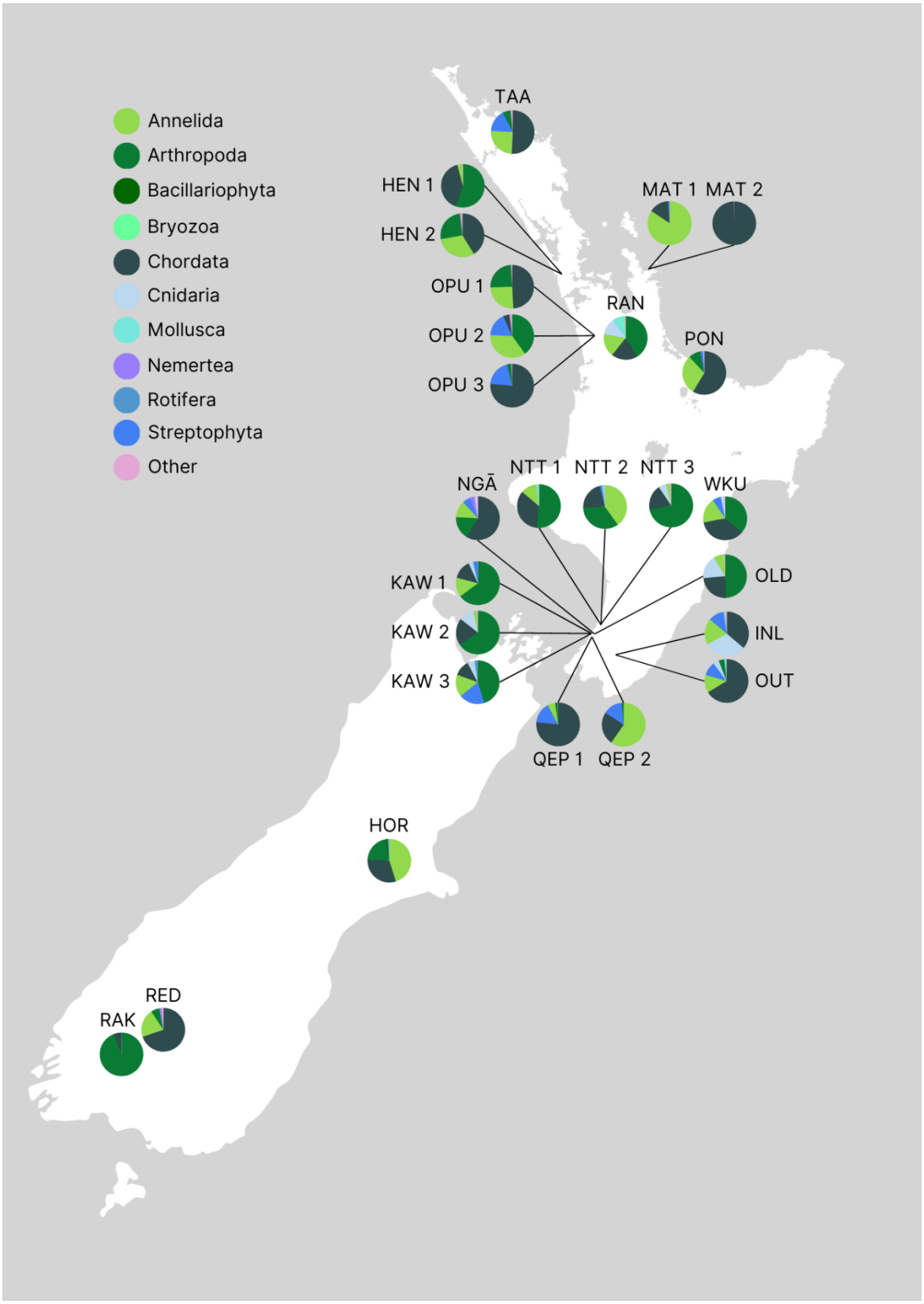


Figure 3.2 Map of each wetland location, with pie graphs indicating the proportion of each phylum represented (phylum proportions <1% were merged into a single 'other' category; these are described in full in Table A3.1).

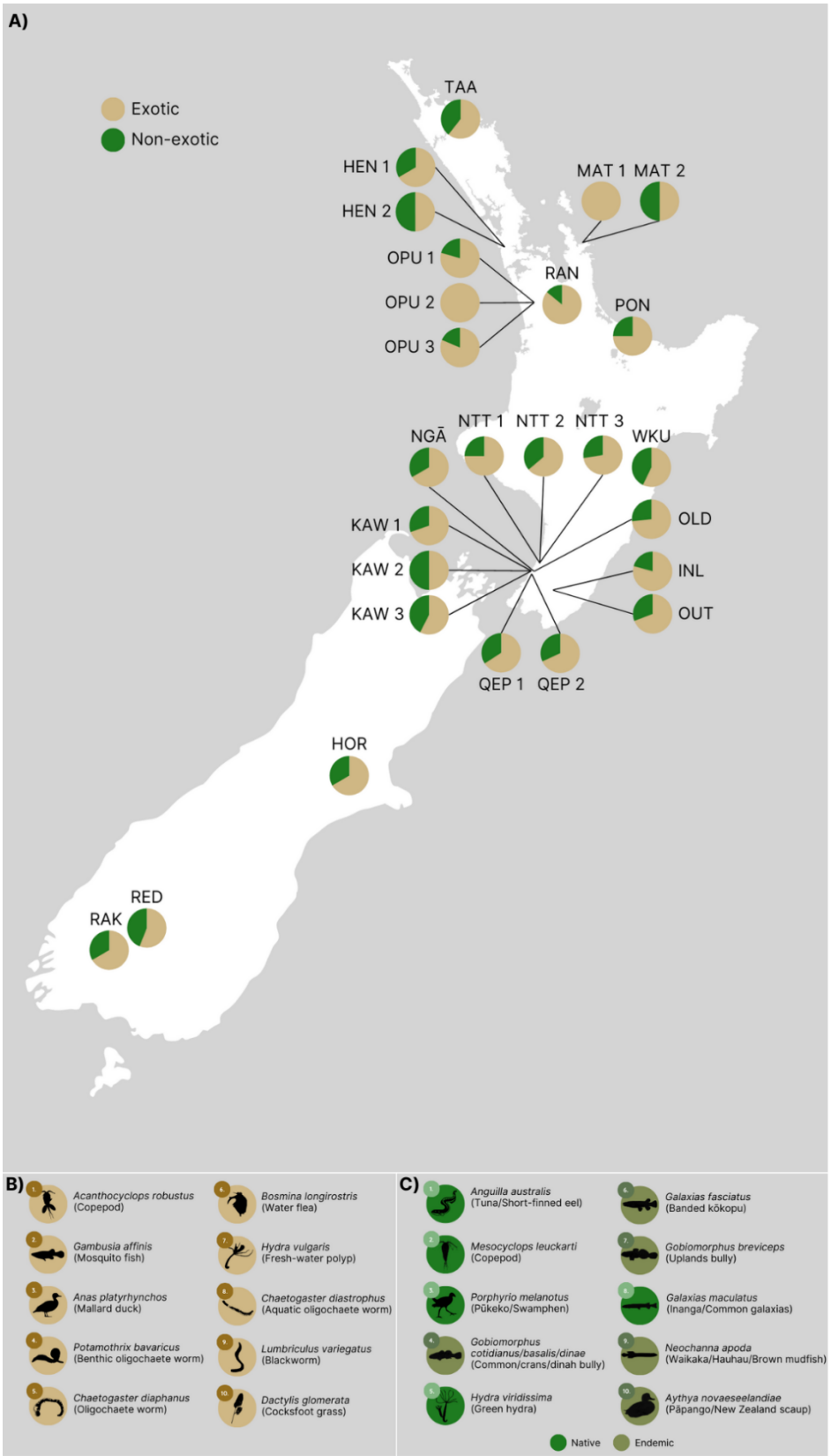


Figure 3.3 A) Map of each wetland location, with pie graphs indicating the proportion of exotic versus non-exotic species. See Figure A3.3 for pie graphs of exotic versus native versus endemic ratio. The top ten **B)** exotic and **C)** non-exotic (i.e., native or endemic) species across all wetland locations.

3.5 Discussion

We explored public eDNA records obtained from 16 locations to better understand spatial biodiversity patterns and the current condition of Aotearoa New Zealand wetlands. We found that DNA sequence composition differed spatially, with low persistence of non-exotic species at all sites.

Johnson and Gerbeaux (2004) recognised nine distinct wetland classes: bog, fen, swamp, marsh, seepage, shallow water, ephemeral wetland, pakihi and gumland, and salt marsh, though multiple classes can occur adjacently (Browne & Campbell, 2005). Variation in environmental features (Johnson & Gerbeaux, 2004) among classes, including water regime, substrate, nutrient status, and pH (Clarkson & Peters, 2012), ultimately determines the flora and fauna that reside in a given wetland by selecting those species that can tolerate the local abiotic conditions (Clarkson & Peters, 2012; Johnson & Gerbeaux, 2004). For example, certain plant species have specialised for wetland environments by adapting to wet and oxygen-deprived conditions (Ausseil et al., 2011b; Sorrell & Gerbeaux, 2004). Thus, environmental differences among sites are a key driver for signals of spatial biodiversity among wetlands.

We examined eDNA samples that had been collected from various wetland types, from swamp to coastal, and found spatial differences even among proximate sites. For example, sites contained varying proportions of the top five phyla (i.e., Annelida, Arthropoda, Chordata, Cnidaria, and Streptophyta), with some dominated by a specific phyla and others containing a plethora of phyla diversity. Similarly, some sites contained equal parts exotic and non-exotic species, while others were completely saturated with exotics. The composition and distribution of the top ten exotic and non-exotic species varied across the sampled sites, with certain species being widespread (e.g., *A. robustus*, *A. australis*), others exhibiting notable read count differences (e.g., *G. affinis*), and some being detected at limited locations (e.g., *G. breviceps*, *N. apoda*). These observations illustrate the complex and dynamic nature of biodiversity across wetland ecosystems.

Ausseil et al. (2008) developed an ecological integrity (EI) index to quantify human pressures on wetland ecosystems. This index considers several measures, including the naturalness of

catchment cover, the artificial impervious cover, nutrient enrichment, introduced fish and woody plants, and drainage. Values of the index range from zero to one, with one denoting pristine or complete integrity. In Aotearoa New Zealand, >60% of studied wetlands had an EI index <0.5, suggesting considerable biodiversity losses (Ausseil et al., 2008). This is consistent with our findings, where all 26 studied sites had at least 50% exotic species proportions in the eDNA samples. Three sites from the present study (Te Henga Wetland – HEN, Opuatia Wetland – OPU, Kawakahia Wetland – KAW) were evaluated by Ausseil et al. (2008) and had EI indices of 0.66, 0.23, and 0.32, respectively. In our study, those sites had varying proportions of Arthropoda, Chordata, and Anneldia. Opuatia Wetland and Kawakahia Wetland samples contained Streptophyta, while Cnidaria and Rotifera were recorded at the latter when considering phyla proportions >1%. Notably, HEN 1 and KAW 2 were among the sites harbouring exactly 50% non-exotic species, while OPU 2 completely lacked any non-exotics. However, the EI index does not include the effects of mammalian pests, herbaceous weeds, and exotic invertebrates due to a lack of spatial data of this nature. This underscores the value of eDNA for building comprehensive long-term databases on exotic species persistence in wetland environments.

In Aotearoa New Zealand, a National Policy Statement for Freshwater Management 2020 (amended 2023; Ministry for the Environment, 2023) sets nationally consistent guidelines for local authorities on how to manage freshwater resources in accordance with the Resource Management Act 1991. Policy 6 within the Policy Statement requires that “there is no further loss of extent of natural inland wetlands, their values are protected, and their restoration is promoted” (p. 10). Although New Zealand legislation requires the protection of wetlands (Myers et al., 2013), the degree of management efforts varies significantly (Denyer & Peters, 2020). Most wetlands are under government management, where they are ranked based on their representativeness to guide the allocation of scarce conservation resources aimed at ensuring biodiversity persistence (Ausseil et al., 2011b). For example, Te Henga Wetland (HEN) is the largest freshwater swamp in Tāmaki Makaurau, Auckland (Tiaki Tāmaki Makaurau Conservation Auckland, n.d.). It holds high ecological value due to

the presence of increasingly rare habitats (Waitakere City Council, 2008), its substantial size, and the high-quality ecosystem it sustains (Tiaki Tāmaki Makaurau Conservation Auckland, n.d.). Management at this site encompasses animal pest and weed control, restoration planting, and educational initiatives, all driven by the local community through various ecological restoration projects (Tiaki Tāmaki Makaurau Conservation Auckland, n.d.; Waitākere Ranges Local Board, 2018). Interestingly, non-exotic species at HEN 1 were among the lowest identified in our eDNA samples (33%), which may indicate the effectiveness of current management practices. Similarly, Kawakahia Wetland/Te Harakeke Swamp (KAW) holds outstanding ecological value and is reasonably intact, partly buffered by regenerating native forests (Smale & James, 2014). In conjunction with landowners, the Greater Wellington Regional Council undertakes active management of pest plants and animals at Kawakahia (Smale & James, 2014) and additional areas of the wetland are also protected by Queen Elizabeth II National Trust Covenants (Riddell, 2014; Smale & James, 2014). The eDNA samples collected at KAW detected 30%-50% non-exotic species. In contrast, restoration at Pongakawa Wetland (PON) began more than four decades ago, with initial plantings occurring along the border of what was once a soggy paddock (Western Bay of Plenty District Council, 2021). This endeavour has been a collective community effort that has evolved into an extension of Pongakawa School's classroom (Western Bay of Plenty District Council, 2021), allowing students to gain a deeper understanding of the vital role wetlands play. The eDNA data showed a 75% dominance of exotic species at Pongakawa Wetland, demonstrating the variability of restoration efforts in creating and restoring wetlands effectively to enhance native biodiversity. However, eDNA collection at this site consisted of a single 205 mL sample that may not provide an accurate estimate of the overall wetland condition. Thus, further sampling at this site would be beneficial and the following of best practice eDNA collection guidelines is recommended more generally (De Brauwer et al., 2022; Smith et al., 2023).

Various resources are available for traditional monitoring and restoring of wetlands in Aotearoa New Zealand (e.g., Wetland Restoration Handbook, Peters & Clarkson, 2010; Wetland Cultural Health Indicator, Robb, 2014). However, with wetlands identified as the most threatened and

degraded ecosystems at the national level in Aotearoa New Zealand (Ausseil et al., 2008), and their extent continuing to decline (Denyer & Peters, 2020), there are calls for reliable, cost-effective, fast-paced tools to monitor ecological trends (Urban et al., 2022), and eDNA is providing new solutions. For example, Wilkinson et al. (2023) recently developed an eDNA-based ecological health monitoring tool, known as a taxon-independent community index (TICI), for Aotearoa New Zealand rivers. The index assigns a quantitative value reflecting the quality and health of the ecosystem based on eDNA samples and is proving to be a powerful way to provide baseline data and characterise spatial change, such as from a native bush reserve downstream to pastoral farming (Drysdale et al., n.d.). Future development of the TICI index for other environments, including wetlands, will require more data to train the model against (Wilkinson et al., 2023). However, in light of our rapidly changing climate, the urgency for biodiversity monitoring measures that go alongside wetland restoration cannot be overstated. Our findings revealed that exotic species remain prevalent in Aotearoa New Zealand wetlands and biological invasion is only forecasted to increase with climate change (Chown et al., 2014; Mainka & Howard, 2010). eDNA biomonitoring, in concert with ongoing efforts to control pest plants and animals, has yielded encouraging results for some of Aotearoa New Zealand's wetlands and holds promise for continued guidance of future restoration programmes.

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Chapter 4.

Spatial and temporal variability of eDNA in an Aotearoa New Zealand wetland

Image of Opuatia Wetland by: Starsha Bird

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Contributions: SB and AM conceived the research project to align with the goals of Waikato Regional Council. JS, ID, Kaitlin Morrison, and Elizabeth Overdyke performed conventional surveys and provided feedback on the draft manuscript. SB collected eDNA samples and analysed the data, with AM, Kat Rowe, Kirsty Vincent, and Linda Tomuli helping with the former. SB wrote the first draft of the manuscript and all authors provided feedback, with SB leading subsequent revision of the final manuscript.

4.1 Abstract

Wetlands are ecologically and culturally significant ecosystems that are experiencing biodiversity declines globally. Biomonitoring techniques that use environmental DNA (eDNA) to detect and monitor biodiversity are well established in lake, riverine, and marine ecosystems. However, their use in wetlands is less advanced due to the presence of sediments that block eDNA filters, limiting water filtration, and a lack of standardised methodology. Here, we examined eDNA dynamics to understand spatiotemporal biodiversity patterns in an Aotearoa New Zealand wetland, and to optimise their application for wetland-specific challenges.

We sampled four sites across Opuatia Wetland at three time points during an austral spring. We conducted conventional taxonomic surveys, tested three different filter sizes (1.2 µm, 5 µm and semi-quantitative dacron filters), and assessed our ability to detect foreign DNA (from kea; *Nestor notabilis*) at different time points and distances post-release.

We found significant differences in DNA sequence composition across time and space, and when using different sized filters. eDNA data generally complemented (versus replaced) conventional survey and identification methods, with certain species only detected by one method or the other in many cases and taxonomic resolution of conventional sampling and identification methods often exceeding that of eDNA. Foreign DNA was detectable 10 m from its release point for up to one week post-release.

Our results provide new insights into the biodiversity dynamics of wetlands and highlight sampling practices that will optimise eDNA detection methods in wetland environments, where rapid biomonitoring techniques are needed to support conservation and preservation.

4.2 Introduction

Despite the ecological and cultural significance of wetlands, they are among the most threatened ecosystems in the world (Davidson, 2014; Fluet-Chouinard et al., 2023), and have diminished in global extent by 3.4 million km^2 since 1700 (Fluet-Chouinard et al., 2023). As well as factors like land-use change and peat extraction (Van Asselen et al., 2013), wetlands are under pressure from invasive plants and animals (Kingsford et al., 2016), which have both economic (Diagnes et al., 2021) and environmental impacts (Doherty et al., 2016).

Monitoring biodiversity and invasive species incursions is an important component of their restoration and preservation. Various resources are available for monitoring ecological conditions for a range of wetland types (e.g., Clarkson et al., 2004; Peters & Clarkson, 2010; Robb, 2014). However, conventional methods (such as netting, visual surveys, and sound monitoring) are typically labour- and time-intensive, and thus can be expensive (Beng & Corlett, 2020; Hervé et al., 2022). They often also require specific taxonomic expertise (Leese et al., 2018), and may involve capturing macroorganisms, with sometimes lethal collections (Saenz-Agudelo et al., 2021).

The uptake of environmental DNA (eDNA; genetic material released by organisms into the environment) as a rapid, non-invasive biomonitoring technique has surged over the past decade owing to its relative ease of use and suitability for a range of applications (Barnes & Turner, 2016; Beng & Corlett, 2020; Harper et al., 2019; Saenz-Agudelo et al., 2021; Stat et al., 2017). For example, eDNA can be used to detect invasive and endangered species; analyse past and present biodiversity patterns, trophic interactions, dietary preferences, and species distributions; and for overall monitoring of ecosystem health (Beng & Corlett, 2020).

Although the span of biodiversity detected using eDNA is often broad, and can exceed that detected by conventional methods for some taxa (Boivin-Delisle et al., 2020; Coutant et al., 2020; David et al., 2021; Pont et al., 2018), environmental factors such as pH, temperature, solar radiation, and microbial activity are known to impact eDNA integrity (Harrison et al., 2019; Strickler et al., 2015). For example, under laboratory conditions (Barnes et al., 2014; Dejean et al., 2011; Goldberg et al.,

2013; Gantz et al., 2018; Sassoubre et al., 2016; Strickler et al., 2015) and in riverine (Deiner & Altermatt, 2014; Nukazawa et al., 2018; Pont et al., 2018) and marine (Ely et al., 2021; Murakami et al., 2019; Thomsen et al., 2012) ecosystems, eDNA can vary in persistence from hours to days (Barnes & Turner, 2016). Moreover, the method by which eDNA is collected from environmental samples can impact the resulting taxonomic diversity recovered. In particular, the volume of water filtered (and the associated filter pore size used) can significantly impact DNA collection (Tsuji et al., 2019), with most macroorganism eDNA captured using filter sizes of 1-10 µm (Turner et al., 2014). Finally, the movement of eDNA through ecosystems, and the temporal and spatial dynamics of eDNA signals, are important drivers of current biodiversity patterns that may also be useful for predicting future changes (Mathieu et al., 2020).

Technical aspects of eDNA sampling, including the impacts of filter size, eDNA decay rate, and sampling pattern and frequency, are well-established in riverine and marine systems (e.g., Ely et al., 2021; Jeunen et al., 2022; Pont et al., 2018; Smith et al., 2023), and best practice eDNA sampling guidelines are frequently becoming available (e.g., De Brauwer et al., 2022; Minamoto et al., 2021). However, the use of eDNA in wetland environments is less defined, due to challenges arising from typically high sediment volumes that can block eDNA filters, limiting the volume of water that can be processed. In Aotearoa New Zealand, substantial losses in wetland extent (historic extent of approximately 2.4 million ha to a current extent of about ~250,000 ha; Ausseil et al., 2011) have reduced biodiversity and impacted the intrinsic relationship between Māori (Indigenous People of Aotearoa) and their repo (wetlands) (Taura et al., 2021). Invasive species are widespread in wetlands nationwide, where they have devastating impacts – altering ecosystem composition, structure, and function (Griffiths et al., 2018).

Here, we explored eDNA dynamics in wetlands, with a view towards understanding spatiotemporal patterns and optimising their application to meet some of the unique challenges posed by the wetland environment. We sampled four spatially-distant sites across Opuatia Wetland (Waikato, Aotearoa New Zealand) across three time points. In conjunction, we performed floristic and

faunistic surveys using conventional collection and morphological identification methods, tested different filter sizes, and examined our ability to detect a foreign DNA source at various time points and distances following its release.

4.3 Methods

4.3.1 Site description

Opuatia Wetland (950 ha) is located in the lower Waikato River catchment, north of Lake Whangape, in Te Ika-a-Māui/the North Island of Aotearoa New Zealand (Fig. 4.1). Hills border the wetland to the north, east, and south, and these are primarily used for crops and agriculture (Barnes et al., 2001; Browne & Campbell, 2005). The overall wetland is a mosaic of fen, swamp, and marsh components (Browne & Campbell, 2005; Reeves, 2011; Waikato Regional Council, n.d.). It features a restiad peat bog – which ranks among the five significant restiad peat bogs remaining in the Waikato Region, and is thus considered a site of high value and national importance (Singers, 2019; Waikato Regional Council, n.d) – and a wide range of hydrological characteristics. The water table is close to or above the surface level and is relatively stable (Browne & Campbell, 2005) – which serves as a general indicator of a healthy wetland (Reeves, 2011) – though water levels are lower during late summer and autumn (February to April) (Browne & Campbell, 2005). A causeway separates sites at the northern edge of the wetland (i.e., SS1 and SS2; Fig. 4.1), with a culvert underneath that prevents winter flooding (Reeves, 2011). The wetland discharges westward via Opuatia Stream into the Waikato River (Barnes et al., 2001; Singers, 2019).

Opuatia Wetland holds ecological importance and also bears special significance to Horahora Marae. Horahora Marae is descended from the great voyaging waka Tainui and the tribes of Ngāti Pou and Waikato-Tainui. The Waikato River is their tūpana (ancestor) and is a source of their tribal identity (Deed of Settlement in Relation to the Waikato River, 2009).

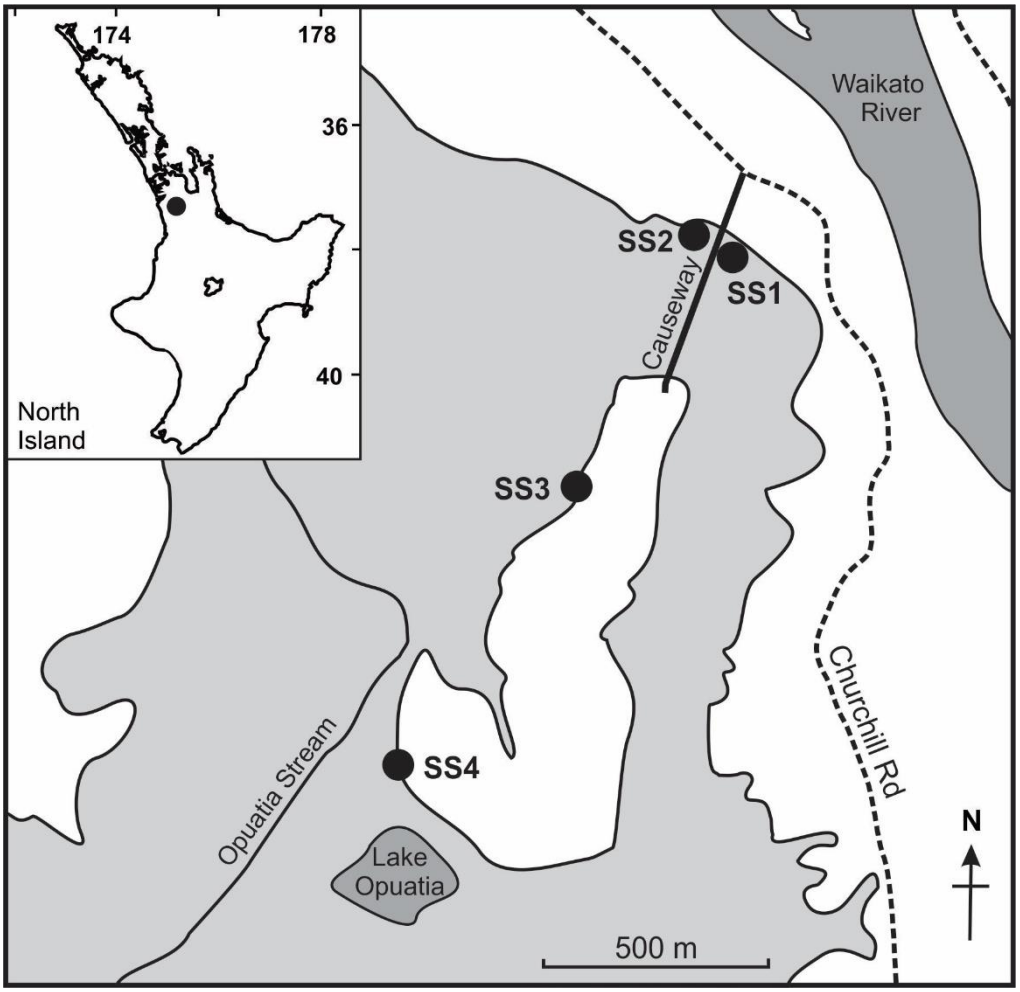


Figure 4.1 Location of Opuatia Wetland and study sample sites (inset: location of Opuatia in Aotearoa New Zealand's North Island). SS1 and SS4 are approximately 1.4 km apart.

4.3.2 Site selection, characteristics, and sampling periods

Four sampling locations ('SS1' to 'SS4') were chosen based on their spatial distance from each other, water availability, and site access, with sampling occurring during spring 2022 (i.e., the start of September to early December) – the period of highest species activity (i.e., breeding season). Wetland types at each location were classified following the Johnson and Gerbeaux (2004) classification system, with SS1 corresponding to a swamp, and SS2-SS4 corresponding to marsh. SS1 contained a mix of indigenous and exotic herbaceous wetland vegetation (Reeves, 2011), and SS2 and SS3 were dominated by introduced crack willow (*Salix fragilis*) and grey willow (*Salix cinerea*), with a sparse mixture of native and introduced species beneath this canopy. Crack willow similarly dominated SS4.

Water quality parameters were measured at each site during each sampling event, including pH (Eutech pH Testr 30), water temperature, dissolved oxygen, conductivity, and salinity (YSI Prosolo). Daily weather was recorded for the entire wetland from AccuWeather, Churchill, Waikato (https://www.accuweather.com/en/nz/churchill/1078713/weather-forecast/1078713; Table A4.1) from approximately one week before sampling commenced up until the final eDNA sample was collected. A rough estimate of water level fluctuations was made during each site visit by marking out a 1 m length on a bamboo stick placed at all sites at the start of spring – the initial water level was noted and then subsequently recorded during each site visit to determine the difference from the initial reading.

4.3.3 Conventional surveys

Conventional survey methods, including zooplankton sampling, gee-minnow trapping, 5-minute bird counts, and botanical surveying, were conducted for comparison with eDNA methods. Among these, the 5-minute bird count and botanical surveys were carried out by Waikato Regional Council staff members. All surveys were conducted after eDNA collection to prevent contamination of the latter. Zooplankton samples and gee-minnow traps were carried out at the start (7-9 September 2022), middle (19-20 October 2022), and end of spring (29-30 November 2022), while the botanical survey was performed in the middle of spring. The 5-minute bird count was conducted at the middle and end of spring on 21 October 2022 and 5 December 2022, respectively.

4.3.3.1 Zooplankton surveys

To collect the zooplankton samples, up to 9 L of surface water was passed through a 40 µm mesh at SS2-SS4. Material retained on the mesh was then washed with filtered tap water into a sample container and preserved with ethanol (>50% final concentration). Samples were enumerated in 5 mL aliquots until a minimum of 300 individuals, or the entire sample, was counted. Individuals were identified to species level wherever possible using standard taxonomic keys (e.g., Shiel, 1995; Chapman et al., 2011).

4.3.3.2 Gee-minnow traps

A total of 12 gee-minnow traps (4 mm mesh) were deployed at SS3 and SS4. Traps were positioned along the shoreline at 3 pm on the day of deployment, with an air pocket at the top due to anoxic conditions, and left overnight before collection the following morning at 9 am (i.e., deployed for 18 hours, total). The water level dropped overnight for all deployments; therefore, the total maximum fishing time was uncertain. All species caught in the traps were identified and recorded, as were species observed outside the traps during the trapping period. While the primary function of a gee-minnow trap is to sample small fish species (Lake, 2013), other taxonomic groups were also recorded (e.g., arthropods, annelids).

4.3.3.3 Five-minute bird counts

Birds were identified to species level using sight or sound over a five minute duration and counted within an approximately 300 m radius of the observer. SS1 and SS2 were examined together due to their close proximity. Environmental conditions (e.g., wind) were noted as they interfere with sound perception. Audio playbacks were performed three times for matuku/Australasian bittern (*Botaurus poiciloptilus*; presumed to be present at Opuatia Wetland) and pūweto/spotless crake (*Zapornia tabuensis*; potential resident) – as these species are secretive and territorial.

4.3.3.4 Botany survey

Ground surveys were conducted within the eDNA sample collection area at SS2-SS4, with species identified to genus and species level where possible.

4.3.4 eDNA

4.3.4.1 eDNA sampling

All eDNA samples were collected using kits from Wilderlab (Wellington, New Zealand). Five to six replicates were collected at each site, including a control sample (1 L distilled water) (Table 4.1), using a composite sampling strategy following decontamination of all equipment with a 10% bleach solution. In brief, this involved using a sampling pole with an attached jug to collect surface water from various points across the site (with the pole extended away from the body and the collector not

entering the direct collection area in order to avoid contamination), which was then pooled into a bucket and left for 10-15 minutes to allow any sediment to settle. Different volumes of water, dependent on filter size (see below) and sedimentation load, were then pushed through a filter designed to trap DNA using a syringe, with a caulking gun used to assist filtration as required. Following this, 60 cc of air was passed through the filter to remove excess water and 300 µL of a DNA/RNA shield preservative was injected. Preserved filters were then sent to Wilderlab for eDNA analysis.

Table 4.1 Summary table of sample collection information for eDNA experiments at Opuatia Wetland, including water quality measurements and the time of recording, and water level fluctuations.

Site	Latitude	Longitude	Collection date	Spring period	Filter size (µm)	Replicates	Volume filtered (mL)
SS1	-37.422857	175.071516	08/09/2022	Start	1.2	5	100 - 205
					5	5	800 - 1,000
					Dacron	6	10,000 - 38,000
			20/10/2022	Middle	1.2	5	150 - 170
					5	6	250 - 450
					Dacron	6	21,000 - 37,000
			02/12/2022	End	1.2	5	205 - 360
					5	6	260 - 600
					Dacron	6	1,000 - 65,750
SS2	-37.422580	175.071082	07/09/2022	Start	5	5	490 -1,000
	-37.422380	175.071010	19/10/2022	Middle	5	5	510 - 1,000
	-37.422580	175.071082	29/11/2022	End	5	6	250 - 640
SS3	-37.427204	175.068028	07/09/2022	Start	5	5	1,000
	-37.426990	175.067830	19/10/2022	Middle	5	5	1,000
	-37.427204	175.068028	29/11/2022	End	5	5	870 - 1,000
SS4	-37.432995	175.063563	07/09/2022	Start	5	5	1,000

	-37.433088	175.063192	19/10/2022	Middle	5	5	1,000
	-37.432995	175.063563	29/11/2022	End	5	5	700 - 1,000

Site	Water temperature (°C)	pH	DO (%)	DO (mg/L)	Conductivity (SPC-µS/cm)	Salinity (SAL-ppt)	Time	Water level difference from initial (cm)*
SS1	11.5	6.9	44.1	4.69	101.3	0	09:03	N/A
	13.8	6.5	2.9	0.3	147.3	0.07	08:30	-18
	18.3	6.9	5.3	0.49	178.3	0.08	09:05	+26

SS2	16.3	6.5	85.3	8.33	104.6	0	14:00	N/A
	21.3	6.2	98.3	8.71	173.6	0.08	13:10	No water
	21	6.8	10.1	0.9	189	0.09	13:07	+33
SS3	17.8	7.3	75.7	7.18	141.9	0	12:24	N/A
	14.5	6.6	15.2	1.51	238.8	0.11	09:03	No water
	19.4	6.9	18.4	1.7	242.2	0.11	11:21	+42
SS4	13.1	9.7	65.4	6.73	136.9	0	10:26	N/A
	15.6	6.5	11.1	1.1	234	0.11	10:52	No water
	18	6.9	10.5	1	235.6	0.11	09:28	+40

*Initial water level at the start of spring: SS1 at 60 cm; SS2 at 70 cm; SS3 at 40cm; SS4 at 40cm.

4.3.4.2 Technical experiments

4.3.4.2.1 Dispersal distance and residence time experiment

To determine the residence time and dispersal distance of DNA at the wetland, DNA from kea (*Nestor notabilis*) faecal samples (since kea is not naturally found in the North Island; Tennyson et al., 2014) was released on 16 September 2022. Though historical interactions between Māori and kea are sparsely documented, they feature in pūrakau (Māori myths and legends) and are a taonga (treasured) species under the Ngāi Tahu Claims Settlement Act 1998. According to the Waitaha Māori legend, kea were kaitiaki (guardians) birds for iwi (tribe) and hapū (sub-tribe) members who traversed Te Wai Pounamu/the South Island mountains in search of pounamu (greenstone). They are said to be the ground hawks who accompanied Māori on their journey (Riley, 2001; Temple, 1996). Kea whakapapa to (share genealogy with) other species across Aotearoa, New Zealand (Temple,1996), including kākā (*Nestor meridionalis*) – a large, forest-dwelling parrot – and kākāpō (*Strigops habroptilus*) – a nocturnal, flightless parrot (Loepelt et al., 2016; Temple, 1996). Using kea DNA here provided an opportunity to explore DNA persistence in wetland environments, supporting the health and well-being of the Waikato River. A karakia (Māori prayer) was performed prior to the DNA release and sampling in accordance with tikanga (Māori customs).

Hamilton Zoo staff collected 140 g of kea excrement from the zoo's colony between 6 and 14 September 2022, from sand/mulch and directly from perches immediately after defecation (i.e., following feeding) and stored this in a chest freezer at -20°C until release. The excrement was then diluted in 1.5 L of water (boiled and cooled before use to minimise and remove contaminants) to form a slurry. Three replicates were collected from the slurry (85 - 167 mL) to quantify the kea DNA counts prior to release.

SS1 was chosen as the slurry release site based on the wetland's hydrology. To first measure water flow at the site, we performed a simple experiment, anchoring two bamboo sticks 1 m apart in the flooded wetland area and placing oranges at one end (Fig. A4.1). The oranges did not move

throughout the day, ultimately determining the timing of our eDNA samples collections post-faecal slurry release.

The kea DNA slurry (total volume = 600 mL) was released at four points within the release area – a horizontal transect approximately 5 m in width (Fig. A4.1). eDNA sampling was performed following composite sampling strategy described above, using 5 µm filters at distances of 1, 10, and 25 m away from the release point for six time points post-release (1 hr, 5 hr, 24 hr, 48 hr, 1 wk, and 3 wks) (Table 4.2; Fig. A4.1). Samples were not collected prior to 24 hr for the 25 m distance based on the findings from the orange experiment, while samples were collected before release (i.e., start of spring collections) to confirm that kea and/or the genus *Nestor* was not already present at Opuatia. Sample collection involved walking on either side of the transect to minimise disturbance within the transect area and followed the composite sampling strategy outlined above.

4.3.4.2.2 In situ residence time experiment

To determine the *in situ* residence time of DNA at its release point in the wetland (i.e., versus dispersal distance post-release; Section 3.4.2.1), DNA from kea faecal samples was released at SS1 on 4 September 2023. As for the dispersal distance experiments above, Hamilton Zoo staff collected 140 g of kea excrement from the zoo's colony (from mid to late August 2023), and this was diluted in 1.5 L of water to form a slurry. Weather was again recorded for the entire wetland from one week before slurry release until 16 September 2023, as described above (Table A4.1). Prior to release, water flow was measured following the orange experiment described above; after 30 seconds, the oranges moved approximately 0.75 m, indicating a flow rate of 0.025 m/s.

The kea slurry was released steadily into the wetland at a single point. eDNA sampling was repeatedly performed at the release site at five time points (2, 4, 6, 9, and 12 days post-release) using a sampling pole with an attached jug to collect surface water from the release point into a bucket, with replicate eDNA samples then collected using 5 µm filters.

Table 4.2 Summary of the dispersal distance and *in situ* residence time experiments, including sampling information, water quality measurements and their time of recording, and water level fluctuations.

Dispersal distance and residence time													
Collection date	**Transect distance (m)**	**Time post-release**	**Replicates**	**Volume filtered (mL)**	**Water temperature (°C)**	**pH**	**DO (%)**	**DO (mg/L)**	**Conductivity (SPC-µS/cm)**	**Conductivity (SPC-mS/cm)**	**Salinity (SAL-ppt)**	**Time**	**Water level difference from initial (cm)***
16/09/2022	1	1 h	6	400 - 650	12.2	6.5	17.2	1.9	-	43	0.1	09:00	-16
	10		5	1,000									
	1	5 h	6	300 - 844	16.3	5.8	57	5.8	-	37.1	0	14:30	
	10		6	370 - 850									
17/09/2022	1	24 h	6	356 - 800	12.4	6.5	14	1.52	-	78.7	0.1	09:26	-17
	10		6	650 - 1,000									
	25		6	390 - 805									
18/09/2022	1	48 h	6	310 - 600	13.1	6	16.4	1.8	-	44.9	0.1	09:23	-17.5
	10		6	457 - 950									
	25		6	200 - 400									
23/09/2022	1	1 wk	6	305 - 720	14.5	6.5	21.5	1.9	150.7	-	0.07	09:02	-15
	10		6	550 - 910	14.4	6.3	17.4	1.56	164.4	-	0.08	09:04	

	25		6	350 - 570	14.3	6.6	15.6	1.48	163.9	-	0.08	09:06	
7/10/2022	1	3 wk	6	400 - 650	14.1	6.3	3.7	0.33	167.2	132.7	0.08	09:37	+45
	10		6	268 - 820	14.8	6.4	11.1	1.06	164.9	132.7	0.08	10:03	
	25		6	300 - 850	14.6	6.4	8.1	0.76	165.7	132.8	0.08	10:08	
In situ residence time													
06/09/2023	*In situ*	Day 2	3	300 -700	15.4	6.7	34.3	3.43	153.3	-	0.07	10:02	N/A
08/09/2023	*In situ*	Day 4	3	230 - 710	12.8	6.6	19.7	2.08	160.2	-	0.08	09:45	N/A
10/09/2023	*In situ*	Day 6	3	255 - 550	12.9	6.8	16.9	1.79	168	-	0.08	09:48	N/A
13/09/2023	*In situ*	Day 9	3	450 -550	13.2	6.8	23	2.41	153.9	-	0.07	09:41	N/A
16/09/2023	*In situ*	Day 12	3	400 - 555	13.1	6.9	23	2.42	148.9	-	0.07	09:47	N/A

*Initial water level at the start of spring at SS1 was 60 cm.

4.3.4.2.3 Filter size comparisons

To determine the optimum filter size for wetland eDNA sampling, we compared 1.2 µm, 5 µm, and qualitative dacron (Aqua One Micro Pad; relatively more coarse than the 1.2 and 5 µm filters) filters at SS1 (Table 4.1). The standard volume processed for the 1.2 µm and 5 µm filters was 1 L and was 100 L for the dacron filter, where an electric pump was used to actively push water through the filter. However, due to turbidity, not all samples met these standard volumes. Thus, water was filtered up to the given limit or until it became clogged for each sample.

4.3.4.3 Biodiversity assessment

4.3.4.3.1 Spatial and temporal variation aspects

Spatial and temporal variation across Opuatia Wetland was examined using the 5 µm filter at sites SS1-SS4 at the beginning, middle, and end of spring sampling periods (Table 4.1). Sampling methodology followed the composite sampling strategy outlined above. In the middle of spring, the water level dropped entirely below ground level in the original sampling areas for SS2-SS4, necessitating minor shifts in sampling locations to where water was accessible as close as possible to each original site (SS2 was 12.8 m NNW of the original sampling area, SS3 was 13 m to the NW, and SS4 was 11.5 m to the WSW; see Table 4.1 for GPS coordinates).

4.3.4.4 Environmental DNA analysis

Environmental DNA followed the protocols outlined in Wilkinson (2023). In brief, lysates were obtained from the sampled filters and stored at -20 °C until DNA extraction. DNA extraction and purification used 200 µl of each sample lysate, with these processed using standard extraction parameters on the Genolution Nextractor NX-48S system. Subsequent steps involving DNA quality/quantity analysis, adapter-fusion, indexing, and amplification were conducted as part of a single-step quantitative PCR process using an Applied Biosystems QuantStudio 1 qPCR instrument. DNA extracts were amplified using eight fusion-tag mitochondrial and nuclear rRNA assays designed for detecting vertebrate, invertebrate, plant, microeukaryote, and microbial DNA. Sequencing libraries

for metabarcoding used these same primers and an Illumina iSeq 100 instrument (150 PE) (Wilkinson, 2023).

4.3.5 Data analyses

To first evaluate the consistency of eDNA replication, an Analysis of Variance was performed using the aov function in R v.2023.06.1+524 (R Core Team, 2023); this showed no significant differences between replicates for each experiment, thus replicates were combined as relevant for further analysis.

4.3.5.1 Technical experiments

To compare the agreement between conventional survey and eDNA results, these datasets were evaluated for overlaps in the presence or absence of species/genera detected. To examine the rate of DNA degradation, the residence time eDNA dataset was searched for the presence of kea (at either species *N. notabilis* or genus *Nestor* levels) at each sampling time point (pre- and post-excrement release) and transect distance. The entire eDNA dataset (excluding the residence time samples) was examined to understand the proportion of sequences that were obtained for each taxonomic rank, as well as the degree of missing data in the eDNA reference database. Nonmetric multidimensional scaling (nMDS) plots were generated to visualise differences among DNA sequences across filter sizes at the start, middle, and end of spring using the metaMDS function from the vegan v.2.6-4 package (Oksanen et al., 2022) in R v.2023.06.1+524 (R Core Team, 2023). Subsequently, a permutational multivariate analysis of variance (PERMANOVA) was performed using the adonis function from vegan to examine statistical support for differentiation among the various groupings. This dataset was also used to calculate the average volume processed for each filter size and temporal period to understand their filtering capacity. Finally, to understand how species richness changed with the number of replicates taken for each filter size, species accumulation curves were generated from the entire eDNA dataset using the specaccum function from vegan. To understand the effects of filter size more thoroughly, species richness was also plotted as the proportion of each taxonomic group against the total number of groups detected.

4.3.5.2 Biodiversity assessment

To understand how biodiversity changed across space and time at Opuatia Wetland, further nMDS analyses examining eDNA patterns across the various sampling and location time points for the entire eDNA dataset and at species and genus-level (as well as just for key wetland species of interest including birds, fish, and plants), were performed using nMDS plots and PERMANOVA. The 5 µm filter results at SS1 were used when comparing spatial variation. To understand how species richness changed with the number of sites sampled, species accumulation curves were generated from the entire eDNA dataset using the specaccum function in vegan. Species richness was also plotted as the proportion of each taxonomic group against the total number of groups detected to further understand the effects of spatial and temporal variation.

4.4 Results

4.4.1 Conventional surveys vs. eDNA

The zooplankton survey and eDNA results showed minimal overlap at the end of spring, except for *Acanthocyclops robustus* and *Synchaeta pectinata*, which were detected using both methods at SS2-SS4, and at SS2, respectively (Fig. A4.2; Table A4.2). However, several organisms were detected in the eDNA data at the genus level, but further identified to the species level using the conventional zooplankton survey methods (e.g., *Brachionus* sp. in the eDNA dataset was identified as *Brachionus angularis* or *Brachionus quadridentatus* by conventional survey; Fig. A4.2). Notably, the rotifer *Parencentrum plicatum* was identified in the zooplankton survey at the start of spring, representing a new record for Aotearoa New Zealand; however, it was not detected in the eDNA results (Fig. A4.2; Table A4.2). Conversely, eDNA sampling detected *Eubosmina coregoni* at SS2, SS3, and SS4 in the middle and/or end of spring – a potentially new record of a non-native species for the country; however, it was not detected in the zooplankton survey.

All fish species captured in the gee-minnow traps at the end of spring were also detected in the eDNA data (Fig. A4.3). However, additional fish species were detected using eDNA that were not

captured in the gee-minnow traps (e.g., brown bullhead catfish *Ameiurus nebulosus*, goldfish *Carassius auratus*, common bully *Gobiomorphus cotidianus*), while the traps caught invertebrates (water spider *Dolomedes aquaticus,* diving beetle *Rhantus suturalis*) that were not detected using eDNA (Fig. A4.3; Tables A4.3, A4.4, A4.5).

There was little overlap between the five-minute bird count and the eDNA results, with both methods detecting different species and only five instances at the end of spring where they each detected the same species at the same site (Fig. A4.4). Notably, *Botaurus poiciloptilus* (matuku/Australasian bittern) – a critically threatened native bird – responded to an audio playback of its birdsong at SS1, but was not detected in the eDNA data. Limited overlap was observed between the five-minute bird count and the eDNA results at the middle of spring (Fig. A4.4; Tables A4.6, A4.7).

Finally, the botanical survey and eDNA results showed little agreement. Out of the sixty-four plant species recorded, only three were identified with both methods: *Galium* sp. (bedstraw) and *Juncus* sp. (rushes) at SS2 and *Plantago* sp. (plantain) at SS3 (Fig. A4.5). Similar to the zooplankton results, most of the eDNA detections were to the genus level, while the botanical survey primarily resolved plants to the species level (Table A4.8). However, greater similarities were observed between methods when comparing at the genus level (e.g., *Azolla*, *Coprosma*, *Glyceria*, *Holcus*, *Iris*, *Juncus*, *Rubus*, and *Ulex* were identified with both methods). Interestingly, SS3 and SS4 are dominated by introduced *S. fragilis* (crack willow) and *S. cinerea* (grey willow), but neither of these species were detected at species level using eDNA. Instead, each was identified at the tribe level (*Saliceae*). Meanwhile, an unusual eDNA detection was *Fuscospora* sp. (beech tree) at SS3 and SS4.

4.4.2 Technical experiments

4.4.2.1 Control samples and taxonomic rank

All field controls detected DNA sequences, some of which were identified as species that are known to inhabit Opuatia Wetland. These controls were filtered on site; thus, a potential contaminant source may have been airborne DNA. However, the overall degree of potential contamination was low (0.6-2.8%). Over 5,300 (55.8%) sequences in the entire data set (excluding the residence time samples)

were unable to be identified to any taxonomic rank. Only 7.1% and 3.0% (970 total sequences) of all sequences could be classified to genus and species levels, respectively (Fig. 4.2).

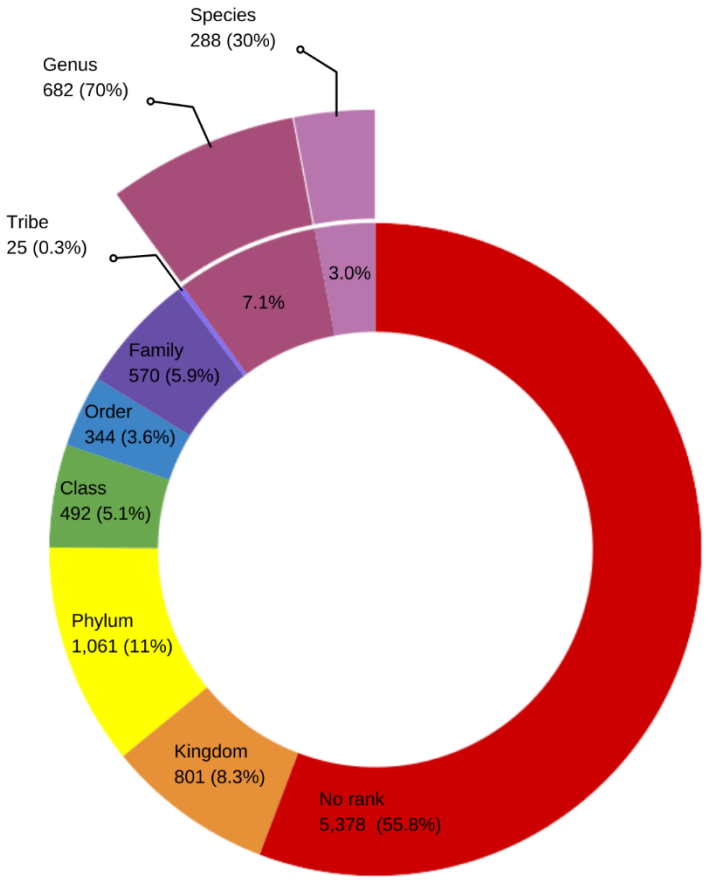


Figure 4.2 The proportion of sequences derived from all sites and filter sizes for each taxonomic rank for the entire dataset, excluding the residence time samples (inner ring), and the proportion of sequences exclusively obtained at the species and genus levels (outer ring).

4.4.2.2 Residence time experiments

Kea DNA was not detected in samples collected before release, suggesting it is unlikely that kea are present at Opuatia and that there was no cross-contamination. The total number of kea DNA counts from the three replicates taken from the faecal slurry (i.e., before release in the wetland) was 76,145, corresponding to 43,479 *N. notabilis* species-level counts and 32,666 counts at the *Nestor* genus level.

The strongest detection of kea DNA in the wetland post-release occurred 1 m from the release point (17,543 counts at species level), followed by 10 m (226 counts at species level) at 1 h and 5 h post-release, respectively. Thus, the species eDNA detection count at the 1 m, 1 hr point had more than halved compared to the level in the starting slurry, while the genus eDNA count had more than doubled (Table 4.3). Overall, we were able to detect kea DNA to the 10 m line for up to one week post-release. However, as time progressed, the taxonomic resolution became dominated by genus versus species level detections. Kea DNA was never detected at the 25 m transect (Table 4.3). The weather was sunny, partly cloudy, with moderate wind for two days prior to the experiment up to 48 h post-release, while occasional showers and low to moderate winds occurred on Days 3-5, and heavy rainfall occurred the night before the 1-week post-release date (Table A4.1). It rained on six of the remaining days before the 3-week experiment end date.

For the *in situ* residence time experiment, kea DNA was only detected at two days post-release, with an eDNA count of nine at the *Nestor* genus level and zero counts detected at the species level. In comparison to the dispersal distance residence time experiment, slight water flow was observed, and light showers occurred on six days over the course of the experiment.

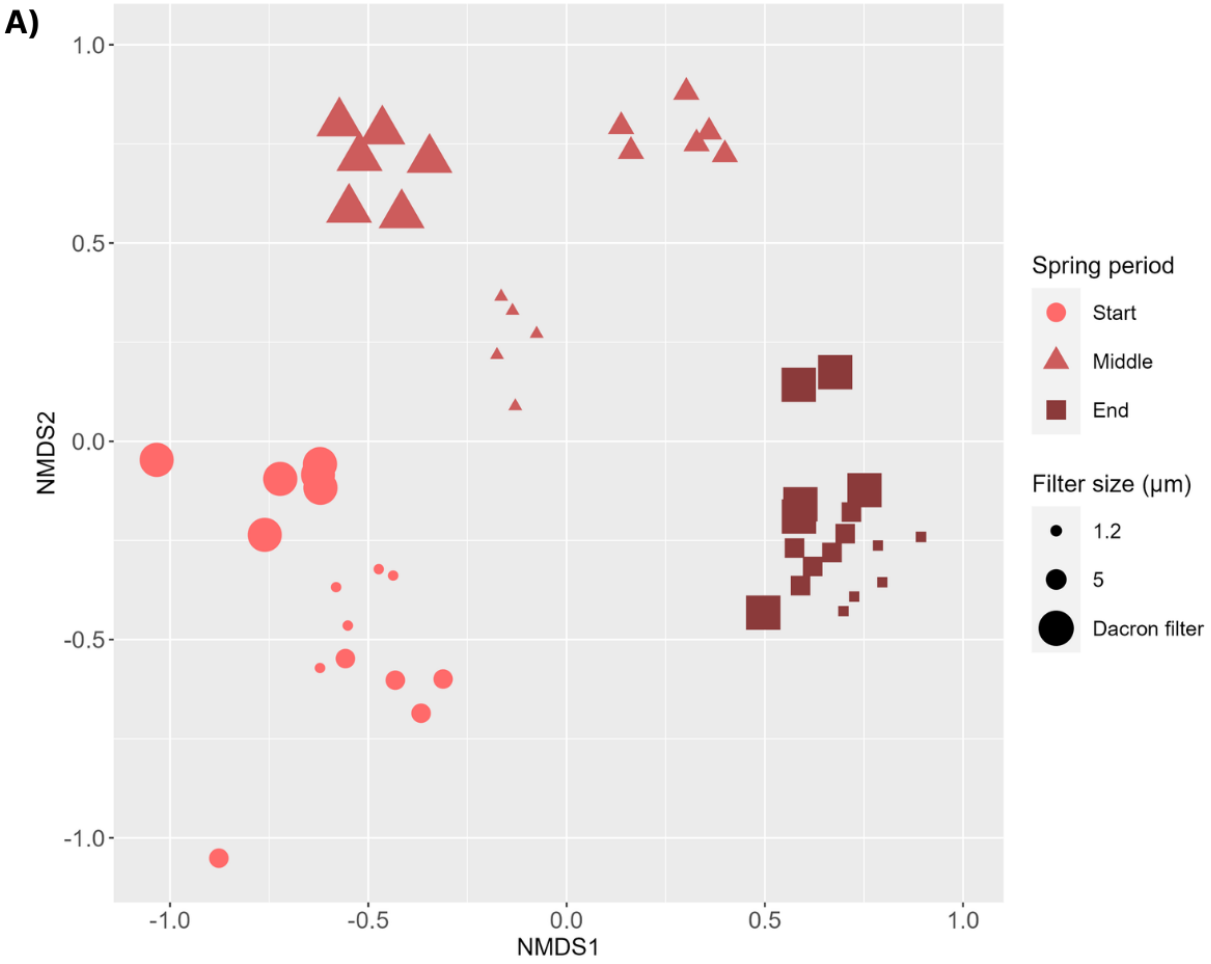
Table 4.3 The residence time experiment eDNA count, where the first value is the eDNA count at the species level and the subsequent value at the genus level; ND = not detected '-' not tested.

Distance from release point	**Time since release**						
	Pre-release	**1 h**	**5 h**	**24 h**	**48 h**	**1 wk**	**3 wk**
1 m	43,479; 32,666	17,543; 69,898	975; 31,738	233; 7,632	22; 4,037	0; 113	ND
10 m		0; 133	226; 8,556	0; 32	0; 78	62; 63	ND
25 m		-	-	ND	ND	ND	ND

4.4.2.3 Filter size experiment

nMDS analysis of the entire dataset revealed significant differences in DNA sequence composition among the 1.2 µm, 5 µm, and dacron filters, as well as differences related to the time of sampling (Fig.

4.3A). For example, eDNA results for the different filter sizes clustered separately at the start ($F_{2,13}$ = 4.757; p = 0.001; PERMANOVA; $F_{2,23}$ = 4.650; p = 0.021; PERMDISP), middle ($F_{2,14}$ = 4.525; p = 0.001; PERMANOVA; $F_{2,14}$ = 8.187; p = 0.008; PERMDISP), and end ($F_{2,14}$ = 2.570; p = 0.001; PERMANOVA; $F_{2,14}$ = 1.545; p = 0.248; PERMDISP) of spring. These patterns were driven by statistically significant pairwise comparisons between 1.2 µm vs. 5 µm filters at the end of spring, 1.2 µm vs. dacron filters at the start and middle of spring, and 5 µm vs. dacron filters at the start and middle of spring (Table A4.9). These patterns were consistent with nMDS analysis of DNA sequence composition at the species and genus levels, as well as for the focused analysis on key species of interest (Fig. A4.6; Table A4.9).



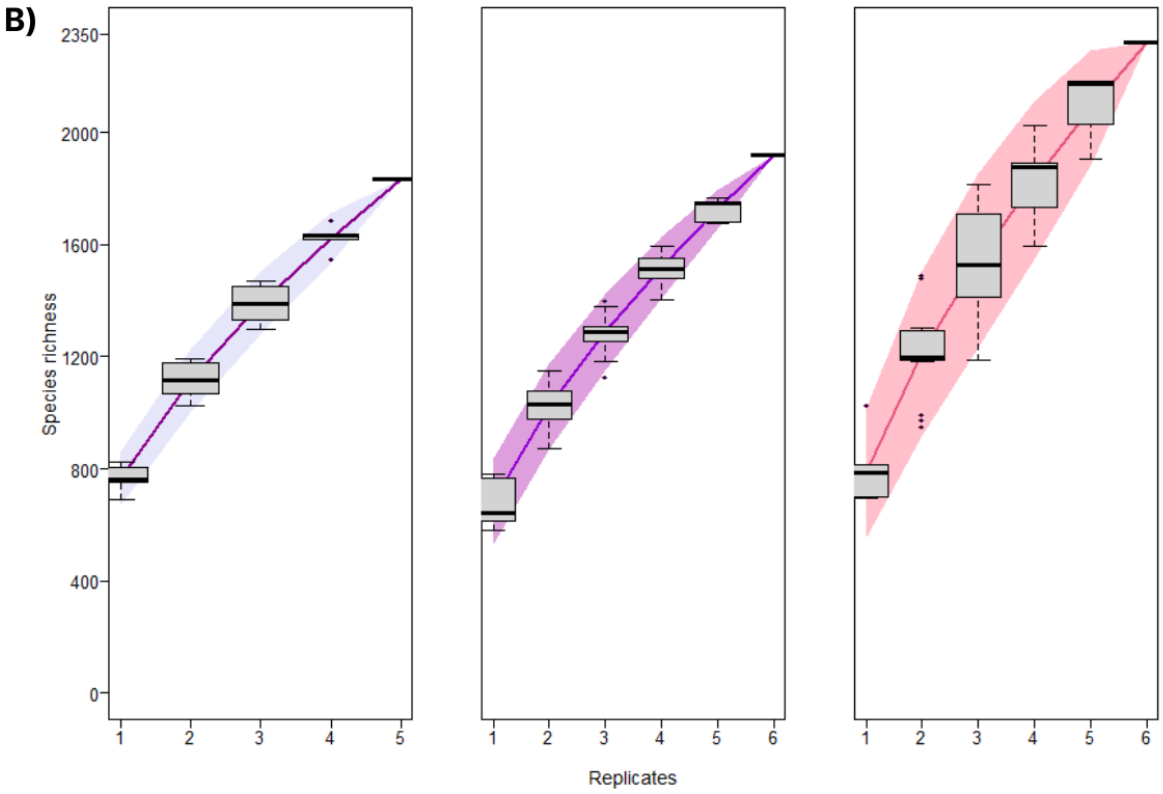


Figure 4.3 Filter comparison: **A)** nMDS plot comparing the difference among DNA sequences between the 1.2 µm, 5 µm, and dacron filters at the start, middle, and end of spring from the entire data set; and **B)** Species accumulation curves overlaid with boxplots of species richness at the end of spring for the 1.2 µm (left), 5 µm (middle), and dacron (right) filters. The solid line indicates the random sampling model of species accumulation provided from the data and the shaded area represents the 95% confidence interval.

The average volume filtered across the three filter sizes at all temporal points fell short of the standard volume (Table 4.4). For example, the dacron filter only processed a quarter of the standard volume. Overall, the dacron filter processed the highest average volume of water (27.8 L; middle of spring), followed by the 5 µm filter (960 mL; start of spring), and the 1.2 µm filter (267 mL; end of spring).

Table 4.4 The average volume filtered (mL) for each filter size at all temporal periods.

Filter size	Start	Middle	End
1.2 µm*	161	155	267
5 µm*	960	350	494
Dacron**	19,200	27,800	24,800

*Standard volume = 1,000 mL; ** Standard volume = 100,000 mL.

4.4.2.4 Species accumulation curves

Species accumulation curves from the entire data set at all three temporal points consistently showed that as the number of replicates increased, so did the species richness for all three filter sizes (Figs. 4.3B, A4.7). The dacron filter (which filters the greatest volume) obtained the highest mean species richness for all three temporal points, followed by the 5 µm filter and then the 1.2 µm filter, except at the end of spring, where the 1.2 µm filter obtained a slightly higher mean species richness (1,834) compared to the 5 µm filter (1,727) at five replicates. See Figure A4.8 for details of each taxonomic group as a proportion of the total number of groups detected for each filter.

4.4.3 Biodiversity assessments

4.4.3.1 Spatial variation

nMDS analysis of the entire dataset revealed differences in DNA sequence composition across the four sites (Fig. 4.4A), with statistically significant differences observed at the start ($F_{3,16}$ = 10.368; p = 0.001; PERMANOVA; $F_{3,16}$ = 3.401; p = 0.042; PERMDISP), middle ($F_{3,17}$ = 8.820; p = 0.001; PERMANOVA; $F_{3,17}$ = 14.471; p = 0.001; PERMDISP), and end ($F_{3,18}$ = 7.190; p = 0.001; PERMANOVA; $F_{3,18}$ = 1.957; p = 0.166; PERMDISP) of spring. Statistically significant pairwise comparisons were detected for SS2 vs. SS3 and SS2 vs. SS4 at the start and middle of spring, for SS1 vs. SS3 at the middle and end of spring, and for SS1 vs. SS4 at the middle of spring (Table A4.10). These patterns were consistent with nMDS analysis of DNA sequence composition at the species and genus levels, as well as for the focused analysis on key species of interest (Fig. A4.9; Table A4.10).

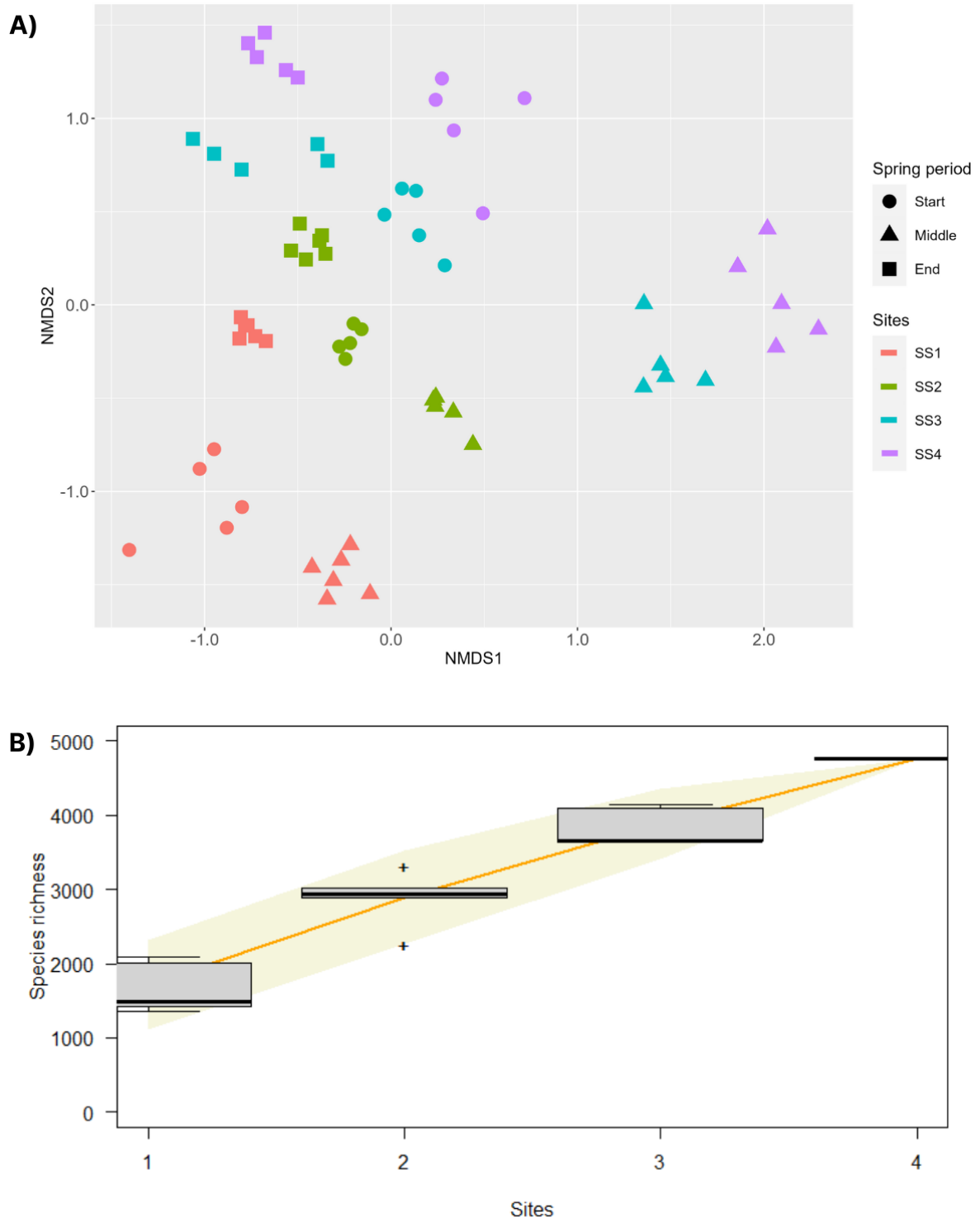


Figure 4.4 Spatial variation: **A)** nMDS plot comparing the difference among DNA sequences across SS1-SS4 at the start, middle, and end of spring from the entire data set; and **B)** Species accumulation curves overlaid with boxplots at the end of spring for the number of sampling sites. The solid line indicates the random sampling model of species accumulation provided from the data and the shaded area represents the 95% confidence interval.

Species accumulation curves from the entire dataset at all three temporal points consistently showed that as the number of sites increased, so did the species richness (Figs. 4.4B, A4.10). The highest mean species richness was recorded at the end of spring (4,756), followed by the middle (3,667) and the start of spring (3,398).

4.4.3.2 Temporal variation

Consistent with spatial results, nMDS analysis of the entire dataset revealed significant differences in DNA sequence composition between the three temporal spring sampling points at SS1 ($F_{2,14}$ = 11.620; p = 0.001; PERMANOVA; $F_{2,14}$ = 7.826; p = 0.004; PERMDISP), SS2 ($F_{2,13}$ = 13.887; p = 0.001; PERMANOVA; $F_{2,13}$ = 3.841; p = 0.051; PERMDISP), SS3 ($F_{2,12}$ = 11.141; p = 0.001; PERMANOVA; $F_{2,12}$ = 8.527; p = 0.008; PERMDISP), and SS4 ($F_{2,12}$ = 9.012; p = 0.001; PERMANOVA; $F_{2,12}$ = 7.829; p = 0.010; PERMDISP) (Fig. 4.4A). Statistically significant pairwise comparisons were detected for the start vs. middle of spring at SS3 and SS4, start vs. end of spring at SS1 and SS2, and middle vs. end of spring at SS1, SS3, and SS4 (Table A4.11). As for the previous comparisons, patterns were consistent with nMDS analysis of DNA sequence composition at the species and genus levels, as well as for the focused analysis on key species of interest (Fig. A4.9; Table A4.11). See Figure A4.11 for details of each taxonomic group as a proportion of the total number of groups detected for each temporal/spatial comparison.

4.5 Discussion

We tested different eDNA field techniques to understand how biodiversity signals vary spatially and temporally in Aotearoa New Zealand wetland environments. We found an overall pattern in which conventional survey methods showed key differences to the eDNA results, elucidated the residence time of DNA at Opuatia Wetland, and identified significant differences between filtering methods, spatial sites, and temporal sampling points.

Understanding the persistence and transport of eDNA signals is important because it indicates the relative age of the deposited DNA (Jo, 2023) and its spatial representativeness (Civade et al., 2016), ultimately assisting the interpretation of eDNA data. Our experiments showed that foreign DNA was

detectable for up to one week following release within a 10 m radius from the source, and for only two days post-release in our *in situ* experiment. The absence of the foreign DNA at the 25 m point in our dispersal distance experiment may have been due to the presence of vegetation between the 10 and 25 m transect lines and/or limited water flow. However, the vegetation did not completely obstruct the flow path, particularly during flooding. Our findings were consistent with the literature, where eDNA concentrations have been shown to decrease with increasing distance from the source point and increasing time (Harrison et al., 2019; Goldberg et al., 2016). In wetland environments, DNA persistence is likely to be significantly impacted by abiotic factors (e.g., pH <5, water temperature >25 °C, limited dispersion; Goldberg et al., 2018). However, different environments have varying intensities of physicochemical parameters and hydrological processes, which may be attributed to varying eDNA decay rates (Jo & Minamoto, 2021; Seymour et al., 2018). For example, Ely et al. (2021) could no longer detect *in situ* foreign DNA after 7.5 hrs in a nearshore rocky reef habitat, while a caged fish experiment in a marine environment showed detection of target DNA up to 1 km from the cage, but recovered no signal just 2 hrs after the cage was removed (Murakami et al., 2019). Meanwhile, in riverine environments, target eDNA could be detected at distances from 9 km (Deiner & Altermatt, 2014) to 100 km (Pont et al., 2018) downstream from the release point.

The type of filter used when collecting eDNA dictates the size of particles that can permeate through, ultimately affecting DNA detection sensitivity (Schabacker et al., 2020). For example, in a stream environment, 5 µm filters have been shown to process larger volumes of water and return higher DNA concentrations than 1.2 µm filters (Banks et al., 2021). In turbid environments – where filter clogging is more apparent – a 20 µm filter has been shown to be effective in capturing eDNA (Cooper et al., 2022; Cooper et al., 2021; Robson et al., 2016). Consistent with this, we found that the dacron filter exhibited the highest mean species richness, followed by the smaller 5 µm and 1.2 µm filters. Thus, unsurprisingly, filtering larger volumes of water using larger filter sizes can increase the mean species richness detected in wetlands, as has previously been shown in river and marine environments (e.g., Banks et al., 2021; Jensen et al., 2022; Jeunen et al., 2022; Macher et al., 2021;

Smith et al., 2023). However, from a taxon-specific perspective, opting for a smaller filter size may be sufficient in some cases while also requiring shorter filtration times. For example, for fish, the 1.2 µm filter yielded a similar proportion of species richness compared to the dacron filter in our study. The number of collected sample replicates is also important in wetland sampling, with species accumulation curves in our study indicating that 5-6 replicates are required to capture the highest biodiversity, as recommended in eDNA sampling guidelines for streams, rivers, and lakes (De Brauwer et al., 2022; Smith et al., 2023).

Understanding of temporal and spatial heterogeneity of eDNA signals is fundamental for providing new insights into shifts in biodiversity signals and defining when and where to sample to attain a comprehensive evaluation of overall biodiversity. Here, we found statistically significant spatiotemporal variation in DNA sequence composition. Despite SS2-SS4 all being classified as marsh wetlands situated within a 1 km proximity at the same wetland site, significant pairwise differences in biodiversity (i.e., DNA sequence composition) were observed among these sites. Less surprising, SS1 – a swamp – exhibited significant differences in biodiversity to the marsh sites (except for SS2, which might be due to their relatively closer proximity), highlighting the value of spatial sampling especially for wetlands where the wetland type varies within the overall site. Other research has also found distinct geographic patterns in species distribution (for fishes, amphibians, and mammals) when sampling a transect from freshwater to marine in coastal wetlands (Saenz-Agudelo et al., 2021) and differences in biodiversity patterns between coastal and temporary inland wetlands (Coleman et al., 2023), while riverine and lake environments also show variation in eDNA samples from different spatial sites (Civade et al., 2016; Hänfling et al., 2016). Here, as the number of sampling sites increased, so did species richness, consistent with previous studies conducted in pond and riverine environments (Evans et al., 2017; Bylemans et al., 2018; Macher et al., 2021). This suggests that sampling at one site within a wetland is unlikely to yield a representative result, underscoring the importance of accounting for even small-scale (~1 km) spatial sensitivity.

Temporal variation was also evident in our study, with the end of spring yielding the highest mean species richness. Compared to spatial variation, relatively few studies have investigated temporal shifts in eDNA, with the research that has been undertaken typically investigating short time scales (i.e., within a year; Mathieu et al., 2020). However, Gabrielsen et al. (2022), used a combination of eDNA and remotely sensed imagery over three years to show that habitat variability at both spatial and temporal scales played a pivotal role in shaping the occurrence and abundance of three amphibian species. Meanwhile, hourly collections over a 32 h period in a marine environment revealed short-term temporal variation in fish and eukaryote richness, with species richness highest during dawn (Jensen et al., 2022). Temporal fluctuations in abundance of scalloped hammerhead (*Sphyrna lewini*) and tiger shark (*Galeocerdo cuvier*) over a 13-week sampling period during summer have also been recorded (Mariani et al., 2021).

To gain insight and validate best practice approaches, eDNA is often compared to conventional biomonitoring methods, where a key finding has been that eDNA should ideally complement rather than replace such methods (Schenekar, 2022). Our findings support this perspective, particularly in the case of the zooplankton surveys, five-minute bird counts, and botanical surveys, where certain species were only detected by one method or the other. For example, previous floral records for Opuatia Wetland (Barnes et al., 2001; Reeves, 2011) showed minimal overlap with the eDNA results observed here for many genera and most species. Meanwhile, greater overlap was observed for bird and fish species, with 8/11 bird species and 10/15 fish species detected in the eDNA versus conventional methods used here (Reeves, 2011; https://nzffdms.niwa.co.nz/). These patterns are consistent with other research, where eDNA has detected more zooplankton species than morphological-based methods in marine and lake environments (Suter et al., 2021; Qiu et al., 2022). Similarly, Mejia et al. (2020) observed low concordance between a botanist survey and eDNA results at Mojave Desert Springs. Conversely, Coleman et al. (2023) observed a similar resolution between conventional methods and eDNA for fish and frog species from wetland habitats. Meanwhile, eDNA has generally been shown to detect all species recorded through conventional fish monitoring (David

et al., 2021; Griffiths et al., 2020; Wang et al., 2021). Consistent with this, our eDNA samples detected all the fish species that were caught in the gee-minnow traps. However, they also detected additional fish species (and insects) that were not trapped. Notably, gee-minnow traps are small and are designed to target small-bodied species, such as rare mudfish (Lake, 2013); using additional traps, such as fykes nets, would likely have caught at least some of the other species detected in the eDNA dataset.

A common theme observed when comparing conventional and eDNA methods here was that conventional methods more often involved taxonomic identifications to the species level. This highlights the dependence of eDNA on reference databases (e.g., the National Centre for Biotechnology Information – NCBI GenBank; and the Barcode of Life Data System – BOLD) for matching unknown genetic sequences, that suffer from a significant amount of missing data (Hotaling et al., 2021). Indeed, more than a quarter of the 3,000 most encountered sequences across eight metabarcoding assays could not be appointed to a phylum in a recent study (Wilkinson et al., 2023), and >50% of sequences were unassigned to any taxonomic rank here. This emphasises the importance of current plans to develop a reference database for biodiversity in Aotearoa New Zealand (https://www.landcareresearch.co.nz/events/national-dna-database-webinar-series/), as well as global sequencing initiatives (e.g., Vertebrate Genome Project; https://vertebrategenomesproject.org/; Bird10K; https://b10k.genomics.cn/index.html; i5K Project; https://i5k.github.io/; Earth BioGenome Project; https://www.earthbiogenome.org/) that may help to address these data gaps.

Notably absent from our eDNA data were the rotifer *P. plicatum*, matuku (Australasian bittern *Botaurus poiciloptilus*), crack willow, and grey willow. Conversely, eDNA detected *E. coregonia* – a non-native water flea potentially new to Aotearoa New Zealand – and *Fuscospora* sp. (beech tree). The absence of *P. plicatum* and matuku in the eDNA detections can be attributed to the current unavailability of their reference sequences in the eDNA reference database – it is possible that eDNA sequences for these species are present in our results but could not be identified at the species level.

The absence of crack and grey willow at the species level is due to current eDNA assays being unable to distinguish *Salix* sp. to species level, and the sharing of identical barcodes between these and other *Salix* and *Populus* species. Meanwhile, the *E. coregoni* detection may actually represent *Eubosmina meridionalis* – a native species for which *E. coregoni* is the closest database match, while the *Fuscospora* sp. detection may represent pollen that travelled into the wetland from a local garden. These detection failures highlight the strengths and limitations associated with eDNA, particularly its variation in effectiveness across taxa that are more or less likely to be detected in a water sample. When used in conjunction, eDNA and conventional methods thus likely offer a more comprehensive view of the overall ecosystem biodiversity.

Nevertheless, eDNA methods are continuing to improve, with development of new assays, decreasing costs of sequencing, and regular additions to the reference database together enhancing their potential for species detection and identification. Indeed, eDNA has revolutionised the way we monitor biodiversity, and significant recent efforts dedicated to protocol development and optimisation (Schenekar, 2022), have enhanced eDNA efficiency and use (Gleeson, 2021). Here, we explored various techniques to optimise eDNA use in wetland environments and demonstrated the complexities associated with turbid environments, while identifying key parameters that should be considered during experimental design. Outstanding questions include whether eDNA can detect macroorganisms in other challenging wetland environments (e.g., high temperature geothermal wetlands and low pH bogs), and whether quantitative measures of ecosystem quality and health (e.g., the taxon-independent community index (TICI); Wilkinson et al., 2023) can be extended into wetlands and other environments.

4.6 Literature cited

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Chapter 5.

Double poutama tukutuku pattern:
The stairway to knowledge. Symbolises whakapapa, collaboration, and levels of learning, continuously striving upward for the better.
Used portion of tuna (short-finned eel) DNA sequence.

Thesis discussion

5.1 General thesis summary

My thesis aimed to demonstrate the utility of environmental DNA (eDNA) as a biomonitoring tool for tracking spatial and temporal patterns in wetland environments and addressing key challenges to inform eDNA methodology. **Chapter 2** shed light on the ecological and cultural consequences of raupatu (confiscation), emphasising the overarching importance of protecting the mauri (life force, spiritual energy) and hauora (health and wellness) of Aotearoa New Zealand's repo (wetlands) for future generations. In **Chapter 3**, I found spatial variation in DNA sequence composition across broad scales and showed that exotic species dominate wetlands across the motu (country). This finding highlighted the significance of eDNA as a tool for establishing long-term spatial data for exotic and non-exotic species to support conservation and restoration initiatives. Finally, in **Chapter 4,** I identified spatial and temporal variation in eDNA patterns across fine scales at Opuatia Wetland and investigated key parameters to consider during eDNA experimental design and data interpretation in wetland environments. In the current chapter, I conclude my thesis by identifying gaps and limitations and presenting recommendations for further development and utilisation of eDNA as a biomonitoring tool.

5.2 Recommendations and future work

5.2.1 Cultural considerations

5.2.1.1 Pairing mātauranga Māori and science

The interface of Indigenous knowledge and science has captured significant interest, particularly in Aotearoa New Zealand. In 2007, the Aotearoa New Zealand government introduced Vision Mātauranga – a science policy framework – designed "to unlock the innovation potential of Māori knowledge, resources and people to assist New Zealanders to create a better future" (Ministry of Research Science and Technology, 2007, p. 2). This policy sets an expectation and obligation for researchers and educators to engage with mātauranga Māori (Māori knowledge) in their practice (Mercier & Jackson, 2019). Since its inception, the pairing of mātauranga Māori and science has

flourished – from a televised series *Project Mātauranga*, which showcased mātauranga Māori working alongside science (Mercier et al., 2014), to its inclusion in secondary school science curriculum from 2024 onwards (Mercier & Jackson 2023).

This interface has also manifested in research. For example, Hudson et al. (2020) showcased how different iwi (tribes) across the motu utilised the Takiwa Geospatial Platform – an application enabling users to interact and analyse data – to organise and visualise scientific and mātauranga data associated with freshwater-decision making. A key step in the data organisation process was the alignment of mātauranga that made sense in the context of scientific data and was underpinned by iwi values. This holistic approach brought together two knowledge baskets within a contextualised and comprehensible framework with the data appropriately managed by Māori. A second example is the united response of researchers and iwi partners to the substantial decline of kuku or kūtai (*Perna canaliculus*, green-lipped mussel) populations in Ōhiwa Harbour (Eastern Bay of Plenty, Aotearoa New Zealand) due to anthropogenic pressures. In this project, taura kuku (mussel spat lines made from traditional Māori materials) emerged as a sustainable solution for seafloor regeneration and biodiversity enhancement while mitigating plastic pollution compared to commercial lines (Paul-Burke et al., 2022). Centralising mātauranga Māori in this research yielded new insights to enhance marine biodiversity and management while also fostering greater local acceptance of the methods being implemented.

Chapter 2 shared intergenerational knowledge of Opuatia Wetland, providing context and significance of the research performed in **Chapter 4**. It serves as an historical record for iwi, hapū (subtribe), and whānau (family) and has been immortalised within this thesis. Indigenous knowledge contributes to a complex matrix, informing the research, setting the scene, and shaping a rounded body of work. While I recommend pairing these two knowledge systems for future endeavours, a thoughtful approach is advised. Science bears some extent of responsibility for the dispossession and marginalisation of Māori on their own whenua (land) and has led to the degradation and destruction of hauanga kai (food gathering sites) (e.g., as discussed in **Chapter 2**). Consequently, science is

perceived as an “agent of colonialism” from the perspective of some Māori (Mercier et al., 2014, p. 71), who may also be sceptical of non-Māori motives (McAllister, 2022) – especially when data has been unethically used in the past (e.g., to perpetuate the harmful stereotype that Māori possess a warrior gene; Perbal, 2013) or when the value of mātauranga has been undermined (e.g., an opinion piece authored by Auckland University academics that mātauranga Māori is not science; Clements et al., 2021). Another challenge is the limited number of iwi kaimahi (iwi workers) who are stretched too thin, creating uncertainty about whom researchers should contact about the possibility of uniting mātauranga Māori and science. To address these issues, better resourcing is necessary, particularly at the start of the research project, where typically a series of wānanga (discussion/meetings) are needed to whakawhanaungatanga (establish relationships) and plan and develop the project scope. Moreover, including Māori at the beginning of the research allows for co-design of the project, more equitable outcomes, and the empowerment of a more meaningful relationship.

5.2.1.2 Data sovereignty

Recent advances in genomic analysis and the decreasing cost of data generation have expanded the capability of molecular ecologists to sequence various organisms (Hudson et al., 2020). This expansion has enabled new opportunities to respond to the biodiversity crisis, inform public health, and monitor ecosystem status and change, and have prompted the ‘open science’ movement – advocating for transparency in field and laboratory methods, DNA sequence data generation, and bioinformatic workflows in order to enhance research translation and reusability, alongside societal benefits (Hudson et al., 2020; Liggins et al., 2022).

While an open science perspective generally aligns with the interests of the research community (e.g., by enhancing collaboration and extending the scope and context of research), it can impinge on the rights of Indigenous Peoples through exclusion, a lack of informed consent and consultation, and/or the misuse of samples and data (Hudson et al., 2020; Liggins et al., 2022; Mc Cartney et al., 2022), and Māori have not been immune to these inequities. For example, publicly available repositories that hold genetic or genomic data of taonga (things that are treasured by Māori)

species – which may include native and endemic species, as well as non-native species that Māori have a connection with – generally do not uphold Māori data sovereignty (Collier-Robinson et al., 2019; and discussed below), despite the fact that Māori are guaranteed te tino rangatiratanga (the right to exercise authority) over their taonga – which directly includes DNA from both people and environments – under Article Two of Te Tiriti o Waitangi (The Treaty of Waitangi; signed 1840; https://waitangitribunal.govt.nz/ treaty-of-waitangi/te-reo-maori-version/).

A current kōrero (conversation) in the eDNA field revolves around the question of data governance and sovereignty, particularly concerning the sequencing of taonga species (e.g., Whāki Webinar – e-DNA Exploring Māori Data Governance and Sovereignty; https://www.waikato.ac.nz/ rangahau/ano-te-pai-thought-leadership/webinars/whaki-webinar-series), and how sequences obtained from environmental samples should be better governed (Bunce & Freeth, 2022). Encouragingly, ongoing efforts to create a national DNA reference library for Aotearoa New Zealand's biodiversity underpinned by Te Tiriti o Waitangi are underway (https://www.landcareresearch.co.nz/ events/national-dna-database-webinar-series/), with several wānanga with various experts and interested parties having taken place throughout 2023. In addition, guidelines (e.g., Te Nohonga Kaitiaki; Hudson et al., 2021) and initiatives (e.g., BioCultural and Traditional Knowledge Notices; https://localcontexts.org/labels/biocultural-labels/) are frequently becoming available that enable the assignment of appropriate cultural licences to DNA data to recognise its Indigenous ownership, rights, and interests. Another emergent mechanism to uphold appropriate data sovereignty is that the submitter of the DNA holds ownership of the data rather than the database itself (this condition was recently revised in the terms and conditions of Wilderlab – a commercial provider of eDNA services based in Wellington, New Zealand; see 5.1: https://www.wilderlab.co.nz/terms).

While I recommend the ongoing application of eDNA methods to support the resilience of biodiversity and understand and monitor ecosystem dynamics – and **Chapter 3** demonstrated the value of public eDNA data (especially that collected by citizen scientists) in providing broader insights that can surpass the objectives of a single study when data is repurposed – an important caveat is that

future research acknowledges and incorporates indigenous perspectives, fostering a revitalised form of communication between researchers and Indigenous communities. This is particularly true for methods like eDNA, where citizen scientists can collect and deposit data to public databases without necessarily being aware of issues like data sovereignty.

5.2.2 eDNA methodologies

5.2.2.1 Expanding the biomonitoring toolbox

Molecular-based techniques are increasingly advocated for biomonitoring applications (Bowers et al., 2021; Wood et al., 2013; Zaiko et al., 2018). While the primary collection of eDNA is currently from water, recent developments have expanded its scope to the detection of airborne DNA (also known as airDNA), invertebrate-derived DNA (iDNA), and other substrates including soil, plant surfaces, scat, and the walls of mammalian traps. However, much of this work is still at the proof of concept stage – for example, demonstrating that retrieval of animal DNA from the air using an electric pump to vacuum air through a filter that collects the DNA is possible (Clare et al., 2021). AirDNA could ultimately prove particularly useful in challenging environments (e.g., caves) that are difficult to access and where visual surveys can be limited. iDNA uses invertebrates like leeches, mosquitoes, sand flies, and carrion flies, which feed on other species either directly through flesh and blood, or indirectly through their scat, and also interact with them through touch (e.g., pollination). Extracting iDNA therefore allows for the extraction of DNA from the species the invertebrates have been interacting with; so far, it has been shown to be able to track mammal populations and detect taxa differences across fine scales (Fernandes et al., 2023), as well as detect invasive and threatened species (Cutajar & Pulsfor, 2023). These techniques are an exciting area of active research in the field and collectively hold promise for expanding the biomonitoring toolbox further and capturing a broader view of ecosystem diversity than is currently captured by water-based eDNA methods.

Another exciting emergent area of research involves a proactive strategy for rapidly collecting data via the implementation of a routine eDNA sampling system across a national grid of collection networks. For example, current work is exploring the use of solar-powered UV light traps for collecting

insect incursions at Aotearoa New Zealand's major ports (https://www.scionresearch.com/about-us/about-scion/corporate-publications/scion-connections/past-issues-list/scion-connections-issue-40,-june-2021/trialling-insect-traps-at-the-port-of-tauranga). Wider deployments of this, and similar networks could encompass other key locations, such as well-known monitoring sites (e.g., along rivers, lakes, terrestrial ecological corridors), and biosecurity checkpoints at airports. In future, such approaches will lead to more rapid reporting of invasive species, more prompt detection of ecosystem changes, and improved measures of ecosystem biodiversity.

A new system currently being considered in Aotearoa New Zealand is the Biodiversity credit system – a legal financing mechanism designed to fund activities dedicated to safeguarding or enhancing indigenous biodiversity sourced from private investors, with the credits recognising positive outcomes resulting from conservation efforts (Ministry for the Environment, 2023). While this initiative sounds interesting, it is unclear how the biodiversity outcomes it relies on will be measured. Thus, I recommend investing in the optimisation of innovative eDNA techniques (i.e., eDNA from non-water substrates and automation of eDNA tools and technologies) to enrich the country's biomonitoring toolbox and extend its detection capabilities across diverse ecosystems.

5.2.2.2 Outstanding technological considerations

Chapter 4 deepened our insight into the technical components to consider for using eDNA as a biomonitoring tool in wetland environments, however it also raised questions for future work. First, I found that species richness increased with both the number of eDNA sample sites (four were sampled in **Chapter 4**) and the number of replicates taken per site (I took a maximum of six replicate samples). However, the species accumulation curves in my study did not reach a plateau – indicating that further sampling would return higher species richness. Thus, the optimum number of replicates or sampling sites required to yield representative results for the biodiversity of wetland sites remains unknown and should be specifically tested in future. Second, the *in situ* and dispersal distance residence time experiments in **Chapter 4** revealed distinct DNA degradation rates (in the *in situ* experiment, DNA was detectable two days post-release, whereas in the dispersal distance residence time experiment, DNA

persisted for up to one week following release within a 10 m radius). The disparity between these two experiments highlights the impact of environmental factors, such as weather and water flow, on eDNA persistence and future work should combine these two factors (i.e. *in situ* and dispersal distances) into a single larger experiment to investigate this issue further. This experiment could also be extended to other wetland types, such as high temperature geothermal wetlands and low pH bogs, to provide a firmer understanding of DNA degradation rates in different wetland conditions. Third, while **Chapter 4** addressed eDNA methodology from a collective biodiversity standpoint, another avenue worth exploring is taxon-specific biodiversity dynamics. For example, I observed minimal differences in the proportion of species detected between filter sizes for fish; generating species accumulation curves for fish at each filter size would reveal the most efficient filter for that taxa and this could be done for all taxonomic groups of interest.

Collectively, these experiments will provide future researchers with even greater insight into the best ways to sample wetland biodiversity, though as noted in **Chapter 4**, eDNA biomonitoring provides a complementary dataset to that provided by conventional biomonitoring methods and we should not be looking to exclude conventional methods from the biomonitoring toolbox any time soon.

5.3 Literature cited

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Appendix A2

Supplementary Information: Chapter 2

Appendix A2.1 - Interview questions

Interview questions asked of interviewees:

- Exploring the past:
    - What did the wetland look like?
    - What species were present?
    - How did iwi/hapū/whānau interact with the wetland? How was it used recreationally?
    - What hauanga kai was collected?
- Describing the present situation:
    - What does the wetland look like now?
    - What are the taonga species?
    - How do iwi/hapū/whānau interact with the wetland? How is it used recreationally?
    - Is the wetland still a source of hauanga kai?
- Identifying future aspirations:
    - What do you wish Opuatia Wetland to look like for iwi/hapū/whānau in 50 years time?
    - Which species do you hope will be present?

Appendix A2.2 - Information sheet for participants (Group)

INFORMATION SHEET FOR PARTICIPANTS

Spatial and temporal variability of eDNA in the context of Aotearoa, New Zealand wetlands

Tēnā koe

You are invited to take part in this research. Please read this information before deciding whether or not to take part. If you decide to participate, thank you. If you decide not to participate, thank you for considering this request.

Ko wai ahau / Who am I?

Ko Pirongia te maunga

Ko Waikato te awa

Ko Tainui te waka

Ko Ngāti Maniapoto me Ngāruahine ōku iwi

Ko Starsha Bird tōku ingoa

I am a Masters student at Te Whare Wānanga o Waikato – The University of Waikato. This interview is part of my thesis research.

He aha te whāinga mō tēnei rangahau / What is the aim of the project?

My thesis research aims to use environmental DNA (eDNA) to explore the spatial and temporal variability of biodiversity patterns in wetlands. A component of my research that I wish to explore is the past, present, and future of Opuatia Wetland from environmental, cultural, and recreational perspectives. I am particularly interested in identifying taonga (treasured) species of Opuatia Wetland. I will compare the obtained data to the eDNA samples I collected in spring 2022, e.g. to see if any taonga species were detected in the biodiversity data. This information will help to indicate the health and well-being of Opuatia Wetland and the surrounding area.

Ka pēhea tō āwhina mai / How can you help?

The semi-structured interview will take 1-2 hours to complete, depending on your answers. The interview will loosely follow the "Ake Ake Model" described in Taura et al. (2017) handbook: Te Reo o Te Repo – The Voice of the Wetland. You will be asked a series of questions and are invited to sketch/draw three time points about Opuatia Wetland:

- Exploring the past
- Identifying the present situation
- Discussing what you wish Opuatia Wetland to look like for iwi/hapū/whānau in 50 years' time

Your participation in this interview is entirely voluntary and you may choose to cease participation without consequence. You have the right to ask any question about the study (before, during, and after). You have the right not to answer any question or do any activity you do not feel comfortable with. You can withdraw from the study at any time, during, and up to 14 days after the data collection without reason, penalty, or repercussion. You can also withdraw all data contributed up until that point. However, once your information from the interview has been integrated (14 days after completion), you will not be able to withdraw your results, though you may cease active participation. If you choose to withdraw, it may not be possible to remove all data as the interview will be conducted in a group setting. You will receive a transcript of your interview that you can review and edit for accuracy. I will follow up with you regarding edits to this transcript within 14 days of its delivery to you.

Ka ahatia ngā kōrero ka tukuna mai / What will happen to the information you give?

The data gathered from the interview will be used to inform the history of Opuatia Wetland and evaluate the ability of eDNA to detect taonga species in wetland environments. This research will form part of my Master's thesis manuscript, a journal article, and conference presentations.

Data will be stored on my private computer and mobile phone, which are both password-protected. You own the data you provide, and I will own my interpretation and analysis of the data. All data will be kept for a minimum of five years after collection before being destroyed, as national laws require this.

Matatapu / Confidentiality

Your privacy and confidentiality are of utmost importance to me. As part of my research project, I would like to inform you that your name will be used in the outputs to acknowledge your valuable contribution. However, I want to assure you that no other personal details will be disclosed, such as contact information, age, or specific identifying information. If you have any concerns or questions regarding this matter, please do not hesitate to contact me.

Mehemea ngā pātai, he raruraru rānei, me whakapā ki a wai / If you have any questions or problems, who can you contact?

If you have any questions, either now or in the future, please feel free to contact:

Student: Starsha Bird

Email: starshabird@gmail.com

Supervisor: Dr Ang McGaughran

Email: angela.mcgaughran@waikato.ac.nz

This research project has been approved by the Human Research Ethics Committee of the University of Waikato under HREC(HECS)2023#34. For any ethical questions or concerns that cannot be resolved in discussion with myself or Dr McGaughran, you can contact the Chair of the Ethics Committee:

Email: hecs-ethics@waikato.ac.nz

Postal address: University of Waikato, Te Whare Wananga o Waikato

Private Bag 3105

Hamilton 3240

Reference

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Appendix A2.3 - Information sheet for participant (Individual)

INFORMATION SHEET FOR PARTICIPANTS

Spatial and temporal variability of eDNA in the context of Aotearoa, New Zealand wetlands

Tēnā koe

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Ko wai ahau / Who am I?

Ko Pirongia te maunga

Ko Waikato te awa

Ko Tainui te waka

Ko Ngāti Maniapoto me Ngāruahine ōku iwi

Ko Starsha Bird tōku ingoa

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- Exploring the past
- Identifying the present situation
- Discussing what you wish Opuatia Wetland to look like for iwi/hapū/whānau in 50 years' time

Your participation in this interview is entirely voluntary and you may choose to cease participation without consequence. You have the right to ask any question about the study (before, during, and after). You have the right not to answer any question or do any activity you do not feel comfortable with. You can withdraw from the study at any time, during, and up to 14 days after the data collection without reason, penalty, or repercussion. You can also withdraw all data contributed up until that point. However, once your information from the interview has been integrated (14 days after completion), you will not be able to withdraw your results, though you may cease active participation. You will receive a transcript of your interview that you can review and edit for accuracy. I will follow up with you regarding edits to this transcript within 14 days of its delivery to you.

Ka ahatia ngā kōrero ka tukuna mai / What will happen to the information you give?

The data gathered from the interview will be used to inform the history of Opuatia Wetland and evaluate the ability of eDNA to detect taonga species in wetland environments. This research will form part of my Master's thesis manuscript, a journal article, and conference presentations.

Data will be stored on my private computer and mobile phone, which are both password-protected. You own the data you provide, and I will own my interpretation and analysis of the data. All data will be kept for a minimum of five years after collection before being destroyed, as national laws require this.

Matatapu / Confidentiality

Your privacy and confidentiality are of utmost importance to me. As part of my research project, I would like to inform you that your name will be used in the outputs to acknowledge your valuable contribution. However, I want to assure you that no other personal details will be disclosed, such as contact information, age, or specific identifying information. If you have any concerns or questions regarding this matter, please do not hesitate to contact me.

Mehemea ngā pātai, he raruraru rānei, me whakapā ki a wai / If you have any questions or problems, who can you contact?

If you have any questions, either now or in the future, please feel free to contact:

Student: Starsha Bird

Email: starshabird@gmail.com

Supervisor: Dr Ang McGaughran

Email: angela.mcgaughran@waikato.ac.nz

This research project has been approved by the Human Research Ethics Committee of the University of Waikato under HREC(HECS)2023#34. For any ethical questions or concerns that cannot be resolved in discussion with myself or Dr McGaughran, you can contact the Chair of the Ethics Committee:

Email: hecs-ethics@waikato.ac.nz

Postal address: University of Waikato, Te Whare Wananga o Waikato

Private Bag 3105

Hamilton 3240

Reference

Taura, Y., Dixon, L., & Turner, M. (2017). The ake ake model – forever and ever. In Y. Taura., C. Van Schravendijk-Goodman & B. Clarkson (Eds.), *Te reo o te repo – The voice of the wetland* (Vol 1., pp. 23-39). Manaaki Whenua – Landcare Research.

Appendix 2.4 - Consent form for all participants

CONSENT TO INTERVIEW

☐ I have read and understood the participant information, and any questions I had have been answered to my satisfaction. I understand that I can ask further questions about the study at any time.

☐ I am participating of my own free will and have not been coerced in any way.

☐ I consent to information or opinions that I have given being attributed to me in any research outputs.

☐ I consent to the interview being voice-recorded.

I understand that:

☐ I have the right not to answer any question or perform any activity I do not feel comfortable with.

☐ I am free to withdraw from the study at any time up to 14 days after completing my interview without penalty or repercussion, and all data connected to me will also be withdrawn, unless the interview was performed in a group setting, it may not be possible to remove all data.

☐ The transcript will be confidential and will not be shared with external parties. I cannot share the transcript with external parties.

☐ All personal data will be destroyed at the conclusion of the study, but de-identified study data will be retained for 5 years in accordance with the University of Waikato research policy. The study data will be used for thesis, research journal publications, and conference presentations. All personal data will remain confidential, but I will be identifiable by name and belonging to Te Horahora Marae in these study outputs.

Please provide your signature to confirm that you understand and consent to the conditions of participation in this research.

Name: _____

Signature: _____

Date: _____/_____/_____

Appendix A3

Supplementary Information: Chapter 3

Figure A3.1 nMDS plot comparing all wetlands, where those with more than one sampling site have been merged into a single location (i.e., n = 16).

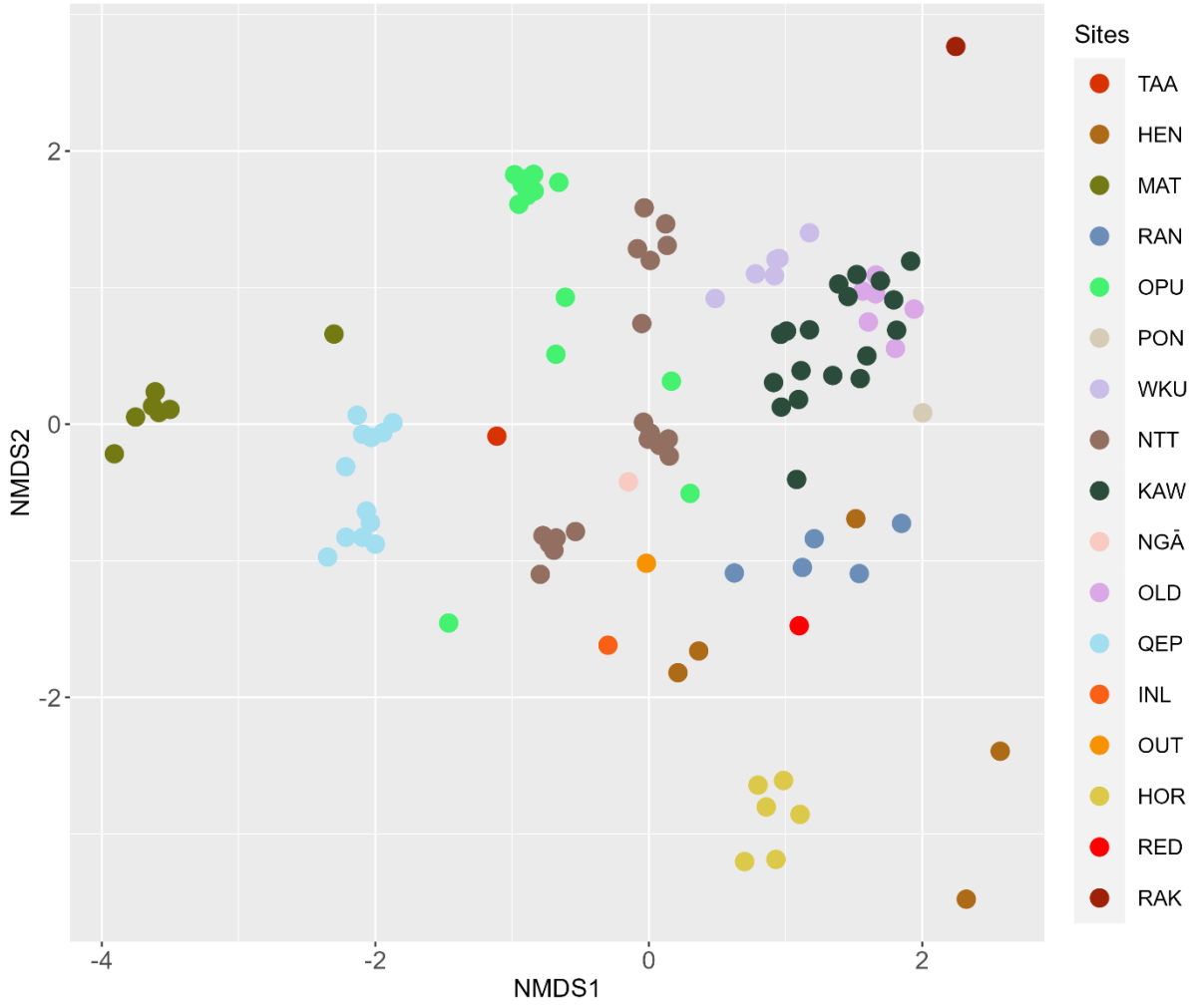
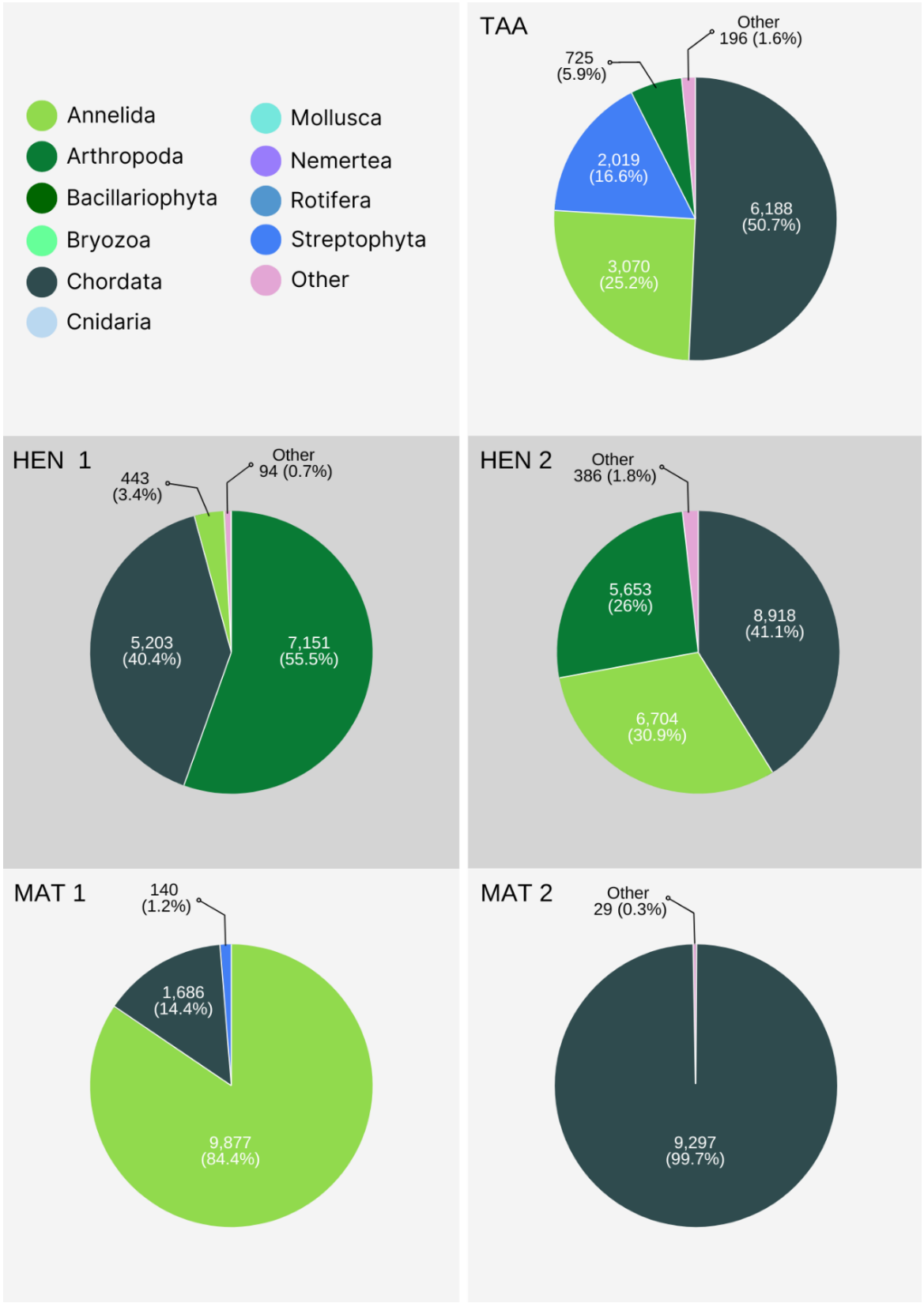
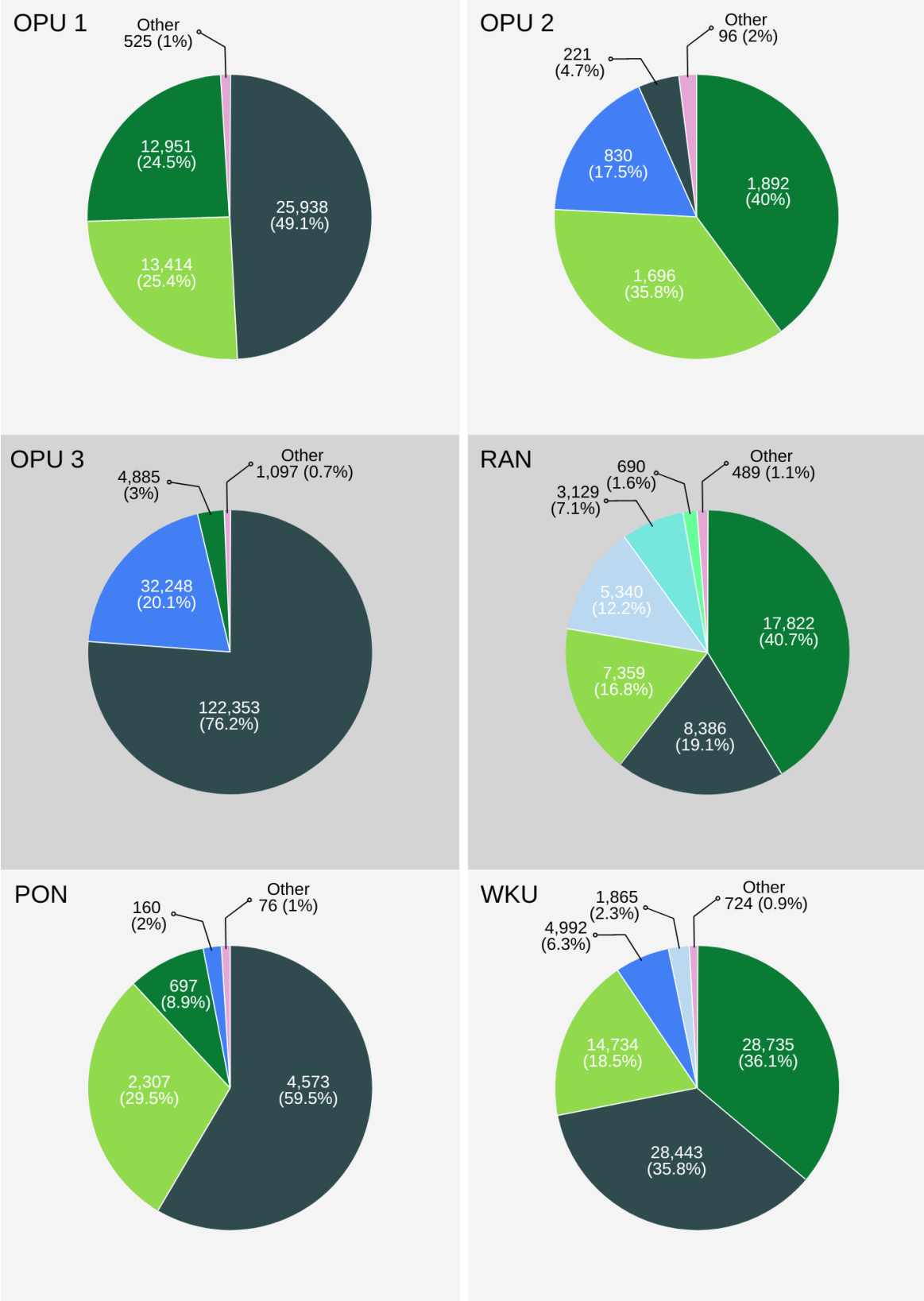
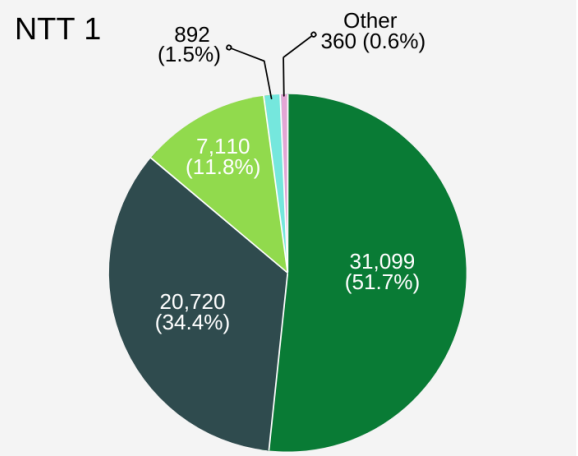
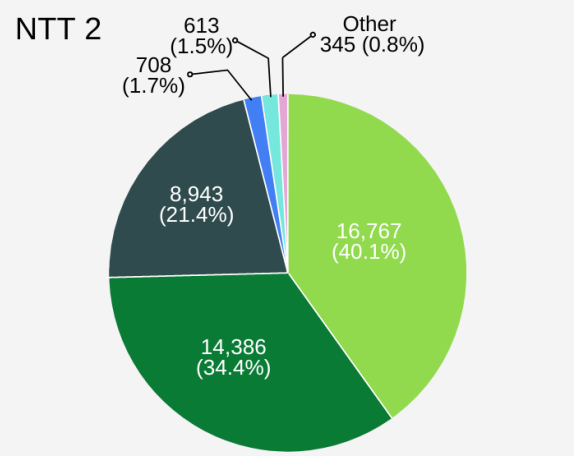


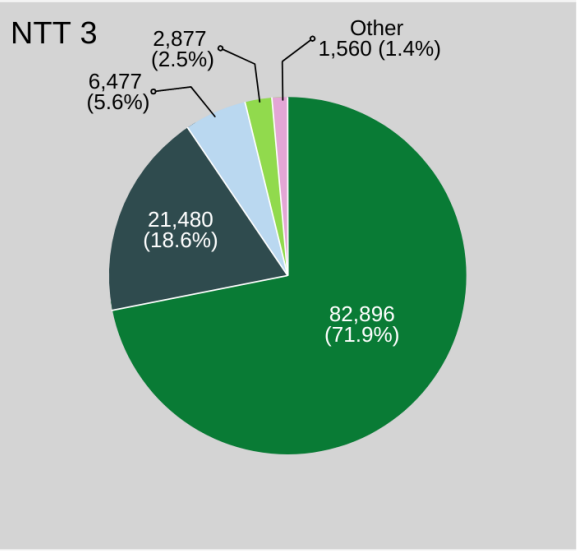
Figure A3.2 Pie graphs of the phylum proportion represented at each wetland location, with locations presented in geographical order (from the top of the North Island to the bottom of the South Island). The read count is listed first, with the overall percentage given in parentheses.

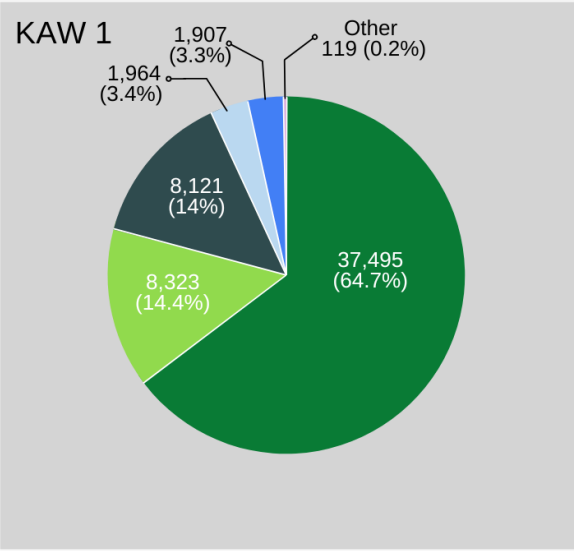


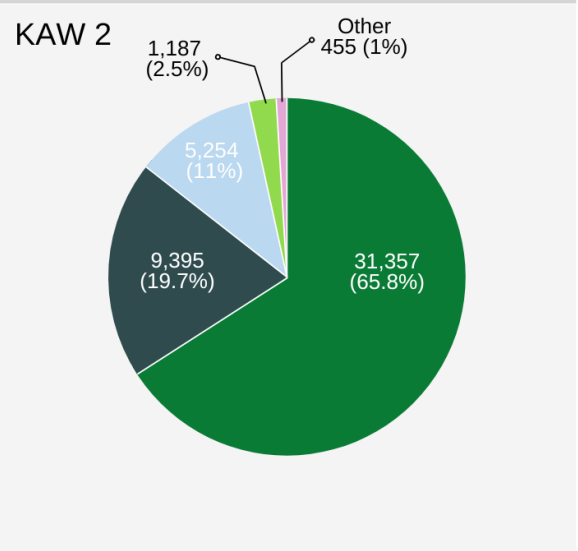

OPU 1
Other
525 (1%)
12,951
(24.5%)
25,938
(49.1%)
13,414
(25.4%)
OPU 2
Other
96 (2%)
221
(4.7%)
830
(17.5%)
1,892
(40%)
1,696
(35.8%)
OPU 3
4,885
(3%)
Other
1,097 (0.7%)
32,248
(20.1%)
122,353
(76.2%)
RAN
690
(1.6%)
Other
489 (1.1%)
3,129
(7.1%)
5,340
(12.2%)
17,822
(40.7%)
7,359
(16.8%)
8,386
(19.1%)
PON
160
(2%)
Other
76 (1%)
697
(8.9%)
2,307
(29.5%)
4,573
(59.5%)
WKU
1,865
(2.3%)
Other
724 (0.9%)
4,992
(6.3%)
14,734
(18.5%)
28,735
(36.1%)
28,443
(35.8%)

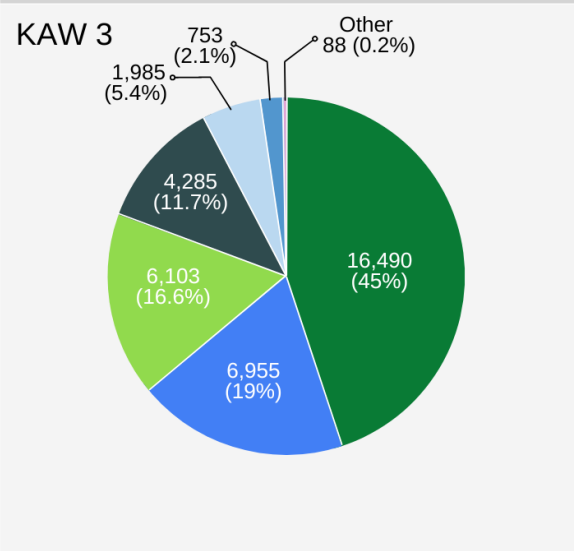

NTT 1
892
(1.5%)
Other
360 (0.6%)
7,110
(11.8%)
31,099
(51.7%)
20,720
(34.4%)

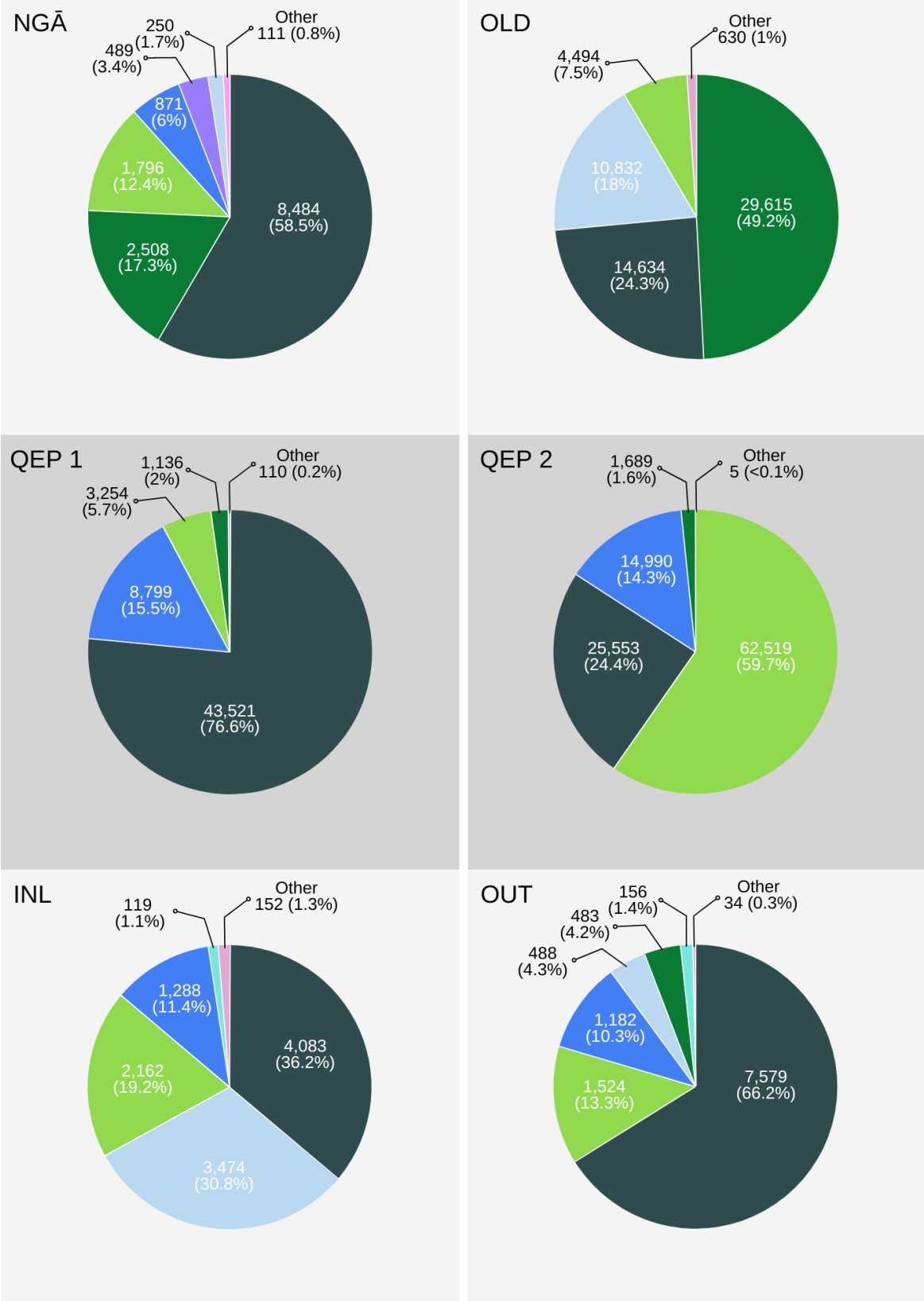

NTT 2
613
(1.5%)
Other
345 (0.8%)
708
(1.7%)
8,943
(21.4%)
16,767
(40.1%)
14,386
(34.4%)

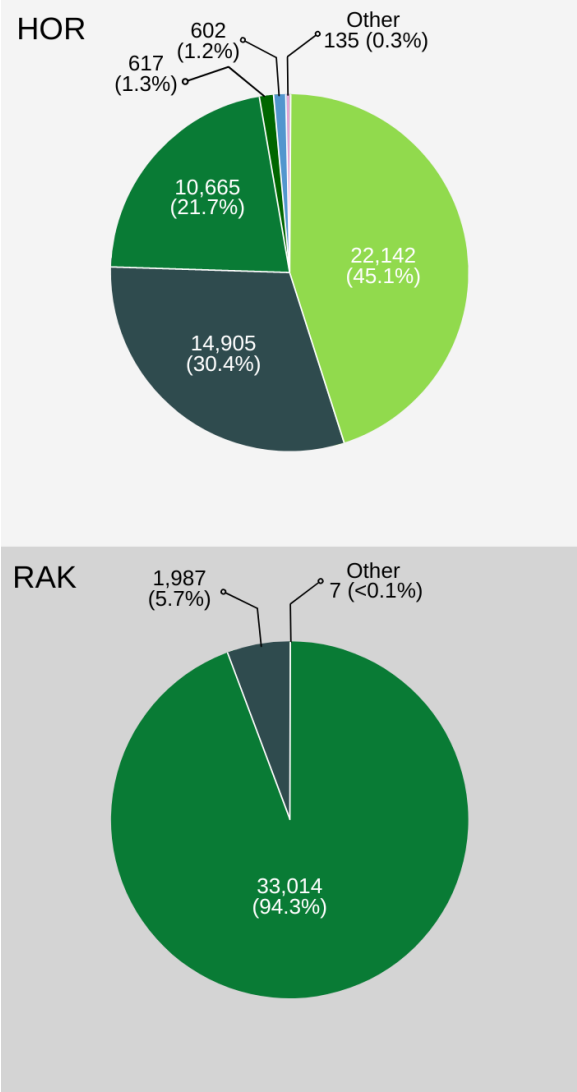

NTT 3
2,877
(2.5%)
Other
1,560 (1.4%)
6,477
(5.6%)
21,480
(18.6%)
82,896
(71.9%)

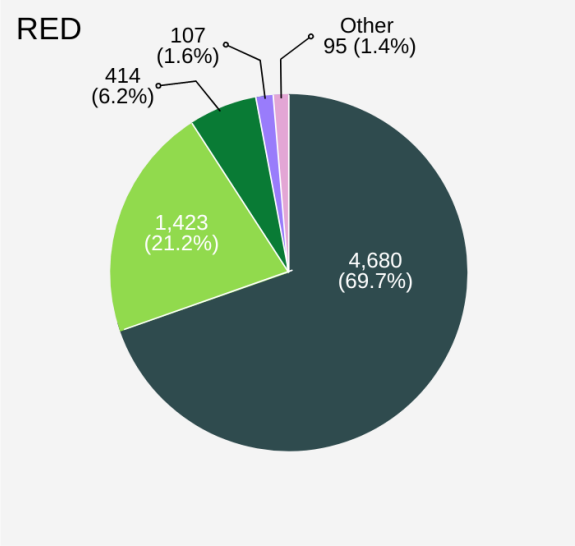

KAW 1
1,907
(3.3%)
Other
119 (0.2%)
1,964
(3.4%)
8,121
(14%)
37,495
(64.7%)
8,323
(14.4%)


KAW 2
1,187
(2.5%)
Other
455 (1%)
5,254
(11%)
9,395
(19.7%)
31,357
(65.8%)


KAW 3
753
(2.1%)
Other
88 (0.2%)
1,985
(5.4%)
4,285
(11.7%)
16,490
(45%)
6,103
(16.6%)
6,955
(19%)

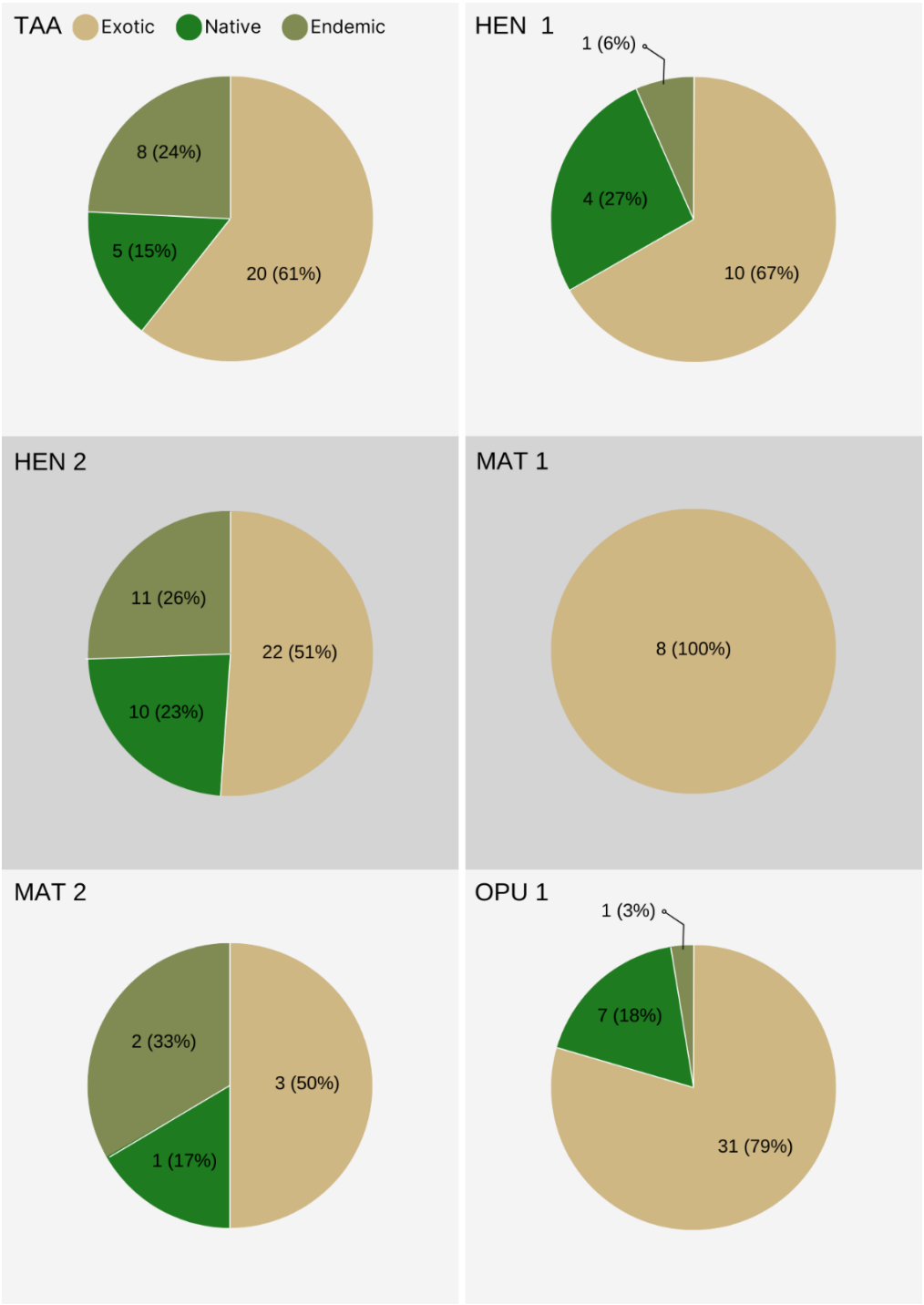

NGĀ
Other
111 (0.8%)
250
(1.7%)
489
(3.4%)
871
(6%)
1,796
(12.4%)
8,484
(58.5%)
2,508
(17.3%)
OLD
Other
630 (1%)
4,494
(7.5%)
10,832
(18%)
29,615
(49.2%)
14,634
(24.3%)
QEP 1
Other
110 (0.2%)
1,136
(2%)
3,254
(5.7%)
8,799
(15.5%)
43,521
(76.6%)
QEP 2
Other
5 (<0.1%)
1,689
(1.6%)
14,990
(14.3%)
25,553
(24.4%)
62,519
(59.7%)
INL
Other
152 (1.3%)
119
(1.1%)
1,288
(11.4%)
4,083
(36.2%)
2,162
(19.2%)
3,474
(30.8%)
OUT
Other
34 (0.3%)
156
(1.4%)
483
(4.2%)
488
(4.3%)
1,182
(10.3%)
7,579
(66.2%)
1,524
(13.3%)

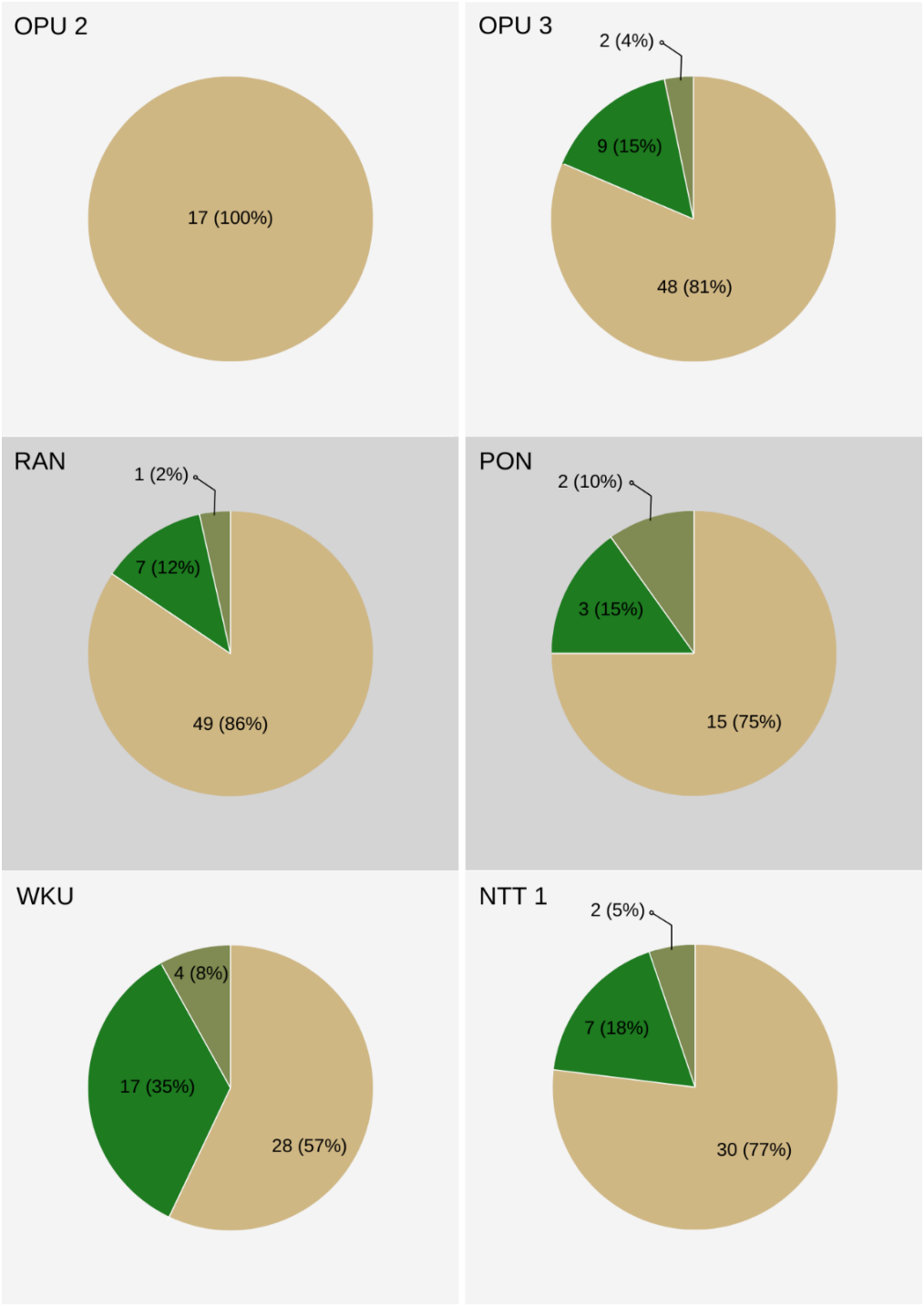

HOR
602
(1.2%)
Other
135 (0.3%)
617
(1.3%)
10,665
(21.7%)
22,142
(45.1%)
14,905
(30.4%)

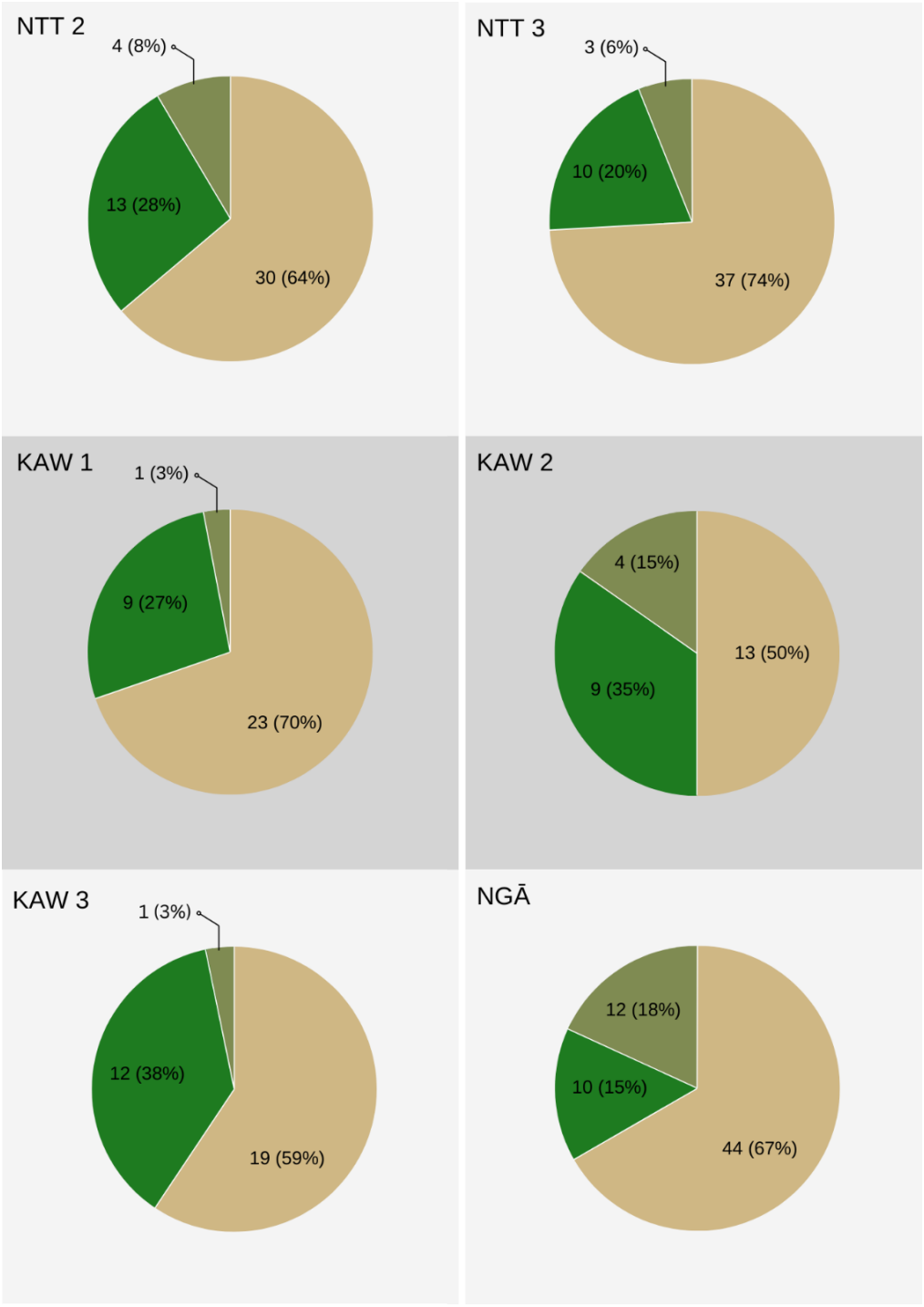

RED
107
(1.6%)
Other
95 (1.4%)
414
(6.2%)
1,423
(21.2%)
4,680
(69.7%)

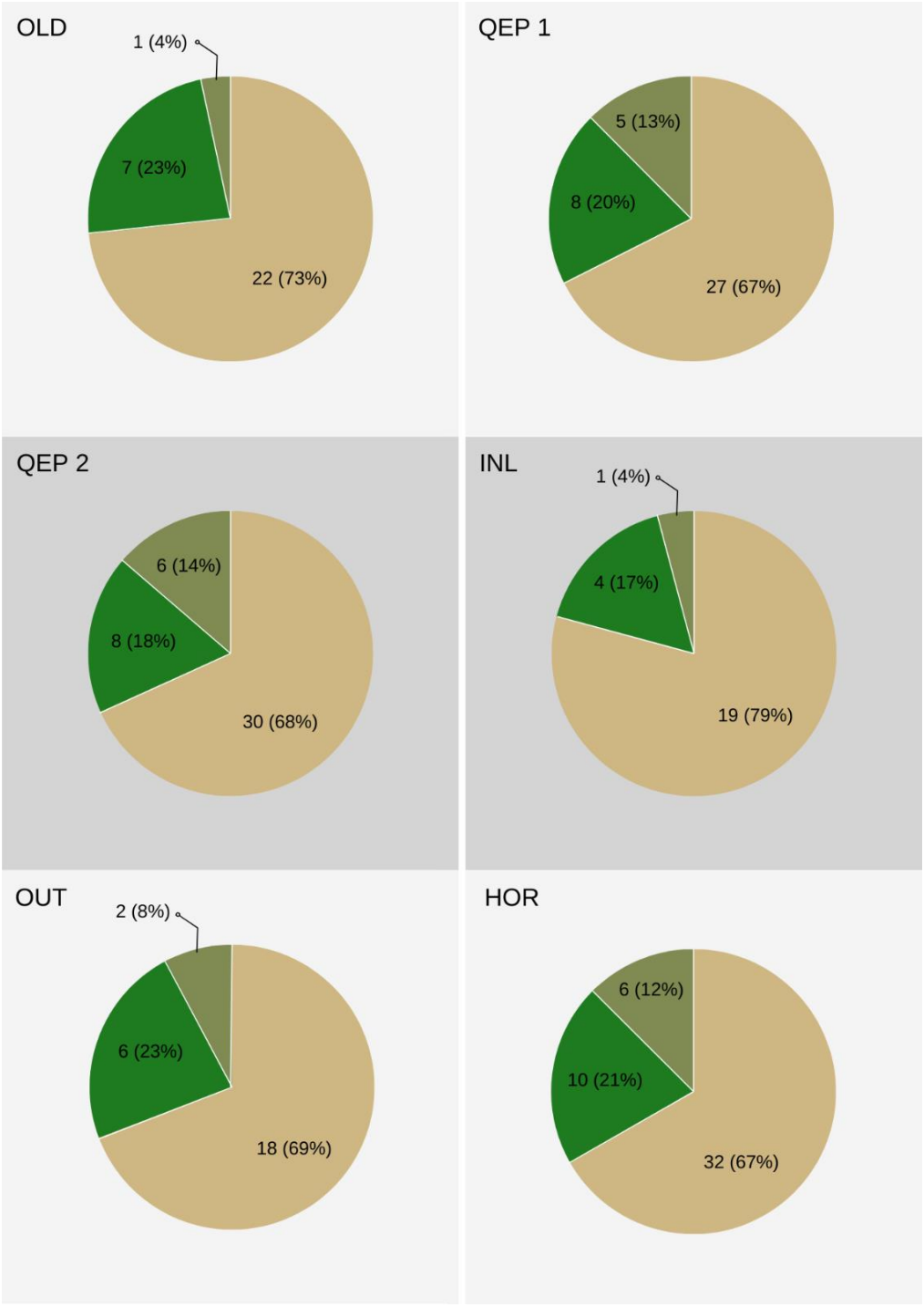
RAK
1,987
(5.7%)
Other
7 (<0.1%)
33,014
(94.3%)

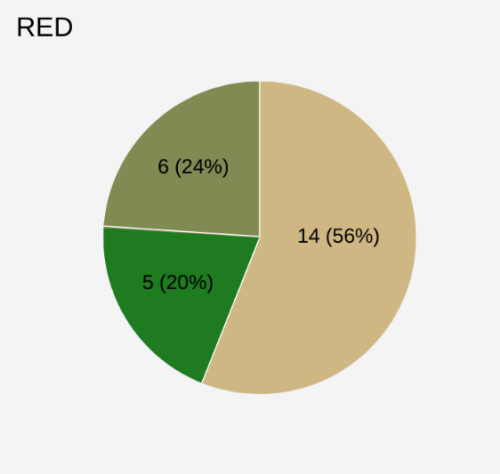
Figure A3.3 Pie graphs of the exotic versus native versus endemic ratio for each wetland location, with locations presented in geographical order (from the top of the North Island to the bottom of the South Island). The number of each species type is listed first, with the overall percentage given in parentheses. Note that one DNA sequence was identified as either *Anas chlorotis* or *Anas gracilis* (brown or grey teal), with the former classed as endemic and the latter as native; these species were thus excluded from the pie graphs.




OPU 2
17 (100%)
OPU 3
2 (4%)
9 (15%)
48 (81%)
RAN
1 (2%)
7 (12%)
49 (86%)
PON
2 (10%)
3 (15%)
15 (75%)
WKU
4 (8%)
17 (35%)
28 (57%)
NTT 1
2 (5%)
7 (18%)
30 (77%)


NTT 2
4 (8%)
13 (28%)
30 (64%)
NTT 3
3 (6%)
10 (20%)
37 (74%)
KAW 1
1 (3%)
9 (27%)
23 (70%)
KAW 2
4 (15%)
13 (50%)
9 (35%)
KAW 3
1 (3%)
12 (38%)
19 (59%)
NGĀ
12 (18%)
10 (15%)
44 (67%)


OLD
1 (4%)
7 (23%)
22 (73%)
QEP 1
5 (13%)
8 (20%)
27 (67%)
QEP 2
6 (14%)
8 (18%)
30 (68%)
INL
1 (4%)
4 (17%)
19 (79%)
OUT
2 (8%)
6 (23%)
18 (69%)
HOR
6 (12%)
10 (21%)
32 (67%)


RED
6 (24%)
14 (56%)
5 (20%)

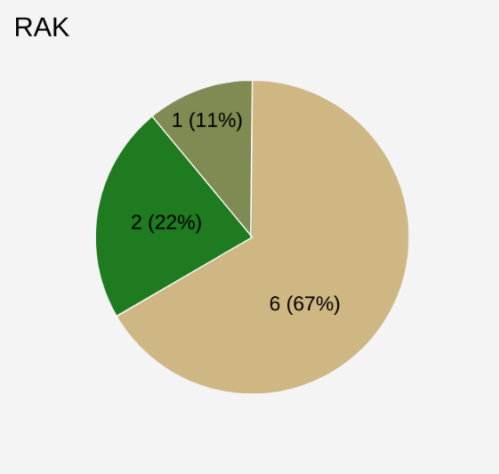

RAK
1 (11%)
2 (22%)
6 (67%)

Table A3.1 Breakdown of the phylum proportions equating to <1% of the total, which were compiled under the category 'other' in Figure 3.2. The read count is listed first, with the overall percentage given in parentheses.

Location code	Annelida	Arthropoda	Bacillariophyta	Bryozoa	Chlorophyta	Ciliophora	Cnidaria	Discosea	Gastrotricha
TAA	-	-	-	-	4 (0.03)	-	70 (0.57)	13 (0.11)	-
HEN 1	-	-	7 (0.05)	-	-	-	-	-	-
HEN 2	-	-	-	-	-	-	34 (0.16)	-	-
MAT 2	-	20 (0.21)	-	-	-	-	-	-	-
OPU 1	-	-	-	-	-	-	-	-	-
OPU 2	-	-	-	-	-	-	-	28 (0.59)	-
OPU 3	747 (0.47)	-	56 (0.03)	180 (0.11)	-	10 (0.01)	14 (0.01)	-	5 (0.003)
RAN	-	-	-	-	44 (0.10)	-	-	-	-
PON	-	-	43 (0.55)	-	-	-	33 (0.42)	-	-
WKU	-	-	25 (0.03)	-	26 (0.03)	449 (0.56)	-	6 (0.01)	-
NTT 1	-	-	-	-	-	5 (0.01)	111 (0.18)	10 (0.02)	-
NTT 2	-	-	157 (0.38)	-	-	-	-	46 (0.11)	41 (0.10)
NTT 3	-	-	-	-	-	25 (0.02)	-	-	-
KAW 1	-	-	-	-	99 (0.17)	20 (0.03)	-	-	-
KAW 2	-	-	-	-	13 (0.03)	64 (0.13)	-	-	-

KAW 3	-	-	10 (0.03)	-	9 (0.02)	35 (0.10)	-	-	-
NGĀ	-	-	-	-	-	-	-	-	-
OLD	-	-	-	-	46 (0.08)	138 (0.23)	-	-	-
Location code	**Annelida**	**Arthropoda**	**Bacillariophyta**	**Bryozoa**	**Chlorophyta**	**Ciliophora**	**Cnidaria**	**Discosea**	**Gastrotricha**
QEP 1	-	-	-	-	101 (0.18)	-	-	-	-
QEP 2	-	-	-	-	-	-	5 (0.005)	-	-
INL	-	57 (0.51)	-	-	-	24 (0.21)	-	-	-
OUT	-	-	-	-	-	26 (0.23)	-	-	-
HOR	-	-	-	-	-	-	9 (0.02)	36 (0.07)	-
RED	-	-	-	-	-	-	34 (0.51)	-	-
RAK	7 (0.02)	-	-	-	-	-	-	-	-

Location code	Mollusca	NA	Nemertea	Oomycota	Platyhelminthes	Rhodophyta	Rotifera	Streptophyta
TAA	65 (0.53)	-	22 (0.18)	-	-	22 (0.18)	-	-
HEN 1	-	-	87 (0.67)	-	-	-	-	-
HEN 2	65 (0.30)	137 (0.63)	150 (0.69)	-	-	-	-	-
MAT 2	-	9 (0.10)	-	-	-	-	-	-
OPU 1	122 (0.23)		16 (0.03)	-	-	-	109 (0.21)	278 (0.53)
OPU 2	37 (0.78)	31 (0.65)	-	-	-	-	-	-
OPU 3	73 (0.05)	7 (0.004)	5 (0.003)	-	-	-	-	-
RAN	-	-	310 (0.71)	-	-	-	7 (0.02)	128 (0.29)
PON	-	-	-	-	-	-	-	-
WKU	170 (0.21)	34 (0.04)	5 (0.01)	-	-	-	9 (0.01)	-
NTT 1	-	15 (0.02)	-	-	-	-	-	219 (0.36)
NTT 2	-	16 (0.04)	20 (0.05)	-	4 (0.01)	-	61 (0.15)	-
NTT 3	50 (0.04)	140 (0.12)	5 (0.004)	21 (0.02)	-	28 (0.02)	297 (0.26)	994 (0.86)
KAW 1	-	-	-	-	-	-	-	-
KAW 2	-	-	-	-	-	-	-	378 (0.79)
KAW 3	24 (0.07)	-	10 (0.03)	-	-	-	-	-
NGĀ	85 (0.59)	13 (0.09)	-	-	-	-	13 (0.09)	-
OLD	-	-	-	-	-	-	-	446 (0.74)

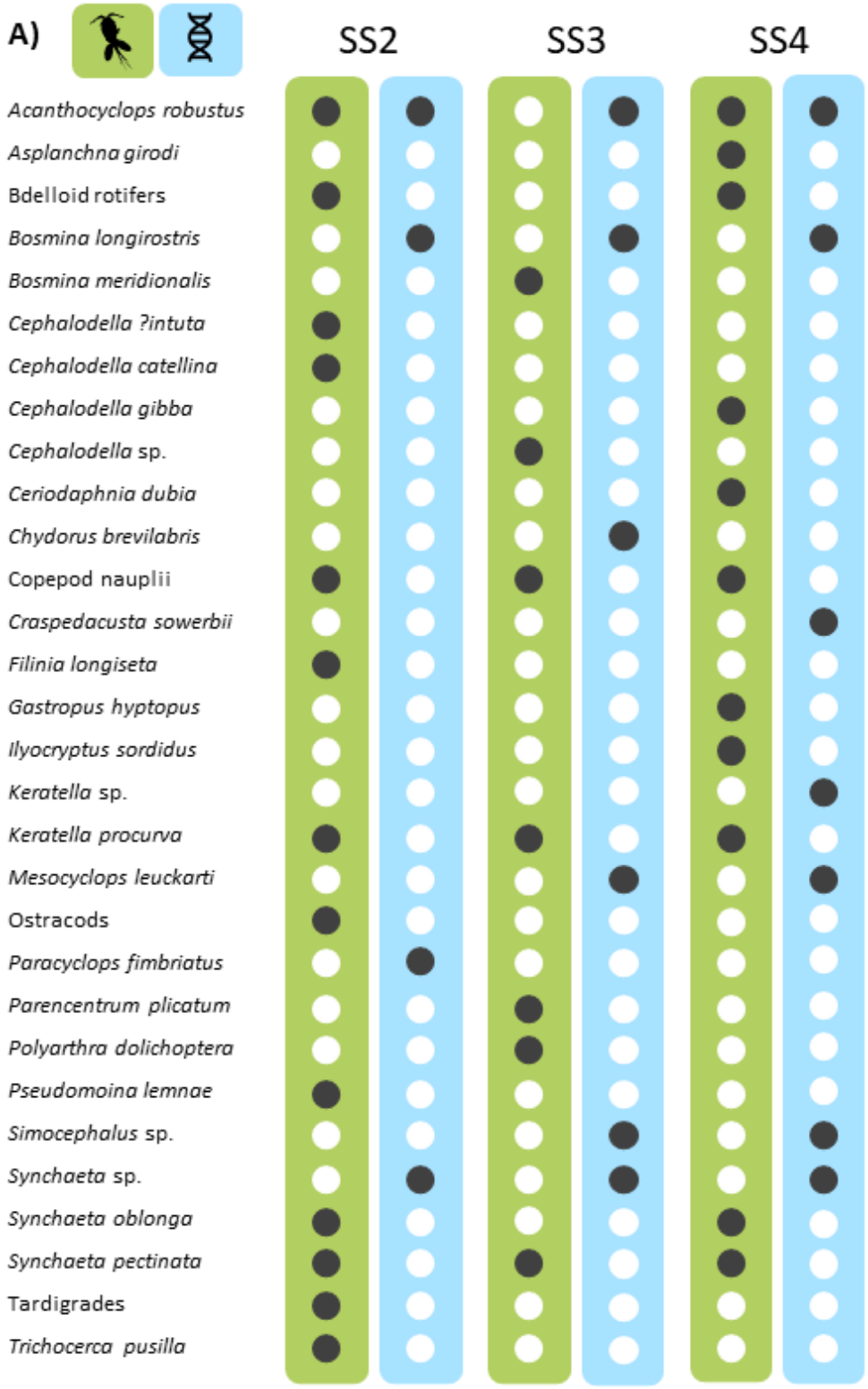
QEP 1	9 (0.02)	-	-	-	-	-	-	-
QEP 2	-	-	-	-	-	-	-	-
INL	-	-	71 (0.63)	-	-	-	-	-
OUT	-	-	-	-	-	-	8 (0.07)	-
Location code	**Mollusca**	**NA**	**Nemertea**	**Oomycota**	**Platyhelminthes**	**Rhodophyta**	**Rotifera**	**Streptophyta**
HOR	67 (0.14)	18 (0.04)	-	5 (0.01)	-	-	-	-
RED	61 (0.91)	-	-	-	-	-	-	-
RAK	-	-	-	-	-	-	-	-

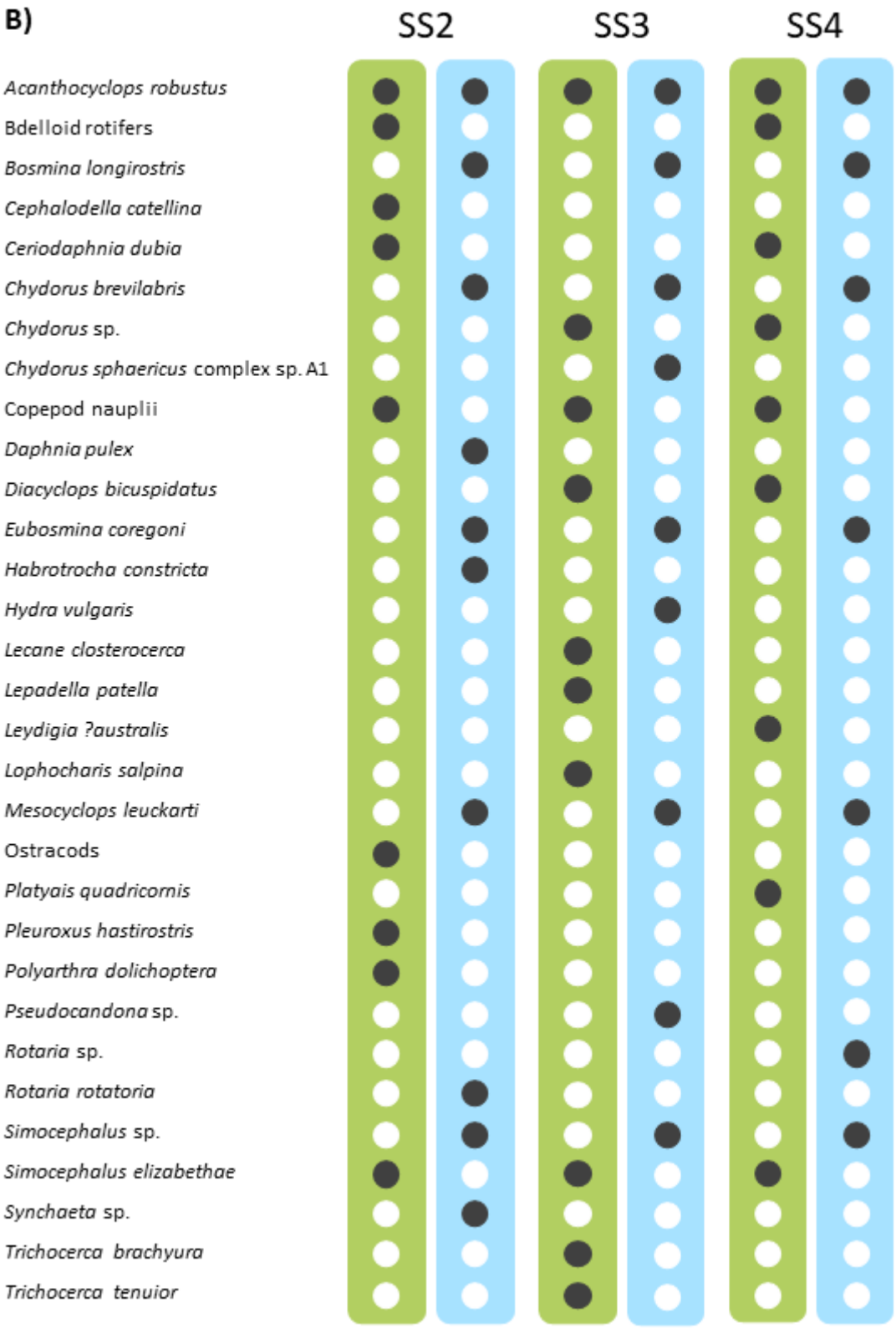
Appendix A4

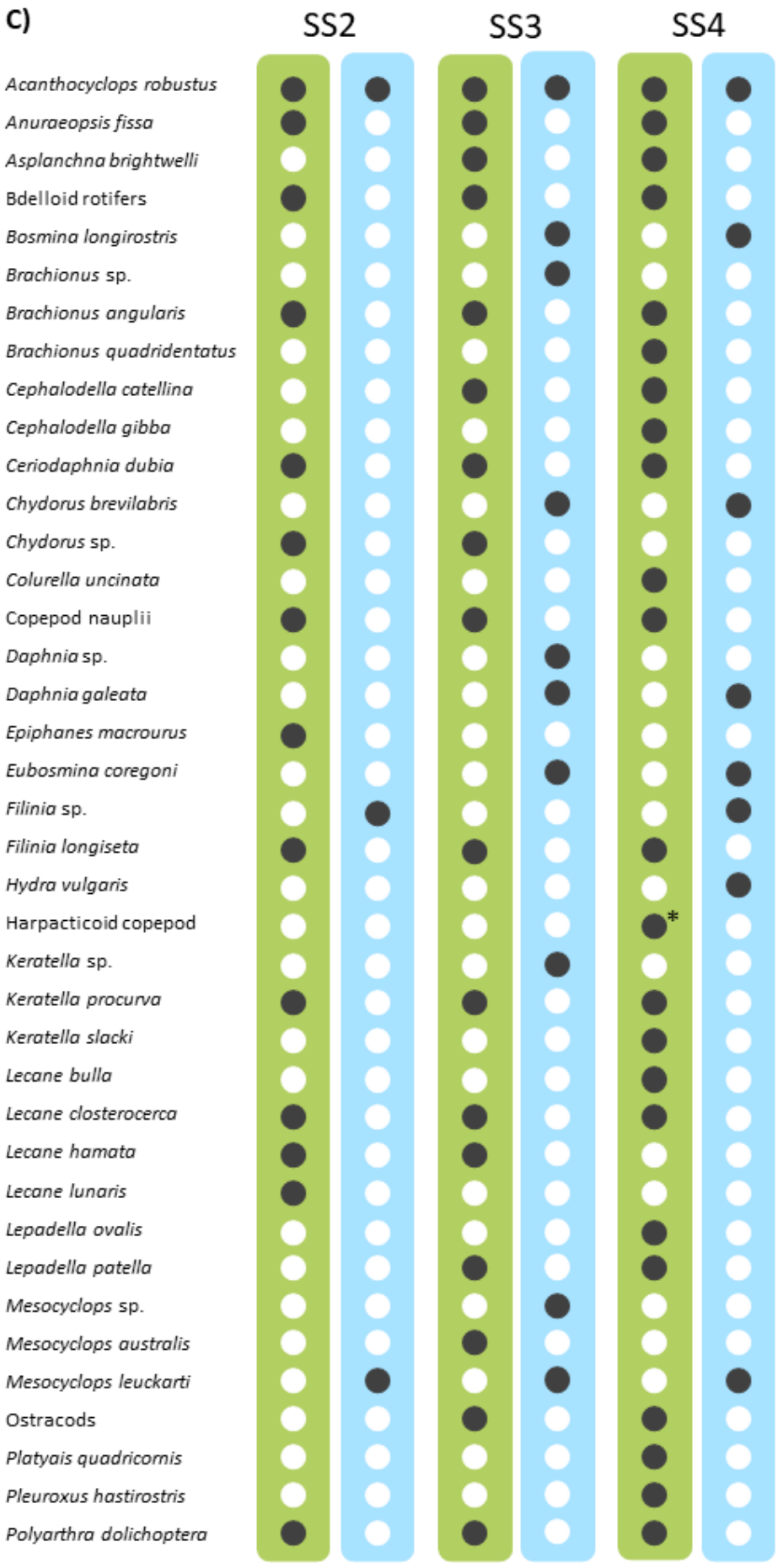
Supplementary Information: Chapter 4

Figure A4.1 Experimental set-up: **(Left)** A photo of the orange experiment, where two bamboo sticks were placed 1 m apart with oranges at one end to measure water flow; and **(Right)** Residence time experimental design, showing the point where the kea DNA slurry was released and the transects sampled at various time points thereafter.

Figure A4.2 Comparison between the zooplankton surveys and eDNA results at SS2-SS4 at the: **A)** start, **B)** middle, and **C)** end of spring. On the left is a list of species detected from both methods. The white circle signals that the species was not detected, and the filled-in circles indicate that the species was detected.




B)
SS2
SS3
SS4
Acanthocyclops robustus
Bdelloid rotifers
Bosmina longirostris
Cephalodella catellina
Ceriodaphnia dubia
Chydorus brevilabris
Chydorus sp.
Chydorus sphaericus complex sp. A1
Copepod nauplii
Daphnia pulex
Diacyclops bicuspidatus
Eubosmina coregoni
Habrotrocha constricta
Hydra vulgaris
Lecane closterocerca
Lepadella patella
Leydigia ?australis
Lophocharis salpina
Mesocyclops leuckarti
Ostracods
Platyais quadricornis
Pleuroxus hastirostris
Polyarthra dolichoptera
Pseudocandona sp.
Rotaria sp.
Rotaria rotatoria
Simocephalus sp.
Simocephalus elizabethae
Synchaeta sp.
Trichocerca brachyura
Trichocerca tenuior


C)
SS2
SS3
SS4
Acanthocyclops robustus
Anuraeopsis fissa
Asplanchna brightwelli
Bdelloid rotifers
Bosmina longirostris
Brachionus sp.
Brachionus angularis
Brachionus quadridentatus
Cephalodella catellina
Cephalodella gibba
Ceriodaphnia dubia
Chydorus brevilabris
Chydorus sp.
Colurella uncinata
Copepod nauplii
Daphnia sp.
Daphnia galeata
Epiphanes macrourus
Eubosmina coregoni
Filinia sp.
Filinia longiseta
Hydra vulgaris
Harpacticoid copepod
*
Keratella sp.
Keratella procurva
Keratella slacki
Lecane bulla
Lecane closterocerca
Lecane hamata
Lecane lunaris
Lepadella ovalis
Lepadella patella
Mesocyclops sp.
Mesocyclops australis
Mesocyclops leuckarti
Ostracods
Platyais quadricornis
Pleuroxus hastirostris
Polyarthra dolichoptera

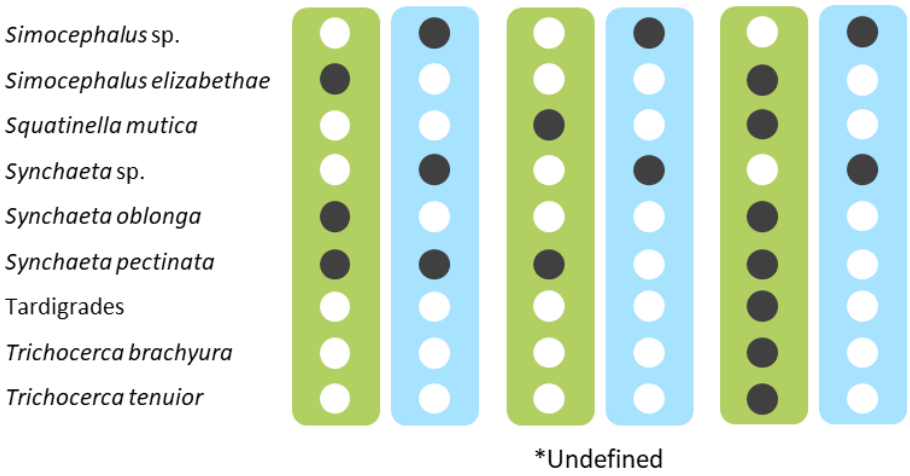
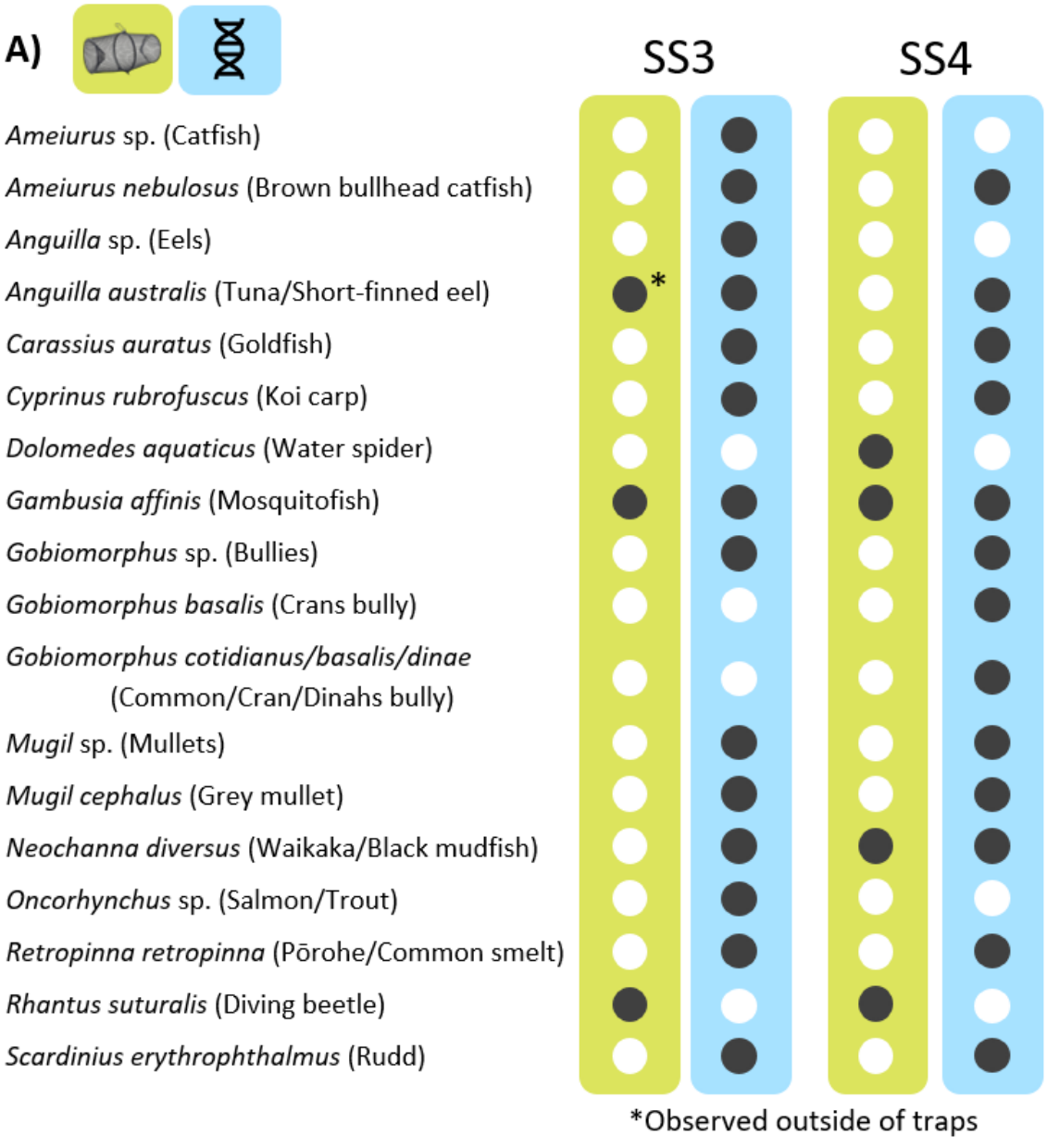
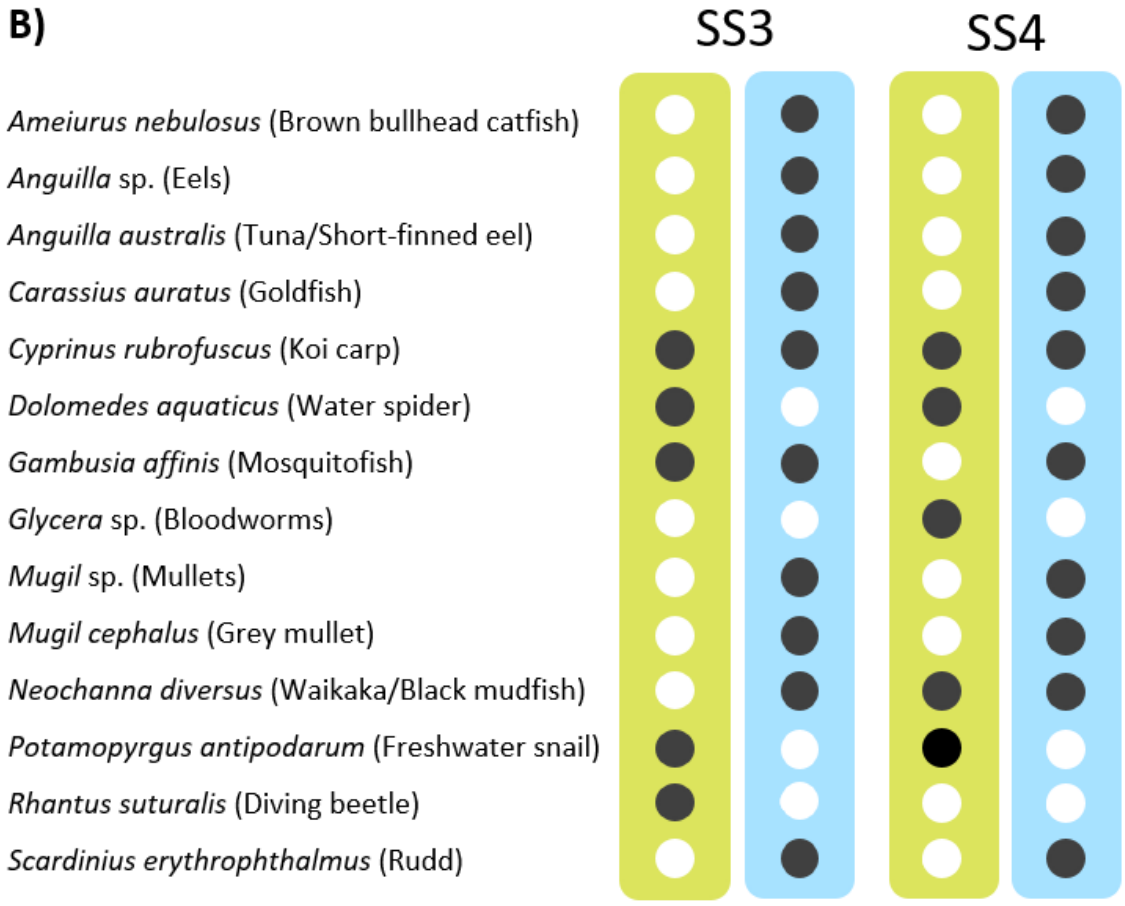

Simocephalus sp.
Simocephalus elizabethae
Squatinella mutica
Synchaeta sp.
Synchaeta oblonga
Synchaeta pectinata
Tardigrades
Trichocerca brachyura
Trichocerca tenuior
*Undefined

Figure A4.3 Comparison between the Gee-minnow traps and eDNA results at SS3 and SS4 at the: **A)** start, **B)** middle, and **C)** end of spring. On the left is a list of species detected from both methods. The white circle signals that the species was not detected, and the filled-in circles indicate that the species was detected.



B)


SS3
SS4
Ameiurus nebulosus (Brown bullhead catfish)
Anguilla sp. (Eels)
Anguilla australis (Tuna/Short-finned eel)
Carassius auratus (Goldfish)
Cyprinus rubrofuscus (Koi carp)
Dolomedes aquaticus (Water spider)
Gambusia affinis (Mosquitofish)
Glycera sp. (Bloodworms)
Mugil sp. (Mullets)
Mugil cephalus (Grey mullet)
Neochanna diversus (Waikaka/Black mudfish)
Potamopyrgus antipodarum (Freshwater snail)
Rhantus suturalis (Diving beetle)
Scardinius erythrophthalmus (Rudd)

C)

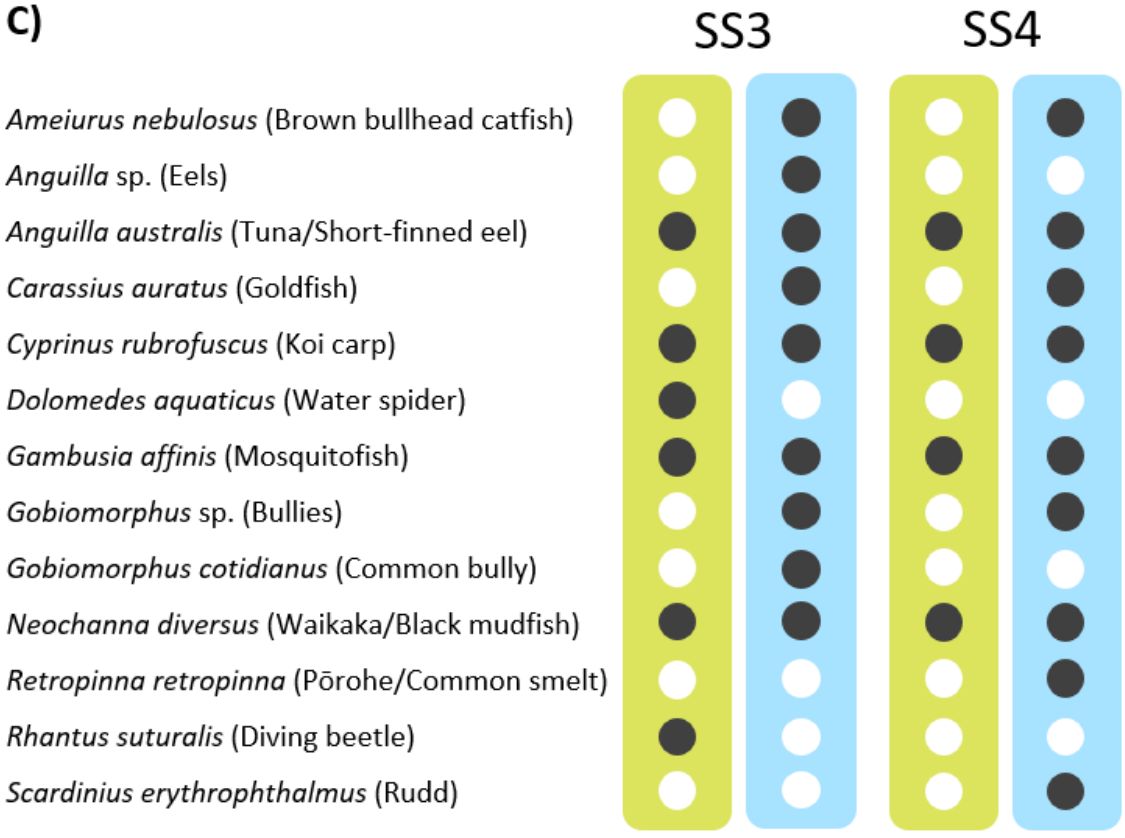

SS3
SS4
Ameiurus nebulosus (Brown bullhead catfish)
Anguilla sp. (Eels)
Anguilla australis (Tuna/Short-finned eel)
Carassius auratus (Goldfish)
Cyprinus rubrofuscus (Koi carp)
Dolomedes aquaticus (Water spider)
Gambusia affinis (Mosquitofish)
Gobiomorphus sp. (Bullies)
Gobiomorphus cotidianus (Common bully)
Neochanna diversus (Waikaka/Black mudfish)
Retropinna retropinna (Pōrohe/Common smelt)
Rhantus suturalis (Diving beetle)
Scardinius erythrophthalmus (Rudd)

Figure A4.4 Comparison between the five-minute bird count and eDNA results at SS1-SS4 in the: **A)** middle and **B)** end of spring. On the left is a list of species detected from both methods. The white circle signals that the species was not detected, and the filled-in circles indicate that the species was detected.

B)

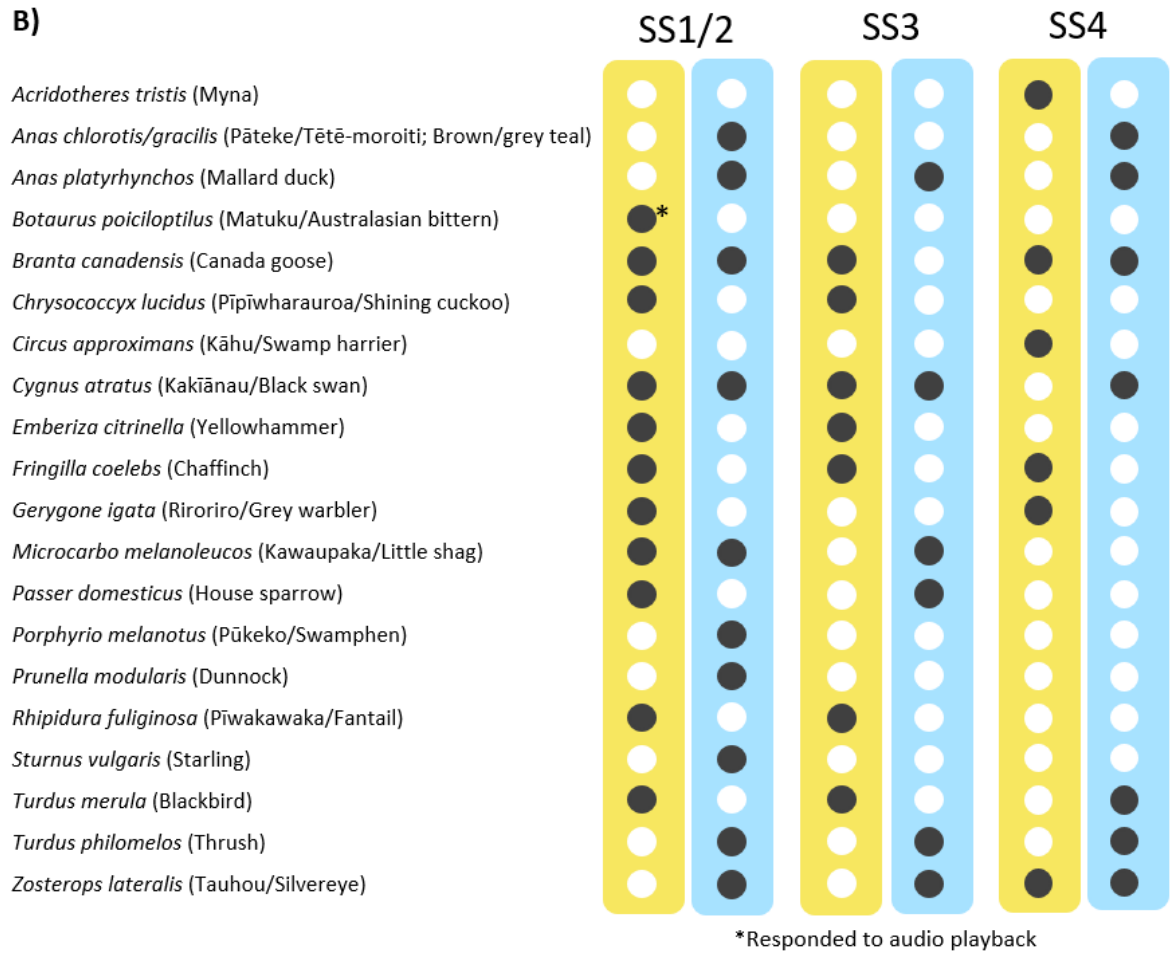
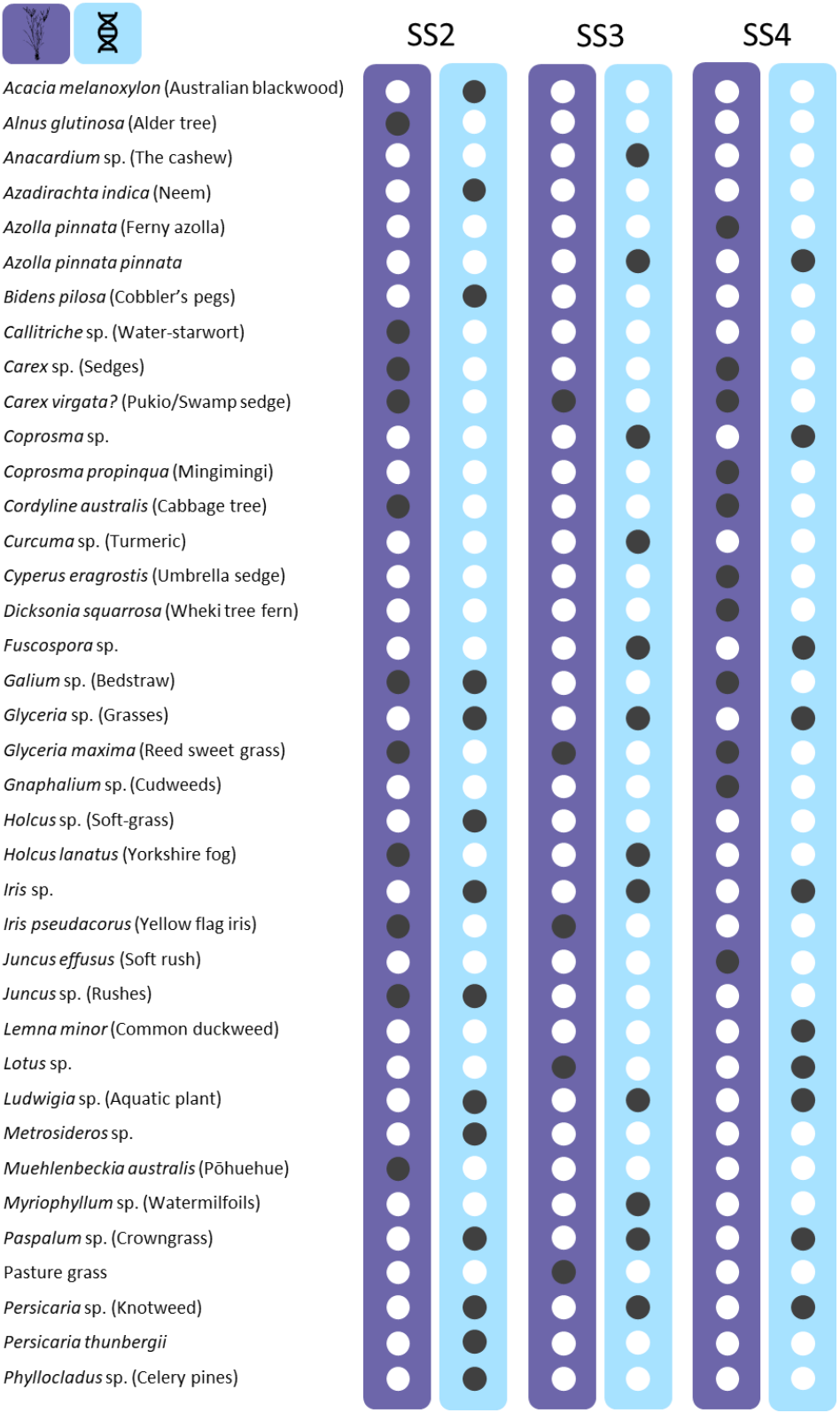

SS1/2
SS3
SS4
Acridotheres tristis (Myna)
Anas chlorotis/gracilis (Pāteke/Tētē-moroiti; Brown/grey teal)
Anas platyrhynchos (Mallard duck)
Botaurus poiciloptilus (Matuku/Australasian bittern)
Branta canadensis (Canada goose)
Chrysococcyx lucidus (Pīpīwharauroa/Shining cuckoo)
Circus approximans (Kāhu/Swamp harrier)
Cygnus atratus (Kakīānau/Black swan)
Emberiza citrinella (Yellowhammer)
Fringilla coelebs (Chaffinch)
Gerygone igata (Riroriro/Grey warbler)
Microcarbo melanoleucos (Kawaupaka/Little shag)
Passer domesticus (House sparrow)
Porphyrio melanotus (Pūkeko/Swamphen)
Prunella modularis (Dunnock)
Rhipidura fuliginosa (Pīwakawaka/Fantail)
Sturnus vulgaris (Starling)
Turdus merula (Blackbird)
Turdus philomelos (Thrush)
Zosterops lateralis (Tauhou/Silvereye)
*Responded to audio playback

Figure A4.5 Comparison between the botanist survey and eDNA results at SS2-SS4 in the middle of spring. On the left is a list of species detected from both methods. The white circle signals that the species was not detected, and the filled-in circles indicate that the species was detected.



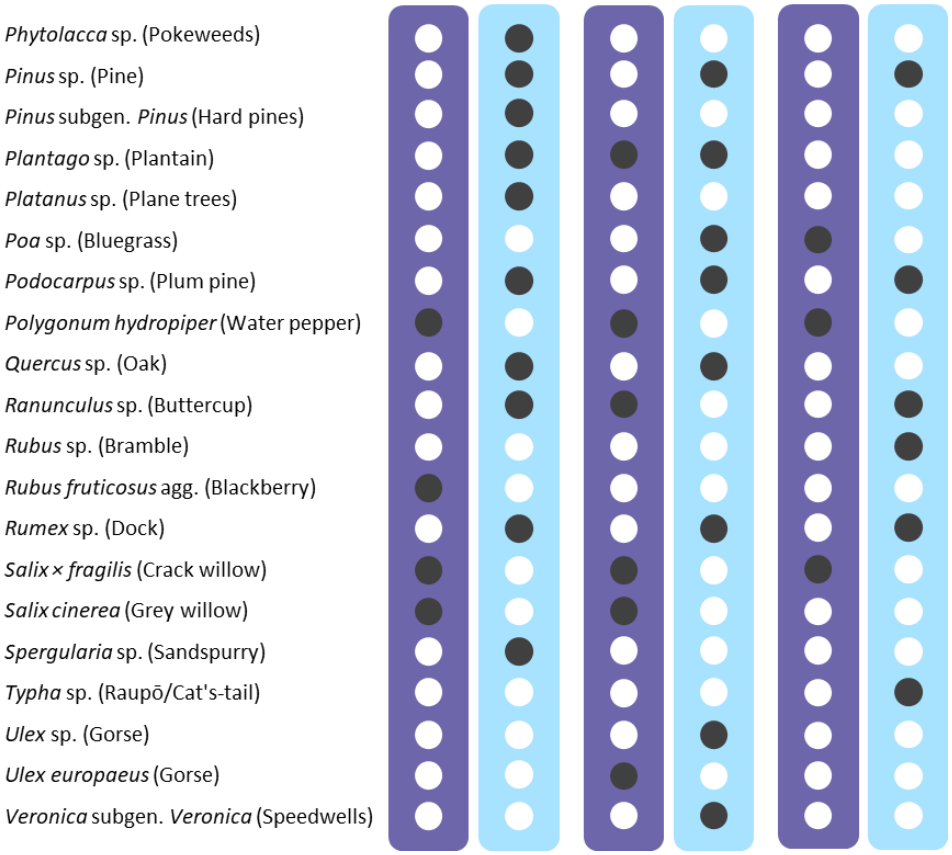
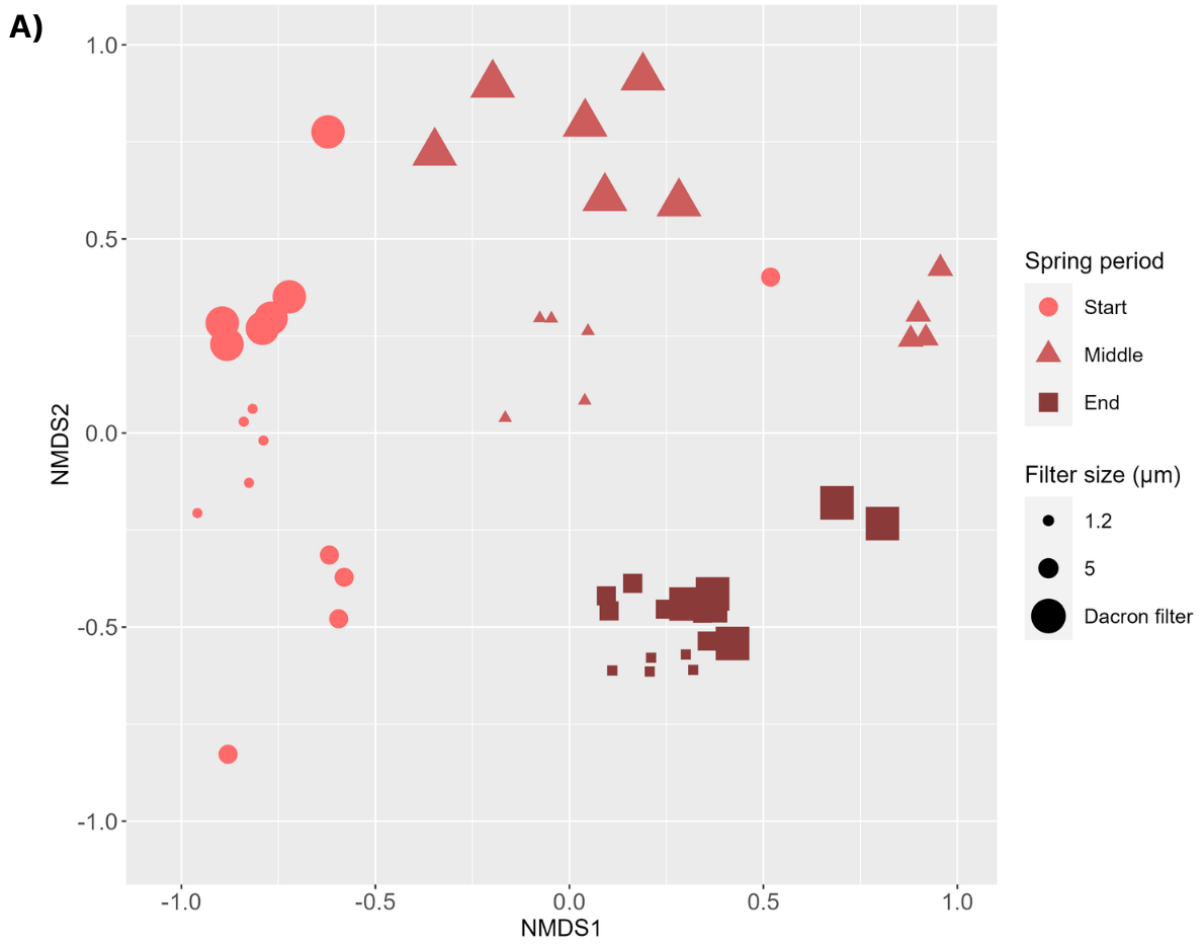

Phytolacca sp. (Pokeweeds)
Pinus sp. (Pine)
Pinus subgen. *Pinus* (Hard pines)
Plantago sp. (Plantain)
Platanus sp. (Plane trees)
Poa sp. (Bluegrass)
Podocarpus sp. (Plum pine)
Polygonum hydropiper (Water pepper)
Quercus sp. (Oak)
Ranunculus sp. (Buttercup)
Rubus sp. (Bramble)
Rubus fruticosus agg. (Blackberry)
Rumex sp. (Dock)
Salix × fragilis (Crack willow)
Salix cinerea (Grey willow)
Spergularia sp. (Sandspurry)
Typha sp. (Raupō/Cat's-tail)
Ulex sp. (Gorse)
Ulex europaeus (Gorse)
Veronica subgen. *Veronica* (Speedwells)

Figure A4.6 nMDS plot comparing the difference among DNA sequences between the 1.2 µm, 5 µm, and dacron filter at the start, middle, and end of spring at **A)** species and genus level and **B)** key wetlands species of interest, including birds, fish, and plants. In A), statistically significant differences were observed at the start ($F_{2,13}$ = 4.385; p = 0.001; PERMANOVA; $F_{2,13}$ = 0.543; p = 0.579; PERMDISP), middle ($F_{2,14}$ = 6.670; p = 0.001; PERMANOVA; $F_{2,14}$ = 0.903; p = 0.452; PERMDISP), and end ($F_{2,14}$ = 2.701; p = 0.001; PERMANOVA; $F_{2,14}$ = 0.460; p = 0.640; PERMDISP) of spring. However, the only statistically significant pairwise comparison occurred at the end of spring between the 1.2 µm vs. 5 µm filters (Table A4.9). In B), statistically significant differences were observed at the start ($F_{2,13}$ = 2.826; p = 0.001; PERMANOVA; $F_{2,13}$ = 0.775; p = 0.471; PERMDISP), middle ($F_{2,14}$ = 3.990; p = 0.001; PERMANOVA; $F_{2,14}$ = 0.009; p = 0.996; PERMDISP), and end ($F_{2,14}$ = 1.967; p = 0.003; PERMANOVA; $F_{2,14}$ = 0.435; p = 0.659; PERMDISP) of spring. No statistically significant pairwise comparisons were observed (Table A4.9).



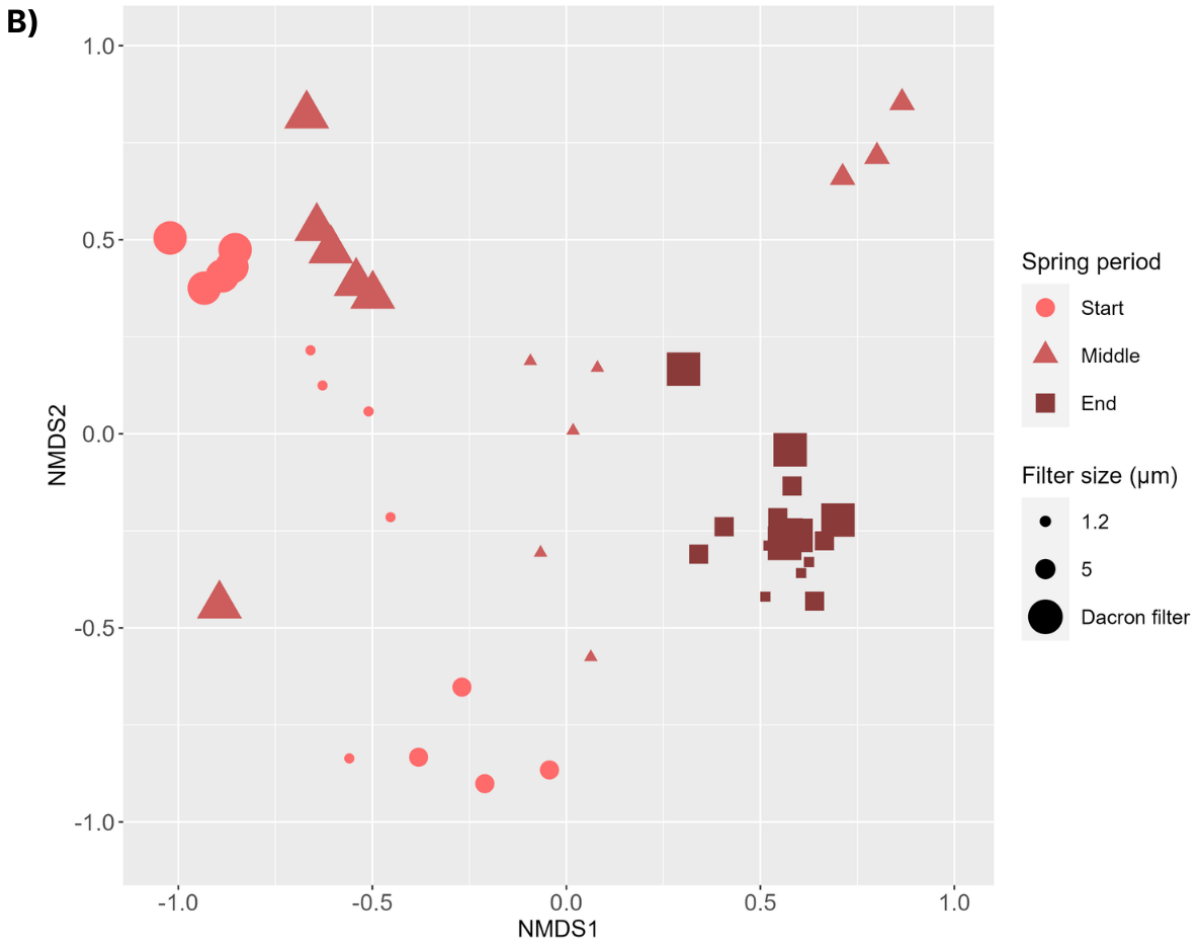

B)
1.0
0.5
0.0
-0.5
-1.0
NMDS2
-1.0
-0.5
0.0
0.5
1.0
NMDS1
Spring period
Start
Middle
End
Filter size (µm)
1.2
5
Dacron filter

Figure A4.7 Species accumulation curves overlaid with boxplots of species richness at the **A)** start and **B)** middle of spring for the 1.2 µm (left), 5 µm (middle), and dacron (right) filters. The solid line indicates the random sampling model of species accumulation provided from the data, and the shaded area represents the 95% confidence interval.

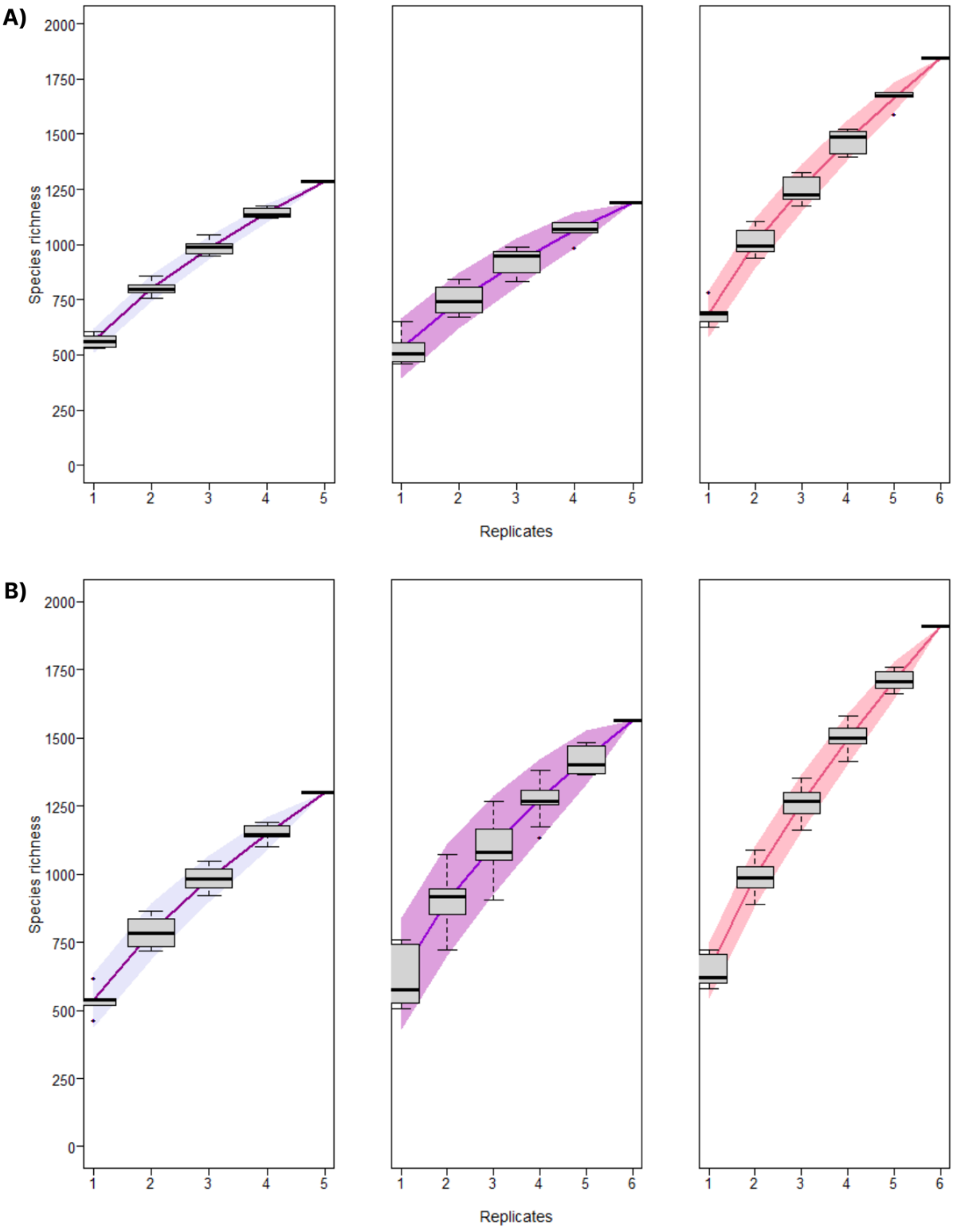
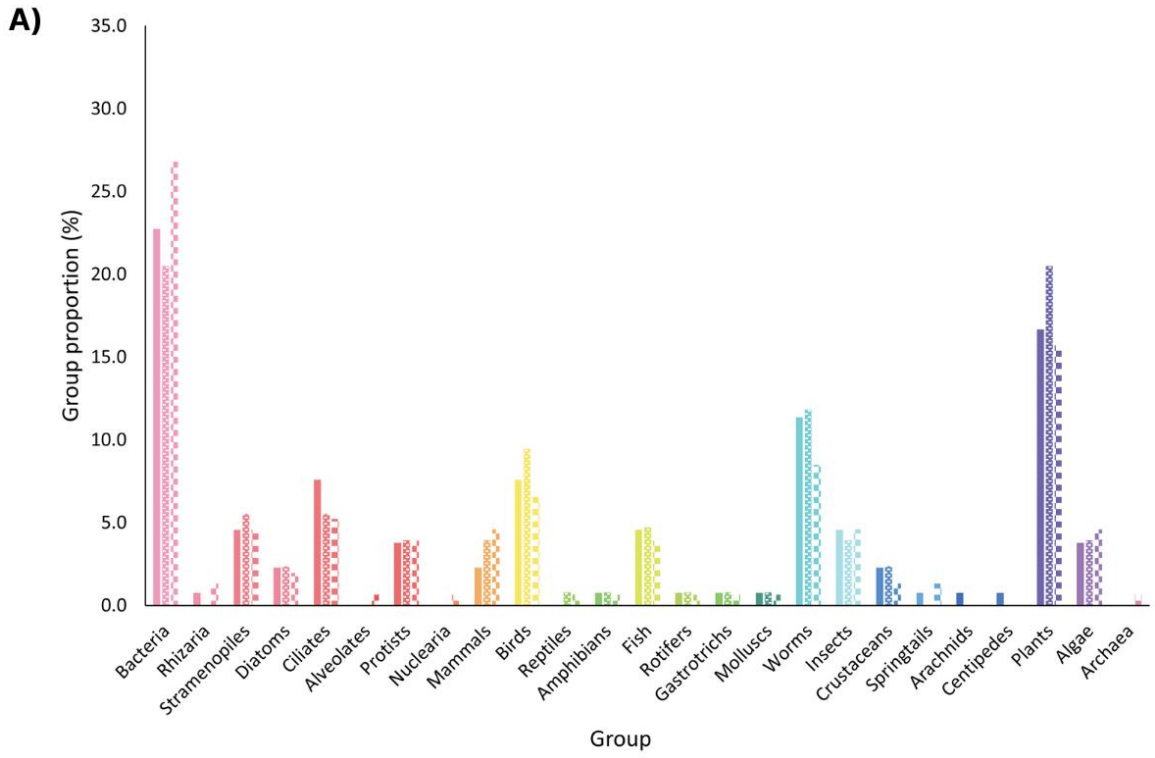
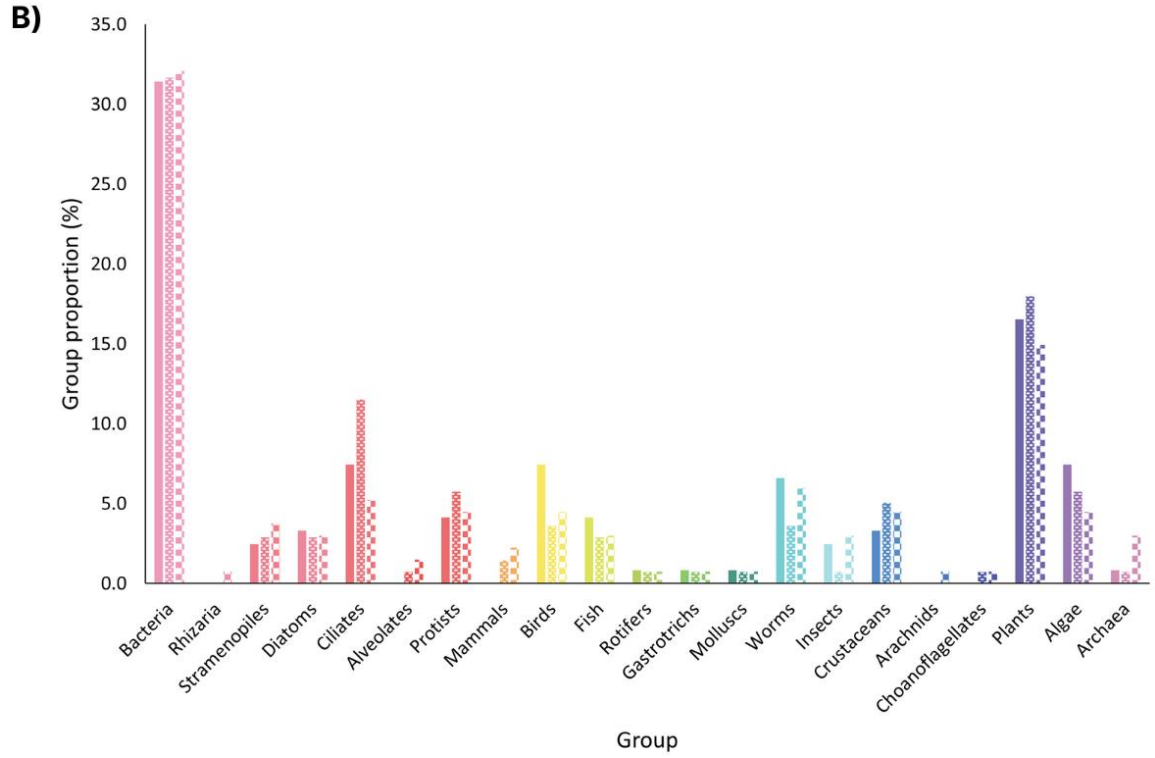


Figure A4.8 Bar graph comparing the three filter sizes at the **A)** start, **B)** middle, and **C)** end of spring. Species groups are along the x-axis, and each group proportion against the group total in percentage along the y-axis. The proportion is based on the number of species detected at the species and genus level.





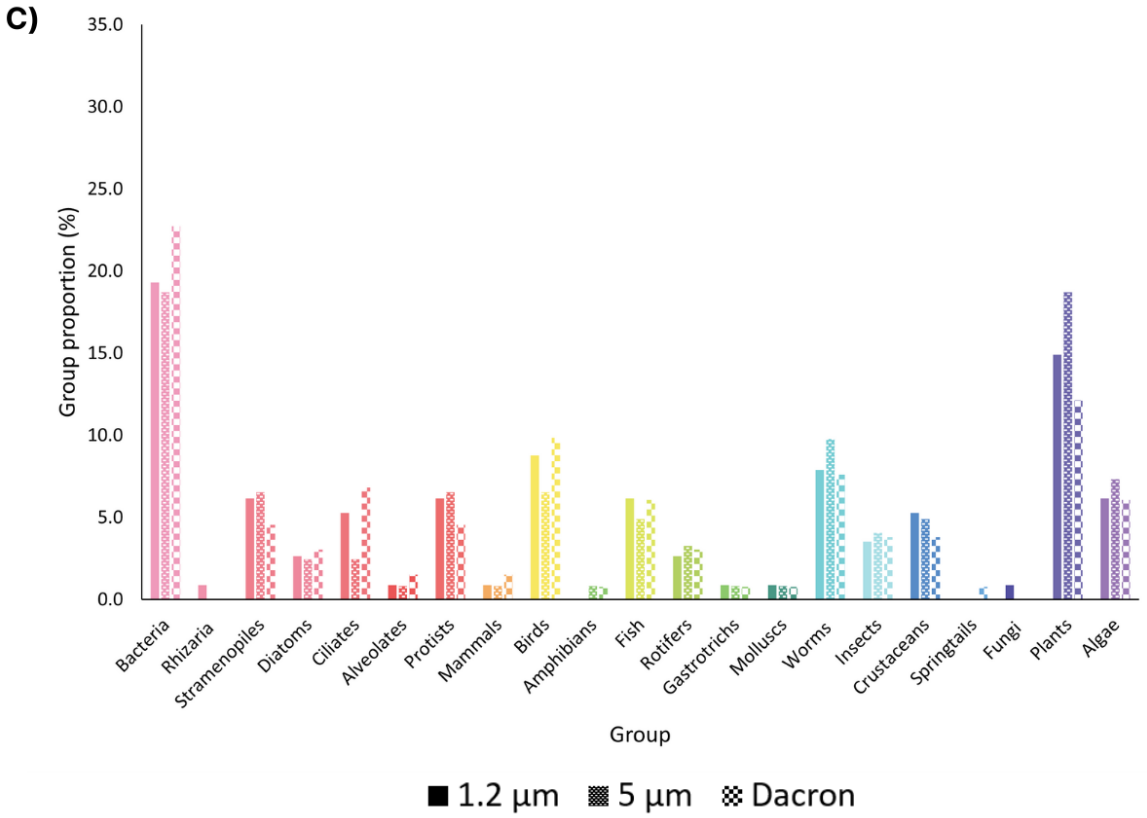
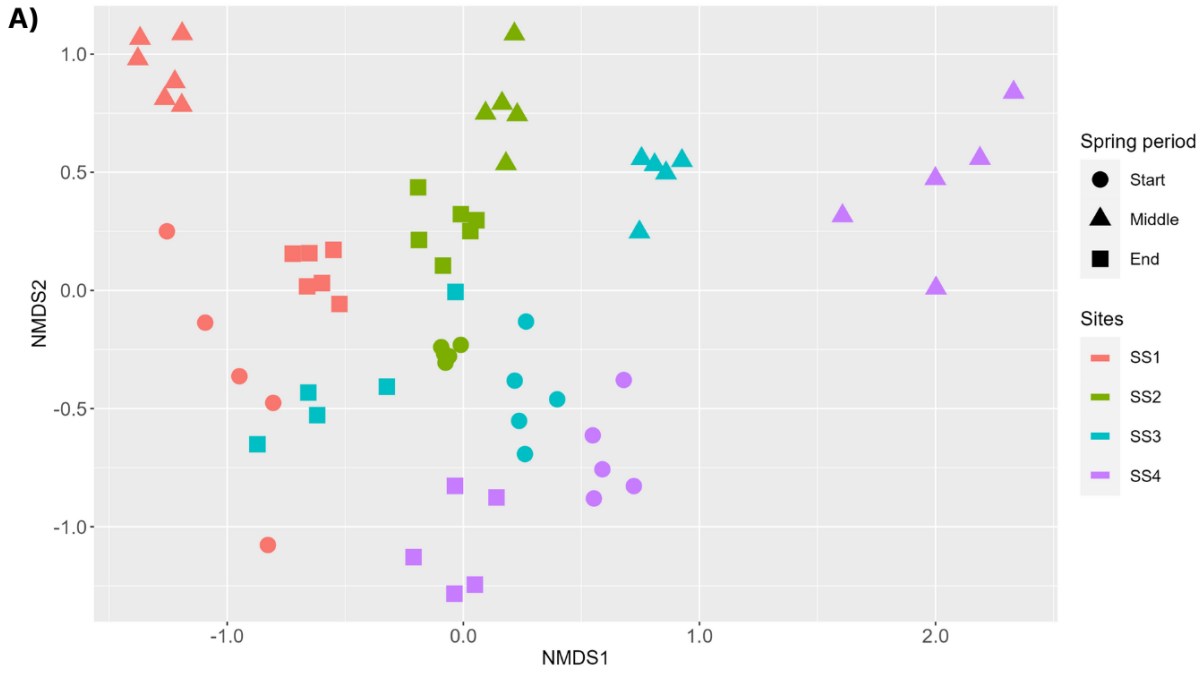


Figure A4.9 nMDS plot comparing the difference among DNA sequences across spatial sites SS1-SS4 at the start, middle, and end of spring at **A)** species and genus level and **B)** key wetlands species of interest, including birds, fish, and plants. In A), statistically significant differences were observed across the four sites at the start ($F_{3,16}$ = 9.590; p = 0.001; PERMANOVA; $F_{3,16}$ = 0.424; p = 0.744; PERMDISP), middle ($F_{3,17}$ = 12.141; p = 0.001; PERMANOVA; $F_{3,17}$ = 12.011; p = 0.001; PERMDISP) and end ($F_{3,18}$ = 12.68; p = 0.022; PERMANOVA; $F_{3,18}$= 3.698; p = 0.022; PERMDISP) of spring. Statistically significant pairwise comparisons were detected at the middle of spring for each site comparison, while SS2 vs. SS3 and SS2 vs. SS4 were statistically significant at the end of spring (Table A4.10). In B), statistically significant differences were observed at the start ($F_{3,16}$ = 5.441; p = 0.001; PERMANOVA; $F_{3,16}$ = 1.859; p = 0.174; PERMDISP), middle ($F_{3,17}$= 9.605; p = 0.001; PERMANOVA; $F_{3,17}$ = 1.449; p = 0.272; PERMDISP), and end ($F_{3,18}$ = 7.150; p = 0.001; PERMANOVA; $F_{3,18}$ = 2.609; p = 0.084; PERMDISP) of spring. Statistically significant pairwise comparisons were detected at the middle and end of spring for SS1 vs. SS2, while SS2 vs. SS3 and SS2 vs. SS4 were statistically significant at the middle and end of spring, respectively

(Table A4.10). These nMDS plots also compare differences among DNA sequences collected at the four sites from a temporal perspective (i.e., start, middle and end of spring) at: **A)** species and genus level and **B)** key wetlands species of interest, including birds, fish, and plants. In A), statistically significant differences were observed at SS1 ($F_{2,14}$= 13.159; p = 0.001; PERMANOVA; $F_{2,14}$ = 0.543; p = 0.6211; PERMDISP), SS2 ($F_{2,13}$ = 15.864; p = 0.001; PERMANOVA; $F_{2,13}$ = 4.454; p = 0.034; PERMDISP), SS3 ($F_{2,12}$ = 11.421; p = 0.001; PERMANOVA; $F_{2,12}$ = 14.257; p = 0.002; PERMDISP), and SS4 ($F_{2,12}$ = 13.226; p = 0.001; PERMANOVA; $F_{2,12}$ = 5.023; p = 0.022; PERMDISP) across spring. Statistically significant pairwise comparisons were detected at the start vs. middle of spring at SS3 and SS4, start vs. end of spring at SS2, and middle vs. end of spring at SS3 and SS4 (Table A4.11). In B), statistically significant differences were observed across temporal points at all sites: SS1 ($F_{2,14}$ = 5.196; p = 0.001; PERMANOVA; $F_{2,14}$= 1.106; p = 0.352; PERMDISP), SS2 ($F_{2,13}$= 12.171; p = 0.001; PERMANOVA; $F_{2,13}$ = 2.337; p = 0.134; PERMDISP), SS3 ($F_{2,12}$ = 6.508; p = 0.001; PERMANOVA; $F_{2,12}$ = 0.363; p = 0.686; PERMDISP), and SS4 ($F_{2,13}$ = 5.182; p = 0.001; PERMANOVA; $F_{2,13}$ = 2.710; p = 0.116; PERMDISP). No statistically significant pairwise comparisons were observed (Table A4.11).



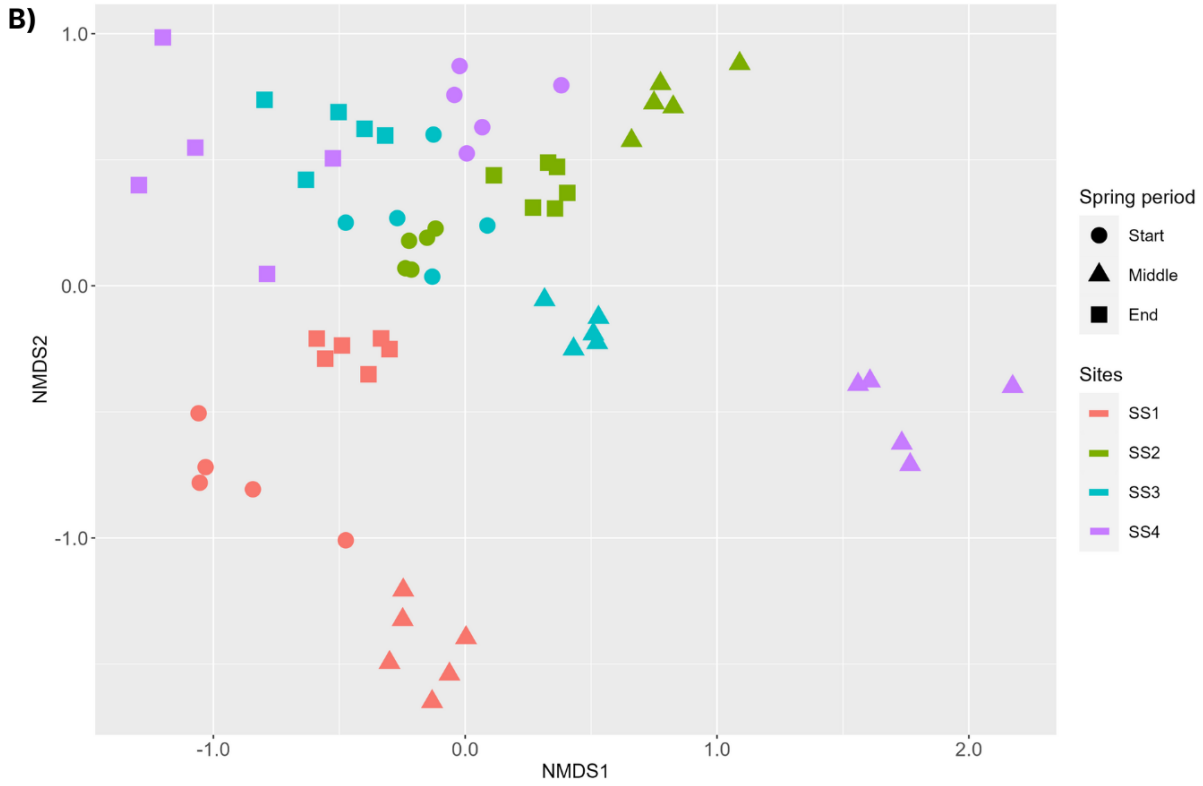

B)
1.0
0.0
-1.0
NMDS2
-1.0
0.0
1.0
2.0
NMDS1
Spring period
Start
Middle
End
Sites
SS1
SS2
SS3
SS4

Figure A4.10 Species accumulation curves overlaid with boxplots at the **A)** start and **B)** middle of spring for the number of sampling sites - along the x-axis - and species richness on the y-axis. The solid line indicates the random sampling model of species accumulation provided from the data, and the shaded area represents the 95% confidence interval.

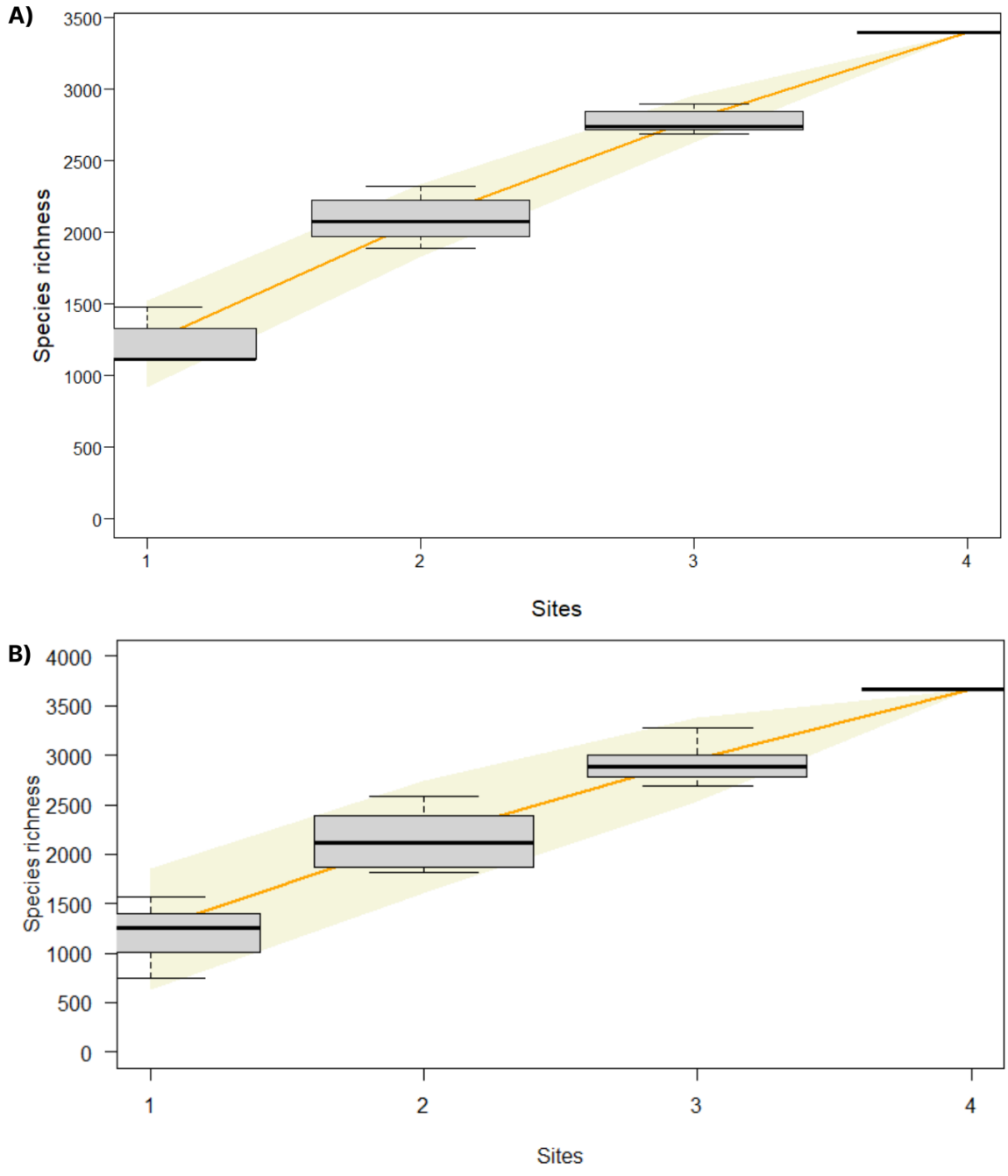
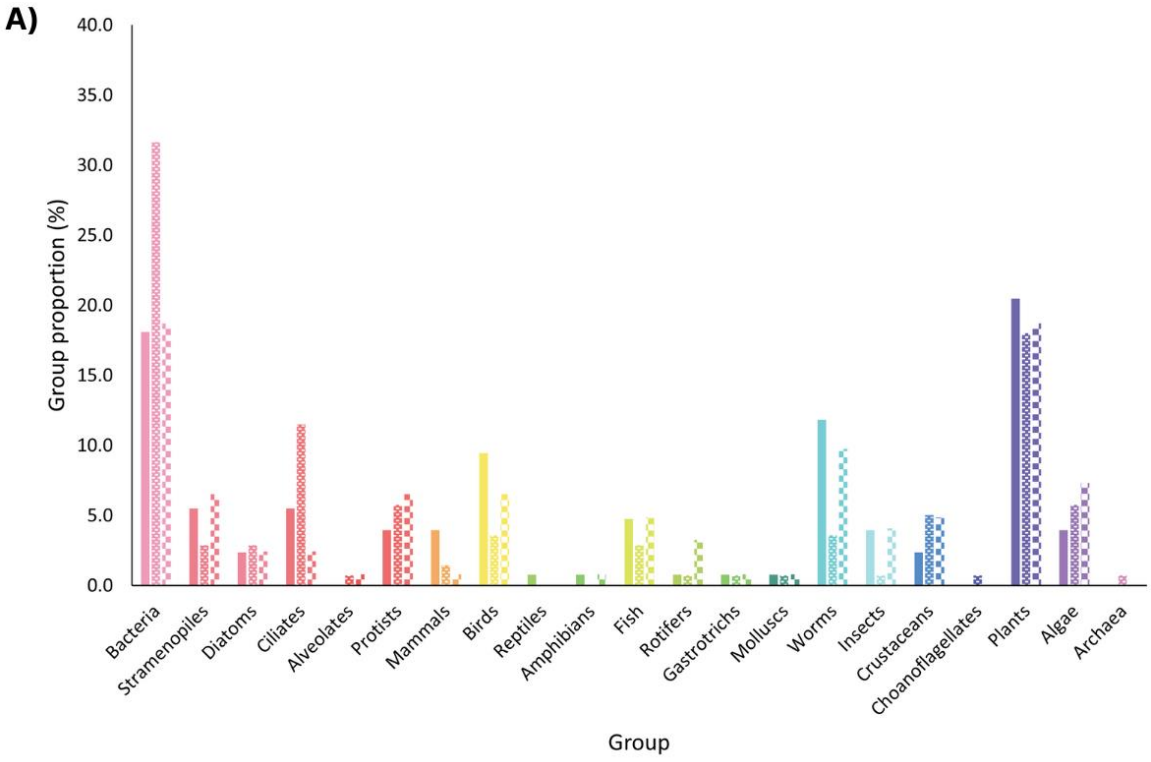
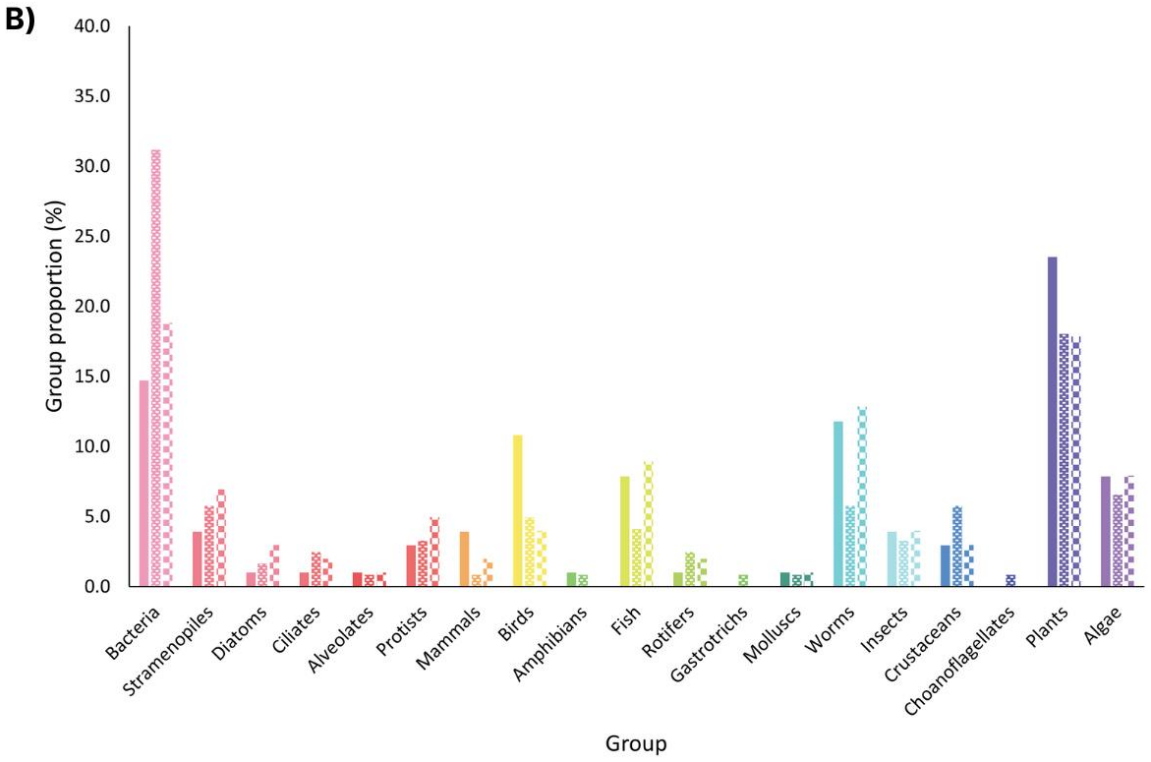
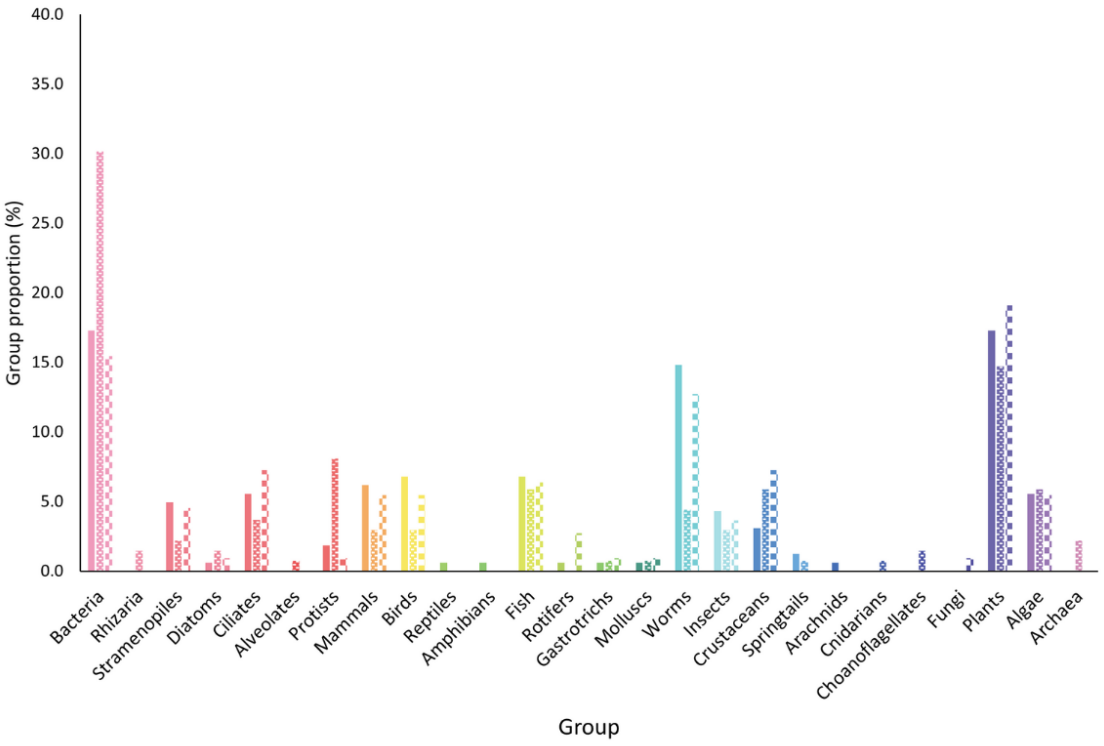


Figure A4.11 Bar graph comparing the start, middle, and end of spring at **A)** SS1, **B)** SS2, **C)** SS3, and **D)** SS4. Species groups are along the x-axis, and each group proportion against the group total in percentage along the y-axis. The proportion is based on the number of species detected at the species and genus level.





C)


40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Group proportion (%)
Bacteria
Rhizaria
Stramenopiles
Diatoms
Ciliates
Alveolates
Protists
Mammals
Birds
Reptiles
Amphibians
Fish
Rotifers
Gastrotrichs
Molluscs
Worms
Insects
Crustaceans
Springtails
Arachnids
Cnidarians
Choanoflagellates
Fungi
Plants
Algae
Archaea
Group

D)

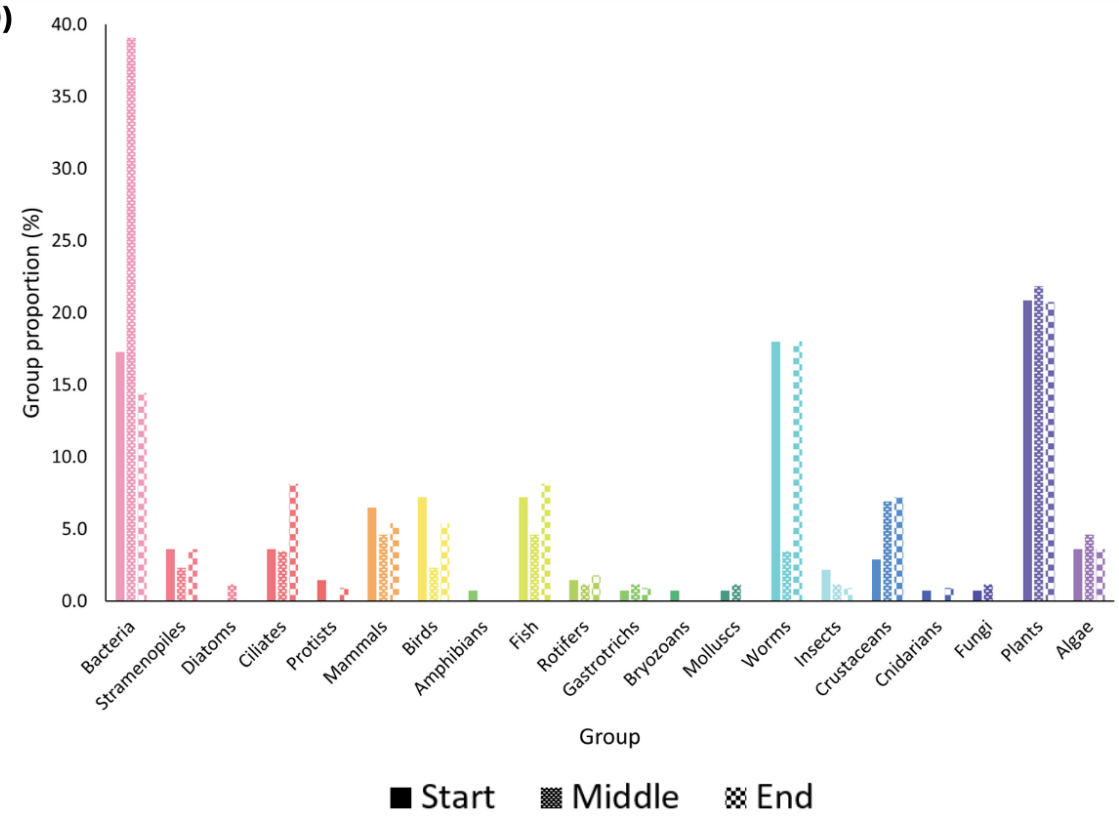

40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Group proportion (%)
Bacteria
Stramenopiles
Diatoms
Ciliates
Protists
Mammals
Birds
Amphibians
Fish
Rotifers
Gastrotrichs
Bryozoans
Molluscs
Worms
Insects
Crustaceans
Cnidarians
Fungi
Plants
Algae
Group
Start
Middle
End

Table A4.1 A description of the weather over the sampling period - obtained from AccuWeather - including the highest day temperature, weather description, max UV index, wind speed, wind gusts, probability of precipitation, probability of thunderstorms, precipitation, hours of rain, cloud cover, and the weather observed when present on site.

Date	Day highest temperature (°C)	Description	Max UV index	Wind (km/h)	Wind gusts (km/h)	Probability of precipitation (%)	Probability of thunderstorms (%)	Precipitation (mm)	Hours of rain	Cloud cover (%)	Observation
30/08/2022	15	Mostly sunny	4 Moderate	ESE 9	24	1	0	0	0	22	-
31/08/2022	16	Increasing cloudiness	5 Moderate	SSE 7	17	1	0	0	0	48	-
01/09/2022	16	Mostly cloudy	2 Low	N 6	17	1	0	0	0	97	-
02/09/2022	16	Clouds and sunshine	3 Moderate	NNW 9	22	2	0	0	0	72	-
03/09/2022	16	Cloudy with rain overspreading the area this afternoon	1 Low	NNW 15	37	86	0	7.3	3	99	-
04/09/2022	12	Some drizzle, sunny cloudy, cool	-	-	-	-	-	-	0		-
05/09/2022	13	Cloudy and cooler; showers, some heavy in the afternoon	1 Low	ESE 11	37	98	20	27.4	3	99	-
06/09/2022	12	Breezy this morning; otherwise, sun and some clouds	5 Moderate	S 15	43	0	0	0	0	43	-
07/09/2022	14	Warmer with intervals of clouds and sunshine	4 Moderate	NW 9	19	4	0	0	0	59	Sunny in morning, grey cloudy afternoon
08/09/2022	16	Partly sunny	4 Moderate	SSE 7	19	25	0	0	0	48	Sunny all day, partly cloudy

09/09/2022	16	Milder with increasing cloudiness	5 Moderate	SE 7	17	25	1	0	0	49	Foggy morning, sunny, partly cloudy
10/09/2022	15	Partly sunny	-	-	-	-	-	-	-	-	-
11/09/2022	18	Partly sunny, light rain in evening	-	-	-	-	-	-	-	-	-
12/09/2022	17	Clouds limiting sunshine	4 Moderate	NW 17	46	100	20	8.8	3	74	-
13/09/2022	18	Variably cloudy with shower; breezy this afternoon	4 Moderate	WNW 22	50	81	16	2.3	1.5	60	-
14/09/2022	15	Sunshine and few clouds	5 Moderate	WSW 11	26	25	0	0	0	28	-
15/09/2022	14	Partly to mostly sunny	5 Moderate	WSW 11	35	25	0	0	0	24	-
16/09/2022	15	Sun and some clouds; breezy this afternoon	5 Moderate	WSW 20	44	3	0	0	0	41	Blanket of light grey clouds, light-moderate wind, very soft spitting
17/09/2022	17	Sun giving way to increasing clouds and milder	5 Moderate	WNW 11	28	1	0	0	0	41	Foggy morning, sunny and partly cloud, light breeze mid-day
18/09/2022	17	Some sun, then increasing clouds	5 Moderate	NNE 9	20	1	0	0	0	63	Foggy morning, sunny and partly cloud, light breeze mid-day
19/09/2022	16	Cloudy; breezy in the morning with a brief shower or two followed by showers in the afternoon	1 Low	NNE 22	46	98	20	11	4.5	99	-

20/09/2022	20	Cloudy and warmer; a shower in spots late this morning followed by a couple of soaking showers this afternoon	3 Moderate	NNW 13	35	78	16	5.1	2.5	100	-
21/09/2022	19	Partly sunny	5 Moderate	NW 15	35	62	12	1.4	1	47	-
22/09/2022	18	Low clouds	1 Low	SE 13	35	25	0	0	0	100	Heavy rain over night
23/09/2022	17	More clouds than sun	-	-	-	-	-	-	-	-	Cloudy, partly sunny
24/09/2022	18	Clouds giving way to some sun	6 High	SW 15	43	25	0	0	0	58	-
25/09/2022	20	Clouds and sun, thickening clouds	4 Moderate	ESE 9	19	25	0	0	0	57	-
26/09/2022	16	Considerable cloudiness	2 Low	E 20	44	2	0	0	0	92	-
27/09/2022	18	Rather cloudy and milder	2 Low	ENE 15	37	8	0	0	0	87	-
28/09/2022	17	Mostly cloudy	3 Moderate	NE 17	37	25	0	0	0	88	-
29/09/2022	18	Mostly cloudy with a passing shower or two	3 Moderate	N 13	33	80	16	1.5	2	84	-
30/09/2022	18	Periods of rain	2 Low	N 11	28	91	18	8.4	5.5	100	-
1/10/2022	17	Periods of rain	2 Low	E 15	37	99	4	19.8	8	100	-
2/10/2022	19	Mostly cloudy, periods of rain	3 Moderate	NE 11	28	97	20	13.6	5.5	87	-
3/10/2022	17	Cloudy with a couple of showers	2 Low	W 19	46	84	17	3.3	2	95	-
4/10/2022	18	More sun than clouds	7 High	NNE 7	25	2	0	0		33	-

5/10/2022	15	Rather cloudy with a couple of showers this afternoon	2 Low	W 19	45	64	13	1.6	1	83	-
6/10/2022	12	Breezy this morning; otherwise partly to mostly sunny	7 High	SW 22	50	25	0	0	0	18	Cold night
7/10/2022	16	Mostly sunny and warmer	7 High	WSW 11	30	0	0	0		24	Very sunny, little clouds, no wind in morning but picked up mid-day
8/10/2022	17	Increasing cloudiness	7 High	N 11	20	2	0	0	0	48	-
9/10/2022	17	Low clouds	2 Low	ENE 13	35	1	0	0	0	93	-
10/10/2022	17	Low clouds	3 Moderate	NE 15	30	25	0	0	0	89	-
11/10/2022	-	-	-	-	-	-	-	-	-	-	-
12/10/2022	18	Cloudy with a brief shower in the afternoon	2 Low	ENE 9	20	58	12	0.9	0.5	100	-
13/10/2022	17	Cloudy; a morning shower in spots, then afternoon showers	2 Low	E 11	30	78	16	1.5	1.5	100	-
14/10/2022	15	Cloudy	2 Low	ESE 15	40	25	0	0	0	100	-
15/10/2022	-	-	-	-	-	-	-	-	-	-	-
16/10/2022	17	After a cloudy start, sunshine returns	8 Very High	SSW 15	45	2	0	0	0	39	-
17/10/2022	19	Intervals of clouds and sunshine with a couple of showers in the afternoon	4 Moderate	WNW 11	30	90	18	6	2	71	-
18/10/2022	18	Partly sunny	8 Very High	WSW 15	40	25	0	0	0	45	-

19/10/2022	16	A morning shower in spots; otherwise, mostly cloudy	6 High	WSW 17	50	60	12	1.1	0.5	75	Cloudy and sunny, moderate wind
20/10/2022	16	Windy this morning; some sun, then turning cloudy	5 Moderate	WSW 22	55	55	11	0.7	0.5	77	Cloudy and sunny, moderate wind. Light rain occurred three times with a duration of approximately 3 minutes
21/10/2022	17	Sun and clouds with a shower in spots in afternoon	6 High	SE 13	30	55	11	0.7	0.5	60	Cloudy and sunny, light to moderate wind
22/10/2022	18	Brilliant sunshine	9 Very high	S 9	20	0	0	0	0	5	-
23/10/2022	-	-	-	-	-	-	-	-	-	-	-
24/10/2022	20	Pleasant with a blend of sun and clouds	4 Moderate	SW 17	45	0	0	0	0	66	-
25/10/2022	-	-	-	-	-	-	-	-	-	-	-
26/10/2022	19	Mainly cloudy	2 Low	NNW 11	25	9	0	0	0	87	-
27/10/2022	21	Pleasant with intervals of clouds and sunshine	5 Moderate	NW 11	25	9	0	0	0	71	-
28/10/2022	22	Some sun, then turning cloudy with a little rain in the afternoon	7 Very high	N 17	40	58	0	1.3	1	83	-
29/10/2022	22	Cloudy	4 Moderate	N 19	45	65	13	1.6	1	95	-
30/10/2022	-	-	-	-	-	-	-	-	-	-	-
31/10/2022	22	Cloudy with a couple of showers, mainly later	4 Moderate	E 9	30	80	16	1.4	1.5	99	-

1/11/2022	23	Pleasantly warm with some clouds, then sunshine	9 Very High	WSW 11	30	1	0	0	0	36	-
2/11/2022	24	Some sun, then turning cloudy and pleasantly warm	6 High	N 15	35	4	0	0	0	75	-
3/11/2022	21	Occasional morning rain and drizzle; otherwise, overcast	2 Low	NNW 15	40	90	0	5.8	2.5	83	-
4/11/2022	21	Partly sunny	10 Very High	W 17	40	25	0	0	0	41	-
5/11/2022	-	-	-	-	-	-	-	-	-	-	-
6/11/2022	20	A little morning rain; otherwise, mainly cloudy	3 Moderate	ENE 17	40	59	0	1.4	1	87	-
7/11/2022	-	-	-	-	-	-	-	-	-	-	-
8/11/2022	-	-	-	-	-	-	-	-	-	-	-
9/11/2022	20	A morning shower in spots; otherwise, clouds breaking for some sun	9 Very high	NE 13	30	49	10	0.6	0.5	47	-
10/11/2022	-	-	-	-	-	-	-	-	-	-	-
11/11/2022	20	Winds gradually subsiding with rain	2 Low	ESE 24	67	100	24	32.3	12	100	-
12/11/2022	23	A little morning rain; otherwise, clouds giving way to some sun	9 Very High	NW 15	41	75	9	6.3	2	58	-
13/11/2022	20	Times of sun and clouds with a couple of showers, mainly early in the day	10 Very High	WSW 19	46	68	14	1.9	1.5	72	-

14/11/2022	23	Partial sunshine with a couple of showers in the afternoon	10 Very High	S 13	24	64	13	1.5	1	39	-
15/11/2022	21	Intervals of clouds and sunshine with a shower in spots	3 Moderate	E 11	30	64	13	2.3	2.3	1.5	-
16/11/2022	21	Considerable cloudiness; a couple of morning showers followed by heavy showers in the afternoon	2 Low	WNW 7	22	95	20	14.4	5	92	-
17/11/2022	19	Rather cloudy with heavy showers	6 High	N 11	28	99	29	22.8	6	87	-
18/11/2022	19	Periods of rain	6 High	NE 11	37	97	17	17.1	6	73	-
19/11/2022	-	-	-	-	-	-	-	-	0	-	-
20/11/2022	18	Mostly cloudy with showers	5 Moderate	NNW 13	32	98	25	18.1	7	88	-
21/11/2022	20	Cloudy with a brief shower or two	3 Moderate	NNW 13	37	83	17	3.4	2	92	-
22/11/2022	22	Variable cloudiness with afternoon showers	9 Very High	N 13	46	100	29	8.1	3	74	-
23/11/2022	18	Windy with a shower in places in the morning; sunshine and a few clouds	11 Extreme	W 22	54	40	8	0.2	0.5	26	-
24/11/2022	17	Variable clouds with showers	9 Very High	WNW 19	44	91	18	3.6	2.5	75	-
25/11/2022	17	A shower in places in the morning; otherwise, intervals of clouds and sunshine	10 Very High	W 17	43	43	9	0.4	0.5	55	-

26/11/2022	-	-	-	-	-	-	-	-	-	-	-
27/11/2022	17	Showers, some heavy in the morning; a thick cloud cover	3 Moderate	N 11	30	94	19	17.3	3	96	-
28/11/2022	18	Low clouds	3 Moderate	WSW 17	44	1	0	0	0	94	-
29/11/2022	-	-	-	-	-	-	-	-	-	-	Cloudy, partly sunny, no to little wind
30/11/2022	19	Clouds giving way to some sun with rain tapering off	7 High	WNW 15	41	96	19	10.2	4.5	69	-
1/12/2022	20	Mostly cloudy	10 Very high	SW 17	48	2	0	0	0	58	-
2/12/2022	19	Breezy this morning; otherwise, clouds giving way to some sun	7 High	SW 22	44	25	0	0	0	65	Cloudy and sunny, moderate wind
28/08/2023	13	Partly sunny	4 Moderate	SW 13	24	25	0	0	0	40	-
29/08/2023	14	Plenty of sunshine	4 Moderate	S 7	17	0	0	0	0	1	-
30/08/2023	15	Increasing cloudiness	4 Moderate	N 7	17	25	0	0	0	45	-
31/08/2023	-	-	-	-	-	-	-	-	-	-	-
1/09/2023	17	After a cloudy start, sun returns	4 Moderate	NE 9	22	0	0	0	0	34	-

2/09/2023	18	Mostly cloudy	2 Low	E 15	39	25	0	0	0	85	-
3/09/2023	17	Clear with periodic clouds	-	-	-	-	-	-	-	-	-
4/09/2023	16	Mostly cloudy and breezy	2 Low	ENE 22	52	25	1	0	0	85	Cloudy, moderate wind, very light intermittent spitting. Overnight rain; predicted 9.8 mm
5/09/2023	16	Cloudy in the morning with a couple of showers, then intervals of clouds and sunshine in the afternoon	2 Low	ENE 15	44	85	17	4.4	2	82	-
6/09/2023	19	Intervals of clouds and sunshine	4 Moderate	NNE 15	32	62	12	1.3	1	53	Cloudy, partly sunny. No wind. 11am started spitting.
7/09/2023	17	Clouds giving way to some sun	4 Moderate	NW 11	28	25	0	0	0	56	-
8/09/2023	19	Clouds giving way to some sun	5 Moderate	E 7	22	1	0	0	0	64	Foggy morning. Sunny, partly cloudy. Soft wind.

9/09/2023	19	Partly sunny and pleasant	4 Moderate	ENE 11	26	1	0	0	0	32	-
10/09/2023	18	Partly sunny with a passing shower or two	5 Moderate	ENE 9	28	80	16	0.6	1	46	Sunny, partly cloudy. Soft wind.
11/09/2023	18	Cloudy with occasional rain in the afternoon	1 Low	NNE 15	39	82	0	8.9	4	99	-
12/09/2023	16	Some sunshine giving way to clouds with occasional rain in the afternoon	5 Moderate	WSW 13	35	59	0	1.4	1	53	-
13/09/2023	17	Breezy this morning; otherwise, partial sunshine	5 Moderate	WSW 19	46	25	0	0	0	41	Mostly cloudy. Light to moderate wind.
14/09/2023	16	Some sun	4 Moderate	WSW 15	41	1	0	0	0	47	-
15/09/2023	16	Breezy with a couple of showers in the morning; otherwise, partly sunny	5 Moderate	WSW 19	48	64	13	1.6	1	44	-
16/09/2023	16	-	-	-	-	-	-	-	-	-	Calm, no wind. Very sunny

Table A4.2 Zooplankton identified from the traditional survey at SS2-SS4 from the start, middle, and end of spring. Taxa are ordered into major groups (Rotifers, Cladocerans, Copepods, and given lines for unidentified Ostracods and Tardigrades). All results are expressed in numbers per L. A total of 32 rotifer taxa, 4 copepod taxa, and 8 cladoceran taxa were found.

Species recorded	Start of spring			Middle of spring			End of spring		
	SS2	SS3	SS4	SS2	SS3	SS4	SS2	SS3	SS4
Rotifers									
Anuraeopsis fissa	0	0	0	0	0	0	0.19	4.33	47.22
Asplanchna brightwelli	0	0	0	0	0	0	0	0.33	1.11
Asplanchna girodi	0	0	0.44	0	0	0	0	0	0
Bdelloid rotifers	0.11	0	0.22	4.6	0	0.11	3.7	0.11	1.94
Brachionus angularis	0	0	0	0	0	0	0.19	0.11	1.94
Brachionus quadridentatus	0	0	0	0	0	0	0	0	0.56
Cephalodella ?intuta	0.11	0	0	0	0	0	0	0	0
Cephalodella catellina	0.11	0	0	0.2	0	0	0	0.33	6.11
Cephalodella gibba	0	0	0.11	0	0	0	0	0	0.56
Cephalodella sp.	0	0.11	0	0	0	0	0	0	0
Colurella uncinata	0	0	0	0	0	0	0	0	0.56
Epiphanes macrourus	0	0	0	0	0	0	4.26	0	0

Filinia longiseta	0.11	0	0	0	0	0	0.37	0.44	3.33
Gastropus hyptopus	0	0	3.33	0	0	0	0	0	0
Keratella procurva	0.33	1.78	0.56	0	0	0	0.37	35.11	3.61
Keratella slacki	0	0	0	0	0	0	0	0	1.39
Lecane bulla	0	0	0	0	0	0	0	0	1.39
Lecane closterocerca	0	0	0	0	0.11	0	0.37	0.11	0.11
Lecane hamata	0	0	0	0	0	0	0.56	0.22	0
Lecane lunaris	0	0	0	0	0	0	0.37	0	0
Lepadella ovalis	0	0	0	0	0	0	0	0	0.11
Lepadella patella	0	0	0	0	0.11	0	0	2.22	0.56
Lophocharis salpina	0	0	0	0	0.11	0	0	0	0
Parencentrum plicatum	0	0.11	0	0	0	0	0	0	0
Platyais quadricornis	0	0	0	0	0	0.11	0	0	3.33
Polyarthra dolichoptera	0	0.11	0	0.4	0	0	2.78	1.33	0.56
Squatinella mutica	0	0	0	0	0	0	0	1	1.39
Synchaeta oblonga	2.67	0	0.22	0	0	0	0.37	0	1.11
Synchaeta pectinata	0.89	4.33	15.11	0	0	0	1.3	0.33	1.39
Trichocerca brachyura	0	0	0	0	0.11	0	0	0	4.72
Trichocerca pusilla	0.11	0	0	0	0	0	0	0	0

Trichocerca tenuior	0	0	0.11	0	0.11	0	0	0	2.22
Copepods									
Acanthocyclops cf. *robustus*	59.89	0	0.89	2.2	1.78	5.33	52.04	1.22	19.44
Diacyclops bicuspidatus	0	0	0	0	0.22	0.56	0	0	0
Mesocyclops australis	0	0	0	0	0	0	0	3.33	0
Indeterminate harpacticoid copepod	0	0	0	0	0	0	0	0	0.28
Copepod nauplii	47	0.56	9.56	21.6	0.33	0.11	3.7	5.11	35.56
Cladocerans									
Bosmina meridionalis	0	0.11	0	0	0	0	0	0	0
Ceriodaphnia dubia	0	0	0.22	0.2	0	2.89	3.89	0.78	0.22
Chydorus sp.	0	0	0	0	6.89	1.33	0.37	0.44	0
Ilyocryptus sordidus	0	0	0.11	0	0	0	0	0	0
Leydigia ?australis	0	0	0	0	0	0.22	0	0	0
Pleuroxus hastirostris	0	0	0	3	0	0	0	0	0.56
Pseudomoina lemnae	0.22	0	0	0	0	0	0	0	0
Simocephalus elizabethae	0	0	0	2.4	85.89	38.89	0.37	0	0.56
Ostracods	0.11	0	0	1	0	0	0	0.11	0.28
Tardigrades	0.22	0	0	0	0	0	0	0	0.28

Table A4.3 The number of fish and invertebrate species caught in each Gee-minnow trap at SS3 and SS4 at the start of spring.

Species recorded	Trap 1	Trap 2	Trap 3	Trap 4	Trap 5	Trap 6	Trap 7	Trap 8	Trap 9	Trap 10	Trap 11	Trap 12
SS3												
Gambusia affinis (Mosquitofish)	5	4	3	18	22	21	11	34	53	1	13	5
Rhantus suturalis (Diving beetle)	0	0	0	0	0	0	5	3	2	2	1	0
SS4												
Dolomedes aquaticus (Water spider)	0	0	1	0	0	0	0	0	0	0	1	0
Gambusia affinis (Mosquitofish)	5	11	1	8	7	3	1	0	11	6	1	13
Neochanna diversus (Waikaka/Black mudfish)	0	0	0	0	0	0	2	0	0	0	0	0
Rhantus suturalis (Diving beetle)	0	4	0	0	2	0	1	1	2	0	1	2

Table A4.4 The number of fish and invertebrate species caught in each Gee-minnow trap at SS3 and SS4 in the middle of spring.

Species recorded	Trap 1	Trap 2	Trap 3	Trap 4	Trap 5	Trap 6	Trap 7	Trap 8	Trap 9	Trap 10	Trap 11	Trap 12
SS3												
Cyprinus rubrofuscus (Koi carp)	11	23	46	24	0	0	0	3	0	13	76	4
Dolomedes aquaticus (Water spider)	0	0	0	0	0	0	0	0	0	0	0	1
Gambusia affinis (Mosquitofish)	0	1	1	2	0	0	0	0	0	0	1	0
Potamopyrgus antipodarum (Freshwater snail)	0	11	0	1	0	7	0	13	0	0	2	0
Rhantus suturalis (Diving beetle)	0	1	0	1	1	0	0	0	0	0	0	0
SS4												
Cyprinus rubrofuscus (Koi carp)	0	0	0	0	0	0	0	0	0	1	0	0
Dolomedes aquaticus (Water spider)	0	0	0	1	0	0	1	0	0	0	1	0
Glycera sp. (Bloodworms)	0	0	0	2	7	0	6	0	0	12	0	0
Neochanna diversus (Waikaka/Black mudfish)	0	1	0	1	0	0	0	0	0	0	0	0
Potamopyrgus antipodarum (Freshwater snail)	0	12	0	0	0	0	5	0	0	6	7	0

Table A4.5 The number of fish and invertebrate species caught in each Gee-minnow trap at SS3 and SS4 in the end of spring.

Species recorded	Trap 1	Trap 2	Trap 3	Trap 4	Trap 5	Trap 6	Trap 7	Trap 8	Trap 9	Trap 10	Trap 11	Trap 12
SS3*												
Anguilla australis (Tuna/Short-finned eel)	0	0	0	0	1	0	0	1	0	0	0	0
Cyprinus rubrofuscus (Koi carp)	7	6	2	0	3	4	3	1	0	0	0	0
Dolomedes aquaticus (Water spider)	0	0	1	0	0	0	0	0	0	0	0	0
Gambusia affinis (Mosquitofish)	29	31	5	5	23	30	11	1	0	2 dead	6	1
Neochanna diversus (Waikaka/Black mudfish)	1	0	0	0	0	1	0	0	0	0	0	0
Rhantus suturalis (Diving beetle)	0	0	1	7	0	0	0	0	0	0	0	0
SS4												
Anguilla australis (Tuna/Short-finned eel)	0	1	0	0	0	1	0	0	2	0	1	3
Cyprinus rubrofuscus (Koi carp)	17	2	22	3	7	0	10	6	5	5	9	34
Gambusia affinis (Mosquitofish)	37	0	29	13	12	8	26	31	0	27	21	27
Neochanna diversus (Waikaka/Black mudfish)	1	0	2	2	1	0	1	0	1	0	2	0

*****Trap 9-12 coarse trap

Table A4.6 The number of birds recorded from the five minute bird count at SS1-SS4 in the middle of spring. The weather was warm and sunny, with light wind and some cicada noise.

Species recorded	**SS1/2, 11:20am**	**SS3, 10:55am**	**SS4, 10:35am**
Acanthis flammea (Redpoll)	0	1	0
Acridotheres tristis (Myna)	0	2	1
Branta canadensis (Canada goose)	0	0	Many
Chloris chloris (Greenfinch)	1	0	0
Chrysococcyx lucidus (Pīpīwharauroa/Shinning cuckoo)	0	1*	1*
Circus approximans (Kāhu/Swamp harrier)	0	1	0
Egretta novaehollandiae (Matuku moana/White-faced heron)	1	0	0
Emberiza citrinella (Yellowhammer)	0	1	0
Fringilla coelebs (Chaffinch)	2	2	1
Gerygone igata (Riroriro/Grey warbler)	2	1	3
Hirundo neoxena (Warou/Welcome swallow)	2	2	0
Porphyrio melanotus (Pūkeko/Swamphen)	0	1	0
Rhipidura fuliginosa (Pīwakawaka/Fantail)	2	0	1
Turdus philomelos (Thrush)	0	1	0
Zosterops lateralis (Tauhou/Silver eye)	1	1	2
Audio playback			
Botaurus poiciloptilus (Matuku/Australasian bittern)	0	0	0
Zapornia tabuensis (Pūweto/Spotless crake)	0	0	0

*Observed outside of 5-minute count time

Table A4.7 The number of birds recorded from the five minute bird count at SS1-SS4 at the end of spring. The air temperature was mild and there was 100% cloud cover, with moderate wind gusts.

Species recorded	**SS1/2, 11:20am**	**SS3, 10:40am**	**SS4, 10:25am**
Acridotheres tristis (Myna)	0	0	3
Branta canadensis (Canada goose)	2	2	Many
Chrysococcyx lucidus (Pīpīwharauroa/Shining cuckoo)	2	1	0
Circus approximans (Kāhu/Swamp harrier)	0	0	1
Cygnus atratus (Kakīānau/Black swan)	1	1	0
Emberiza citrinella (Yellowhammer)	1	3	0
Fringilla coelebs (Chaffinch)	3	3	4
Gerygone igata (Riroriro/Grey warbler)	1	0	2
Microcarbo melanoleucos (Kawaupaka/Little shag)	1	0	0
Passer domesticus (House sparrow)	1	0	0
Rhipidura fuliginosa (Pīwakawaka/Fantail)	2	2	0
Turdus merula (Blackbird)	1	1	0
Zosterops lateralis (Tauhou/Silver eye)	0	0	1
Audio playback			
Botaurus poiciloptilus (Matuku/Australasian bittern)	1	0	0
Zapornia tabuensis (Pūweto/Spotless crake)	0	0	0

Table A4.8 Summary notes from the botanist survey at SS2-SS4 in the middle of spring. Some plants were unable to be identified without seed heads or flowers.

Site	Summary notes
SS2	Off causeway. Fairly open canopy of dead willow with some scattered grey and crack willow and cabbage trees present. Groundcover included reed sweet grass, yellow flag iris, *Glyceria maxima*, *Carex virgata*?, *Galium* sp, *Callitriche* sp, Yorkshire fog, *Polygonum hydropiper*, *Muehlenbeckia australis*, *Juncus* sp and *Carex* sp, with alder trees and sprayed blackberry nearby. Fantail seen at this site, deer excrement observed.
SS3	A more open site with scattered crack willow canopy and sparse groundcover amongst large areas of pooled water, recent high water levels evident. Grey willow and gorse were present on the margin of pasture grasses along with *Lotus* sp, Plantain, buttercup etc. Both willow species were flowering at the time of survey. Species noted included: Yellow flag iris, reed sweet grass, *Polygonum hydropiper*, *Carex virgata*? (no seed head found).
SS4	Crack willow (flowering at the time of survey) and a few scattered cabbage trees formed a fairly dense canopy over a mosaic of pooled shallow water and ground cover vegetation (at the time of visit, although recent deeper inundation was evident from water lines and dieback of ground vegetation). Pasture grass was encroaching from the wetland margin at the base of the slope and reed sweet grass was also present under the willow. Groundcover included scattered sedges, rushes, herbaceous species and a few shrubs. Species noted were: *Carex virgata*, *Polygonum hydropiper*, *Juncus effusus*, *Galium* sp, *Carex* Sp, *Coprosma propinqua*, *Cyperus eragrostis*, *Azolla pinnata*, cabbage tree, wheki treefern, *Gnaphalium* sp, *Poa* sp. Deer and geese excrement were noted.

Table A4.9 Observed p-values for pairwise comparisons between 1.2 µm, 5 µm, and dacron filters at the start, middle, and end of spring across the entire dataset, at species-genus level, and the key species of interest.

Filter pairwise comparison	Start	Middle	End
Entire dataset			
1.2 µm vs. 5 µm	0.852	0.224	0.039
1.2 µm vs. Dacron	0.010	0.047	0.185
5 µm vs. Dacron	0.045	0.002	0.580
Species-genus			
1.2 µm vs. 5 µm	0.340	0.117	0.050
1.2 µm vs. Dacron	0.397	0.761	0.850
5 µm vs. Dacron	0.845	0.363	0.556
Key species of interest			
1.2 µm vs. 5 µm	0.144	0.850	0.117
1.2 µm vs. Dacron	0.751	0.995	0.941
5 µm vs. Dacron	0.421	0.917	0.527

Table A4.10 The observed p-values for pairwise comparisons between SS1-SS4 at the start, middle, and end of spring across the entire dataset, at species-genus level, and the key species of interest.

Spatial pairwise comparison	Start	Middle	End
Entire dataset			
SS1 vs. SS2	0.209	0.487	0.228
SS1 vs. SS3	0.420	<0.001	0.014
SS1 vs. SS4	0.208	<0.001	0.097
SS2 vs. SS3	0.025	0.005	0.446
SS2 vs. SS4	0.012	0.002	0.867
SS3 vs. SS4	0.520	0.394	0.185
Species-genus			
SS1 vs. SS2	0.934	<0.001	0.031
SS1 vs. SS3	0.930	<0.001	0.627
SS1 vs. SS4	0.518	<0.001	0.915
SS2 vs. SS3	0.995	<0.001	0.030
SS2 vs. SS4	0.291	<0.001	0.029
SS3 vs. SS4	0.190	<0.001	0.551
Key species of interest			
SS1 vs. SS2	0.125	0.018	0.041
SS1 vs. SS3	0.495	0.966	0.964
SS1 vs. SS4	0.096	0.772	0.911
SS2 vs. SS3	0.235	0.046	0.109
SS2 vs. SS4	0.768	0.197	0.042
SS3 vs. SS4	0.182	0.787	0.904

Table A4.11 The observed p-values for pairwise comparisons between the start, middle, and end of spring across the entire dataset, at species-genus level, and the key species of interest.

Temporal pairwise comparison	SS1	SS2	SS3	SS4
Entire dataset				
Start vs. Middle	0.502	0.216	0.005	0.010
Start vs. End	0.009	0.021	0.914	0.649
Middle vs. End	0.002	0.203	0.005	0.004
Species-genus				
Start vs. Middle	0.388	0.268	<0.001	0.037
Start vs. End	0.546	0.026	0.354	0.579
Middle vs. End	0.676	0.096	0.002	0.047
Key species of interest				
Start vs. Middle	0.169	0.063	0.342	0.127
Start vs. End	0.408	0.090	0.705	0.628
Middle vs. End	0.547	0.849	0.697	0.104