The potential for submerged macrophyte recovery from the seed bank of the shallow coastal Whakakī Lake, Aotearoa

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Abstract

Shallow lakes provide a range of ecosystem services, including habitat for waterfowl, fish, aquatic plants, and invertebrates, and have significant recreational, aesthetic, and cultural value. Eutrophication is one of the leading causes of shallow lake ecosystem degradation globally. Excess input of nutrients, especially phosphorus and nitrogen, can cause a regime shift where the ecosystem switches from a macrophyte-dominated system to one driven by primary production from phytoplankton. The naturally occurring communities dominated by aquatic macrophytes as primary producers undergo a drastic change, flipping to an algae-dominated state that can result in the degradation or disappearance of natural plant and animal communities. Whakakī Lake is a shallow coastal lake in northern Hawke's Bay. This lake is in a highly degraded, hypertrophic state that no longer supports a community of submerged macrophytes. Previous work on the macrophyte community of Whakakī Lake in 1992 and 2007 provides an idea of the original condition of the macrophyte communities and the gradual decline in abundance and diversity that preceded the current conditions. Sediment coring at four sites along a transect was conducted in Whakakī Lake to quantify and characterise the seed and oospore bank of submerged macrophytes. A diverse and abundant seed bank was identified with 12 species of macrophytes and charophytes found throughout the lake. The highest abundance of seeds and oospores was located on the northern edges of the lake shore, near the Tuhara Stream inlet. Germination trials using the seeds and oospores collected from the seed bank were run over three months under controlled

conditions to assess the viability of the Whakakī Lake seed bank. Species geminated under three salinity treatments: zero, low and moderate salinity levels. Light availability was altered to assess the impact of reduced light (photosynthetically available radiation) on species germination. The lack of germination success of seeds under severely reduced light levels and complete darkness demonstrated how high turbidity and lack of light is hindering seed germination within Whakakī Lake. With improvements to water quality, specifically the reduction of external and internal nutrient loads and increased water clarity, it is possible a submerged macrophyte community could re-establish within Whakakī Lake based on seeds and oospores available within the seed bank.

Acknowledgments

This project would not have been possible without the support of many people. Firstly, a huge thank you to my co-supervisor Dr Andy Hicks (HBRC), for your guidance, support, encouragement, and endless great ideas. I am proud of and grateful for my time working with you. Thank you to my supervisor, Professor Russell Death (Massey University), for your guidance and advice throughout this project. I acknowledge the generous financial support from the Hawke's Bay Regional Council, which gave me the opportunity to pursue a master's degree on the side of a full-time job. I gratefully recognise the help of Shaun Wilkinson and the team at Wilderlab for the eDNA analysis you provided and for your willingness to entertain my crazy ideas. To Mary de Winton, whose previous work at Whakakī Lake was the building blocks for this research – thank you for your assistance in planning and expertise on macrophytes and charophytes in the early stages. I couldn't have managed without the help of some friends. Thanks to Jordan Ellmers and Dan Fake for the early mornings, late finishes, heavy lifting, and continuous moral support. To the rest of my HBRC team, Vicki Lyon, Sandy Haidekker, Andrew Horrell, Jane Gardiner, and McKay Smiles, for enduring tanks and tents filled with miscellaneous aquatic plants spread around the office, and always meeting thesis-related fretting with encouragement and enthusiasm. To Anna Madarasz-Smith, Becky Shanahan, Anton Kelly, and Eliah Murdoch-Pike, thank you for your statistical wisdom, willingness to help and unconditional support. Immense gratitude to Cole for his patience, support, and only minor protest when I returned home with a tank full of aquatic plants, ready to add to the family.

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Chapter I

Eutrophication in Shallow Lakes

1.1 Shallow lake eutrophication - a global issue

Intensification of agriculture globally has had detrimental impacts on non-agricultural terrestrial and aquatic ecosystems worldwide. A doubling of agricultural food production during the past 35 years is associated with a 6.87-fold increase in nitrogen fertilisation, a 3.48-fold increase in phosphorus fertilisation, a 1.68-fold increase in the amount of irrigated cropland, and a 1.1-fold increase in land in cultivation (Tilman, 1999). Marine and freshwater systems will likely be impacted the most by this agricultural intensification, experiencing eutrophication from the high nitrogen and phosphorus release rates from agricultural fields. Eutrophication of freshwater environments can lead to loss of biodiversity, outbreaks of pest species, shifts in food chain structure and impacted fisheries (Tilman, 1999, Lijklema,1994).

Shallow lakes are one of the most abundant lake types, usually occurring in lowland areas and often where the most there has been intensification of land use has occurred. Shallow lakes are less than 10 m deep, are generally well mixed throughout the year, and are highly productive environments (Scheffer, 2004, Scheffer *et al.*, 2007). Shallow lakes provide a disproportionate contribution to biodiversity, providing habitat for waterfowl, fish, aquatic plants, and invertebrates and provide ecosystem services such as carbon sequestration, nutrient cycling, and food production (Beklioğlu *et al.*, 2016). Due to the depth of shallow lakes, they are sensitive to changes in water level, land-use change and other natural and anthropogenic influences. Shallow lakes worldwide are experiencing the effects of climate change, intensification, eutrophication, and other pollution, leading to biodiversity loss and changes in their ecosystem functions. The increased potential for droughts associated with climate change will put further pressure

on shallow lake ecosystems and may exacerbate water level reduction, intensifying eutrophication and salinisation (Beklioğlu *et al.*, 2016).

Eutrophication is one of the leading causes of global degradation of shallow lake ecosystems, where biological processes are altered by increased nutrient supply of nitrogen and phosphorus (Gluckman, 2017). The "Fifth Global Environmental Outlook" report by the United Nations Environmental Program found more than 40% of global water bodies are impacted by moderate or heavy eutrophication (Xia *et al.,* 2016). Eutrophication creates an ecological system dominated by primary producers (phytoplankton, cyanobacteria, aquatic plants), leading to hypoxic or anoxic conditions, loss of biodiversity, poor water quality and detrimental impacts on human health, recreational and aesthetic values. Lake eutrophication has been observed globally in industrialised countries as early as the start of the twentieth century; however, a global focus on climate change and the health of freshwater ecosystems has made lake eutrophication an important social and political issue (Xia *et al.,* 2016). Agriculture and urban areas are the primary sources of these nutrients in freshwater (Carpenter *et al.,* 1998, Lijklema,1994). Simplification of species' communities due to eutrophication structure can drive lake ecosystem instability. Once a critical turbidity threshold is reached, a clear water lake with a primary producer community dominated by macrophytes may flip to an alternative stable state dominated by phytoplankton (Scheffer *et al.*, 2007). Turbid water conditions reduce the amount of light (photosynthetically available radiation), shading out rooted plant species and leading to macrophyte extinction. The loss of sediment stabilising root systems can further contribute to high turbidity in shallow lakes through increased resuspension of lake sediments.

Lake sediments are an essential aspect of water quality in shallow lakes and act as a sink for nutrients from the surrounding catchment. Vertical stratification in shallow lakes is absent or intermittent, and there is potential for direct transfer of nutrients from these sediments to the lake's surface at any time of year. Internal phosphorus loads can be high in shallow lakes and may exceed external loads on an annual basis in lakes with highly enriched sediments due to high nutrient legacy loads (Jeppesen *et al.*, 2007). With little or no macrophytes to stabilise sediment, wind-driven resuspension of the lakebed can cause high benthic stress and push nutrient-enriched sediment into the water column, further boosting phytoplankton growth and production. Other forms of nutrient release from lake sediments include the anoxia-mediated release of phosphorus, and bioturbation (Welch and Cooke, 1995), e.g., from burrowing fish species. Despite significant research into lake eutrophication in recent times, it remains an important concern worldwide, with more than 40% of lakes affected by eutrophication and algae blooms (Yang *et al*., 2008). Climate change poses a further threat to intensifying eutrophication in lakes globally, with greater mean temperatures, higher internal loading associated with deoxygenation caused by warm temperatures, reduced lake depth from droughts, and high nutrient loads from storm flows (Moss 2011).

1.2 Eutrophication of shallow coastal lakes in New Zealand

New Zealand has approximately 900 known shallow lakes that are < 10 m deep and within 25 km of the coast. Under natural conditions, most of New Zealand's shallow coastal lakes were likely clear water lakes that supported rich macrophyte populations (Drake *et al.*, 2009). Nutrient load from New Zealand's increasingly intensive agricultural land and the presence of pastoral land in a catchment has been shown to

correlate with regime shifts in New Zealand lakes (Schallenberg and Sorrell, 2009), (Abell, *et al.,* 2011). Land-use change, clearing of natural vegetation, alterations for drainage, and poor agricultural practices are contributing to the decline of coastal lakes around the country (Drake *et al.*, 2010).

Excess phosphorus is the most common cause of lake eutrophication in New Zealand. Phosphorus binds with soil and dissolves slowly over time and in most cases, does not readily leach through the soil profile. Excessive fertiliser use can cause phosphorus to accumulate in soils (Bennett *et al*., 2001); during rainfall events, these enriched soils are washed into lakes from the surrounding catchment through runoff and erosion (Parfitt *et al.,* 2008). Even soils with good phosphorus retention can only hold a limited amount, and excessive fertiliser use can supersaturate soils, at which point the phosphorus is readily leached. Phosphorus is more likely to leach through organic or peat soils, which is the soil type that commonly occurs around shallow lakes. Once phosphorus has entered a lake, it can stimulate algae growth and cause toxic cyanobacteria blooms. New Zealand has experienced considerable land-use changes in the past ~100 years, and a high proportion of land has been converted to high-intensity pastoral land (Ewers *et al.,* 2006). In 2019, an estimated 7,151 tonnes of phosphorus fertiliser was applied in Hawke's Bay, and an average dairy farm operation will apply more than 500 kg of phosphorus most years. Orchards and crops require more phosphorus than pasture ("Fertilisers – nitrogen and phosphorus | Stats NZ").

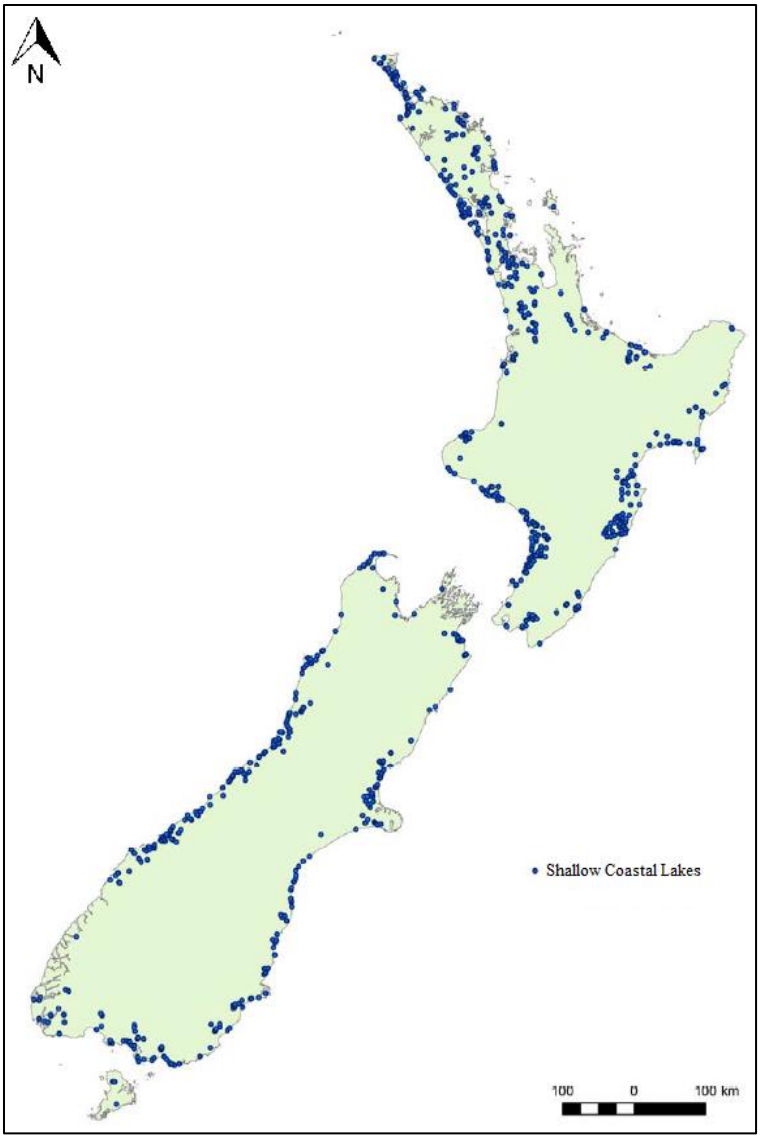

Figure 1. 1 Locations of approximately 900 known shallow, coastal lakes in New Zealand (lakes in the subantarctic islands and the Chatham Islands are not shown)

Excess nitrogen contributes to lake eutrophication in New Zealand through nitrogen-fixed legumes, fertiliser (Parfitt *et al*., 2008) and high-intensity land use such as beef, dairy, and pastoral farming (Ledgard *et al.,* 2019). Most of the nitrogen lost from pastoral systems is through urine from stock, not fertiliser. Agricultural Intensification

has meant more stock per hectare, which has led to more urine production. The nitrogen in stock urine is highly concentrated, equivalent to applying ~1000 kg of nitrogen per hectare (McCauley, 2020). Shallow lake ecosystems can become nitrogen enriched through applied fertiliser or atmospheric pollutants, as nitrogen can be fixed from the atmosphere – which may promote further phosphorus limitation in lake systems. Coastal lakes have been frequently overlooked in environmental science, monitoring, and restoration. These ecosystems provide essential habitat for native fish species, invertebrates, birds, and macrophytes and often have rich cultural histories and significance to tangata whenua.

Shallow coastal lakes are a commonly overlooked component of New Zealand's freshwater resource, and less is known about the natural conditions of shallow coastal lakes in New Zealand compared to other freshwater systems. However, a study by Kelly *et al.* (2011) found that lakes in disturbed catchments generally had a high TLI (trophic level index), higher pH, reduced light, less submerged macrophyte cover, smaller food webs, lower rotifer diversity and higher proportions of introduced fish species. The pressures faced by shallow coastal lakes in New Zealand are reflected in their high trophic level indices.

Located 15 km north of Wairoa, Whakakī Lake is a severely impacted ecosystem, which was once part of an extensive wetland system with high ecological, recreational, and cultural values. Today, Whakakī Lake is a hypertrophic, algae dominated shallow coastal lake with the highest known TLI (trophic level index) of any other in New Zealand (Fig 1.2).

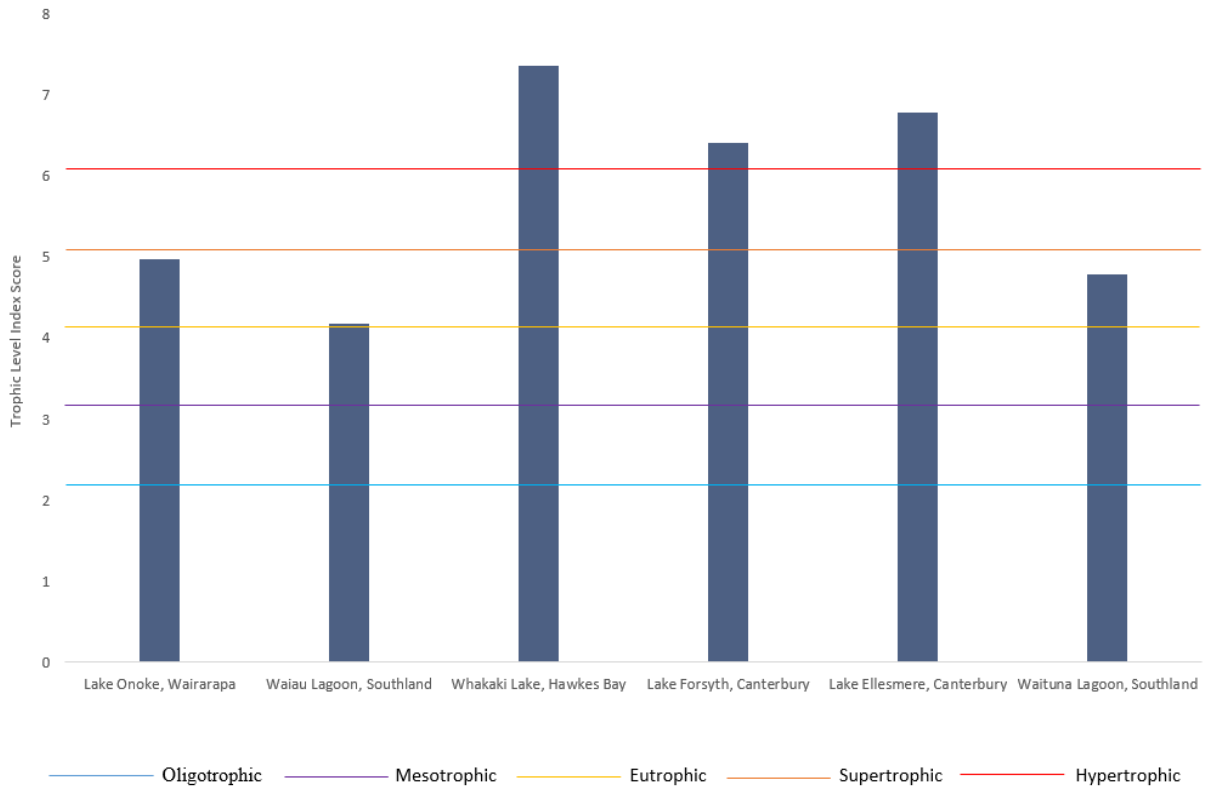

Figure 1. 2 Trophic Level Index Scores (TLI) from six coastal ICOLL lakes in New Zealand. Average TLI reported. Data sourced and modified from LAWA (lawa.org.nz/explore-data/lakes) Data sourced over 15-year period

1.3 Thesis structure

My thesis research characterises the submerged macrophyte seed bank of Whakakī Lake and assesses the viability of the seeds under current and manipulated conditions. This research sought to test several hypotheses, which are described in more detail in the specific chapters. Chapter II will examine the historical management of Whakakī Lake, and the impacts of this on water quality, chapter III will explore an in-

depth analysis to characterise the seed and oospore bank of Whakakī Lake through sediment coring, while chapter IV will examine a series of experiments to assess the viability of the seed and oospore bank.

During the seed bank analysis, my objectives were to 1) quantify the seeds found at each site within the lake 2) describe and characterise species composition and abundance at each site, and 3) compare species abundance and distribution throughout the lake. I expected seeds and oospores would be evenly distributed throughout the lake, and species composition would be relatively similar between all four sites. I expected to find seeds representative of the species last described as being present in Whakakī Lake by de Winton & Champion (2008).

During my 2-month germination trials, I hypothesised that if seeds germinated, they would do so only under full light conditions and not under limited or no light. I also expected that if seeds and oospores germinated, they would germinate under all salinity conditions, but the species composition would vary between salinities based on individual species requirements. My thesis includes a chapter describing my study area (chapter II), touching on essential details of the historical management of the area and its significance to the community; this chapter gives context to the importance of the restoration of this lake. The thesis ends with a general discussion (chapter V) which draws together my findings from the experimental chapters, considers the limitations of my research and provides advice for further research into the macrophyte community of Whakakī Lake and the potential steps required for restoring a healthy and functioning macrophyte community.

Chapter II

Te roto o Te Whakakī

2.1 Study Area – Whakakī Lake

Whakakī Lake is a large 450 ha, shallow coastal lake (< 2 m deep) separated from the sea on its southern shore by a narrow strip of gravel dunes, with an additional 200 ha of adjacent wetland margin. The lake is the largest remaining water body of a once extensive 6,000 ha wetland system (Fig. 2.1) east of Wairoa and is the largest coastal lake on the North Island's East Coast (Hawke's Bay Regional Council, 2018).

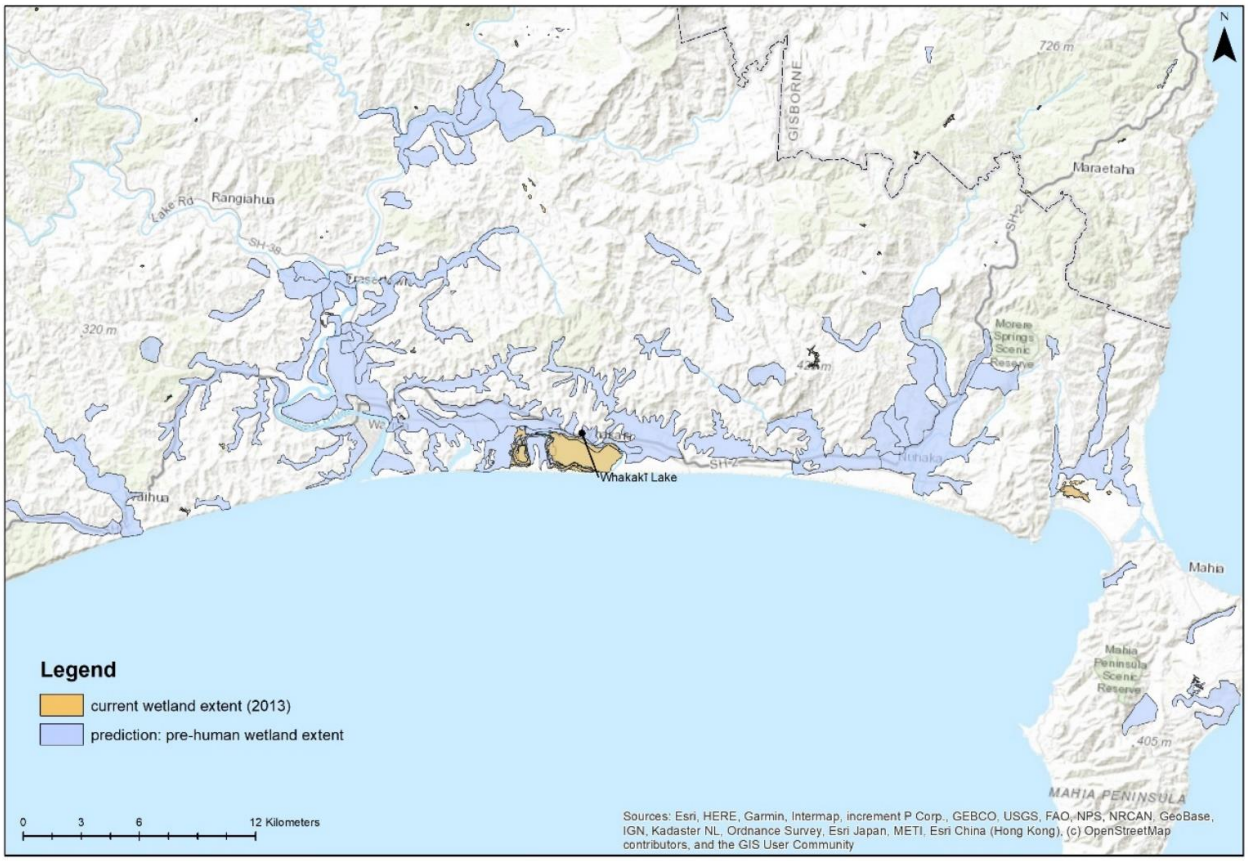

Figure 2. 1 Map depicting the current (2013) and pre-human (predicted based on soil information from the New Zealand Land Resource Inventory (NZLRI) and a 15m digital elevation model (DEM) to refine soil boundaries) wetland extent in the Wairoa - Nuhaka regions surrounding Whakakī Lake. Data sourced and adapted from LINZ https://data.mfe.govt.nz

Table 2. 1 Characteristics of Whakakī Lake extracted from HBRC and LINZ database. Data range of water quality data sourced between 4/06/2015 – 7/07/2021

Lake surface area (km^2)	4.75
Depth (m)	≤ 1.5m
Volume (x1000 m^3)	1582
Catchment area (km^2)	32
Average Secchi (m)	0.13
Average Turbidity (FNU)	115.03
Average Conductivity (spc)	5150.63

Drainage and flood works have reduced the wetlands and disrupted the natural hydrological connections, reducing the system to only 10% of its original area (HBRC, 2018). Whakakī Lake is part of a much larger wetland complex that includes the Ngamotu, Ohuia, Waihoratuna, Wairau, Te Paeroa, and Patangata Lagoons. Whakakī Lake is an intermittently closed and open (ICOLL) system, with natural drainage through the Rāhui channel to the east of the lake. These intermittent openings directly to the Pacific Ocean make Whakakī Lake a unique habitat type in New Zealand and globally because of the variations in water level, temperature, and salinity (HBRC, 2018). Whakakī lake is classified as a Waituna-type lake (2a), a large, shallow, coastal lake barred from the sea by a barrier or beach that is generally closed to the sea unless opened artificially (Hume *et al.,* 2016). These systems are typically freshwater and fed by small inlet streams. Drainage to the sea is generally by filtration through the barrier. Periodic openings occur when water levels build a sufficient hydraulic head in the lake to breach the barrier, such as river inflows and severe storm swell overtopping. Wind waves and wind-induced currents are important for mixing the water column. Observations of

historical lake ridges suggest that these agents were even more critical in pre-human times when the depth and fetch of the water bodies were greater than today. Subclass 2a is recognised as coastal plain depressions and is the most common type occupying depressions on low-lying coastal land that were typically coastal embayment's during the early Holocene but have since been isolated from the ocean by barriers (Hume, 2016). Twelve other systems were classified in New Zealand as a 2a system, including the neighbouring lagoons to Whakakī lake that were once part of the extensive wetland complex (Fig 2.2).

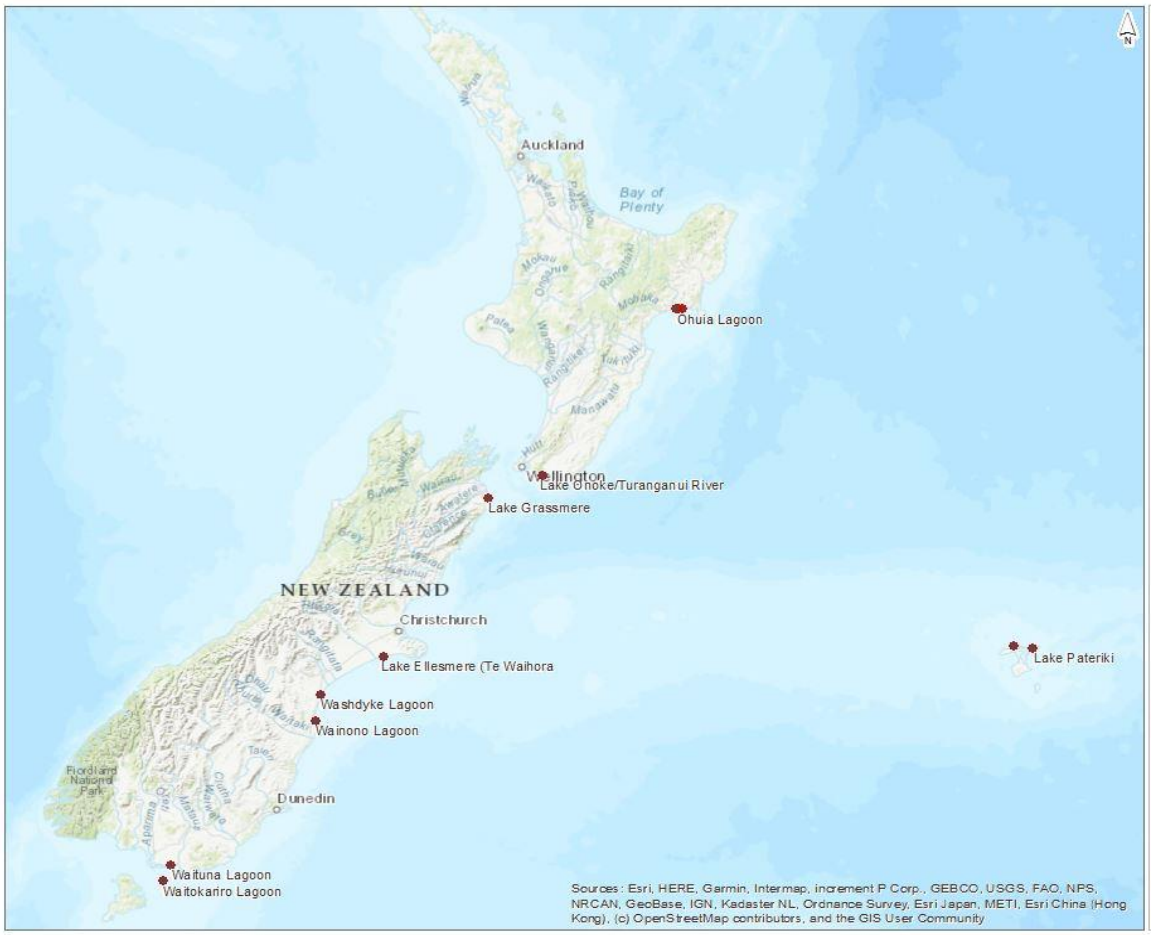

Figure 2. 2 Map depicting all 2a hydrosystems in New Zealand as described by NIWA (2016). Data sourced and modified from NIWA (2016) A classification of New Zealand's coastal hydrosystems.

Water levels in the lake have been actively managed by the creation of direct-to-sea openings by regional authorities for flood management and drainage of adjacent farmlands, roads, and railways since the late 1950s (de Winton *et al.,* 1992, Woods *et al.*, 1993). Whakakī is an area of cultural and spiritual significance for local iwi and hapū and is considered taonga tuku iho, cultural property and heritage (Fig. 2.3). The lake is important to tangata whenua for mahinga kai, with tuna being an important harvest historically and today. Once a life source, Te roto o Te Whakakī is now key to hapū identity (Forster, 2012).

Figure 2. 3 Māori village and canoe, on Whakakī Lagoon. Williams, Edgar Richard, 1891-1983: Negatives, lantern slides, stereographs, colour transparencies, monochrome prints, photographic ephemera. Ref: 1/1-025561-G. Alexander Turnbull Library, Wellington, New Zealand /records/22902900

Ngāti Kahukura, Ngāti Kirituna and hapū of Te Whakakī Nui-a-Rua have cultural associations with the lake. Whakakī Lake and its extended wetland complex are important wildlife and fish habitat, being rated as a nationally significant wildlife habitat in the Wetlands of Ecological and Regional Importance (WERI) database by the Department of Conservation in 1986. The lake supports fish populations of long and shortfin eels, inanga, common bully, flounder, and mullet.

2.2 Historical management

The history of land drainage around Whakakī Lake began before the 1900s and accelerated between 1945 and 1975, with the hillside in the catchment deforested and developed by the early 1940s (Woods *et al.,* 1993). The water level in the lake has been periodically controlled since the early 1900s by Māori through the Patangata Lagoon via the Rāhui Channel at a site known as Paakaa. From 1907 the Whakakī Drainage Board and Wairoa County Council funded and undertook the openings at this site. The establishment of the Whakakī Drainage Board and work completed by the Ministry of Works saw dramatic changes to the landscape surrounding Whakakī Lake, such as the confinement and channelization of streams, and the conversion of swampy wetland areas to dry land (Woods *et al.,* 1993).

Both tangata whenua and local government recognised the desire to address flooding around the Whakakī community; however, the focus of the District Commissioner was to drive land drainage and promote development. Some believed the Rāhui Channel could not remain open for long enough to provide the required drainage, partly due to the gradual silt deposition in the lakebed near the Patangata outlet, and that

a direct-to-sea option would provide a more effective way of maintaining low water levels within the lake. Tangata whenua recognised the need to address flooding and issues created by road and catchment changes, however, were never happy with the idea of a direct-to-sea opening. Promises were made that summer water levels would be maintained if the direct-to-sea drainage option was to proceed (Woods *et al.*, 1993).

The first direct opening occurred in August 1956. Two sites along the lake's southern shore were created, Te Awa Waahi – the 'Winter' site and the 'Summer site' to drain the lake directly to the sea. The Wairoa Country Council and Whakakī Drainage Board maintained openings direct to sea until 1963, when the Hawke's Bay Catchment Board took over. By the 1970s, concern from tangata whenua was raised over the new direct drainage site due to low water levels and the impacts on wildlife and plant communities. Tangata whenua had little to no involvement in the openings of Whakakī Lake, and letters from the Catchment Board suggest an unclear understanding and no process for managing the openings at either the 'summer site' or Paakaa. Occasional openings at the Paakaa site took place over the early 1970s in conjunction with openings at the summer site. Increasing development of farmland around the lake contributed to demand for openings to reduce flooding and retain highly productive land, resulting in the lake being further drained to lower levels. Between November 1976 and August 1992, there were 66 direct-to-sea openings, ranging from one to six per year. Since 1957 there have been several written accounts of complete dewatering after openings to the sea; however, records do not detail the extent or duration of dewatering. Prolonged dewatering would clearly disrupt the lake ecosystem and stress aquatic species significantly.

Over the late 1960s through to the late 1980s, the direct opening continued to be used, focusing on setting maximum and minimum water levels in the lake at RL 10.50-11.80 (Woods *et al.*, 1993). From 1989 Hawke's Bay Regional Council (HBRC) took over the management of the lake openings (Woods *et al*., 1993). Artificial openings through the direct outlet were abandoned in 1997, and natural connections through the Rāhui Channel were reinstated due to lobbying from the Whakakī Lake Trust. Currently, the lake is periodically opened in autumn and winter for flood control and drainage through the Patangata Lagoon via the Rāhui Channel. Since 1997 the water level has remained more consistent, and plans are underway to install a weir on the Rāhui Channel to maintain a stable water level within the lake.

2.3 Whakakī lake water quality

Whakakī Lake is currently in a highly degraded state, with a trophic level index (TLI) score above 7 for the past six years (Fig 2.4). A TLI score of ≥ 7 puts Whakakī Lake well into the realm of hypertrophic, where the lake has very high amounts of phosphorus and nitrogen with poor water clarity and excessive algae growth (Burns *et al.*, 1999).

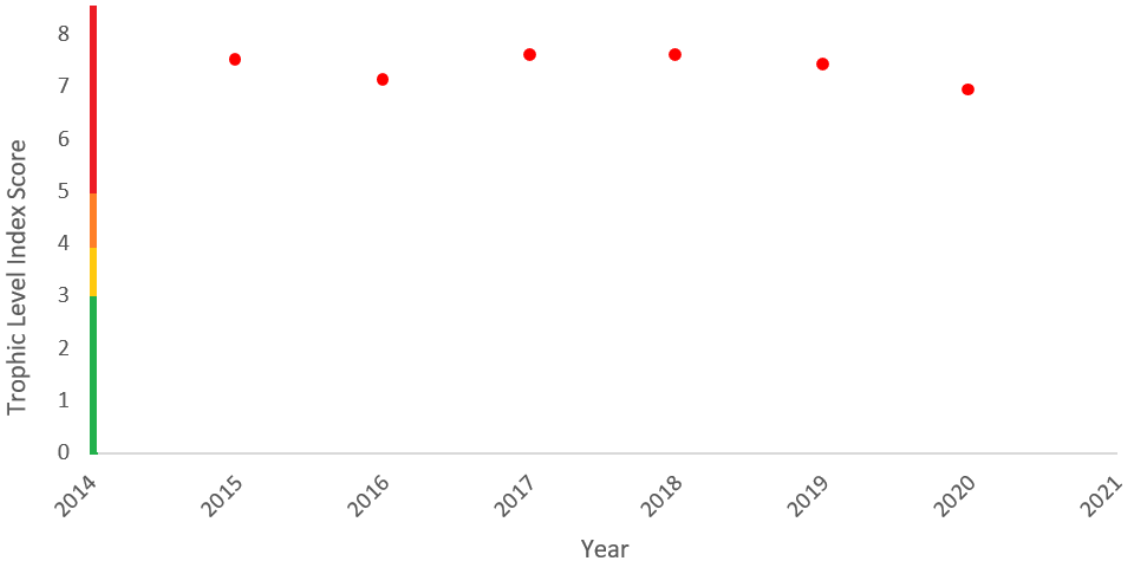

Figure 2. 4 Trophic Level Index (TLI) score for Whakakī Lake from 2015 - 2021. Data sourced and modified from LAWA. A TLI of 0-1 = very good or microtrophic, 2-3 = Good or oligotrophic, 3-4 = Fair or mesotrophic, 4-5 = Poor or eutrophic, 5-6 = very poor or supertrophic. >6 = hypertrophic or extremely degraded

The Hawke's Bay Regional Council currently undertakes monthly water quality sampling at Whakakī Lake, which began in 2015. Monthly monitoring results for critical water quality parameters such as total phosphorus, total nitrogen, chlorophyll-a, cyanobacteria, and water clarity indicate that all parameters often or always fall below the National Bottom Line outlined in the National Policy Statement for Freshwater Monitoring 2020 (NPSFM). Under regulations outlined in the NPSFM 2020, if a regional council identifies that an FMU or part of an FMU is degraded or degrading, they must take action to halt or reverse the degradation. The water quality results below in Fig. 2.5 outline the lake's continuously high total phosphorus and total nitrogen levels. These

high nutrient levels can be kept in the sediment within the lake and continuously made available through the mixing of the lake, reinforcing the cycling effect nutrient-enriched lakes often find themselves in. Persistent high levels of phosphorus and nitrogen only act to enhance algae and cyanobacteria growth (Serediak *et al.*, 2014; Vézie *et al.*, 2002). Total Phosphorus and Total Nitrogen in g/m^2, are presented in Fig. 2.5 which are the substrate bound forms of these nutrients and are less bioavailable than the dissolved forms, DIN and DRP. High levels of chlorophyll-*a* above the NPSFM national bottom line and frequently high levels of potentially toxic cyanobacteria in Fig. 2.6 A and B demonstrate the influence high levels of nutrients have on the lake phytoplankton. The concentration of chlorophyll-*a* in the water column is a measure of the biomass of phytoplankton in the lake (Vézie *et al.*, 2002). Along with potentially devastating ecological effects, high levels of cyanobacteria and chlorophyll-*a* impact the recreational and aesthetic values of Whakakī Lake, making it unsafe to swim (Wood *et al.*, 2018) and potentially impacting the harvesting of tuna and mullet from the lake for mahinga kai.

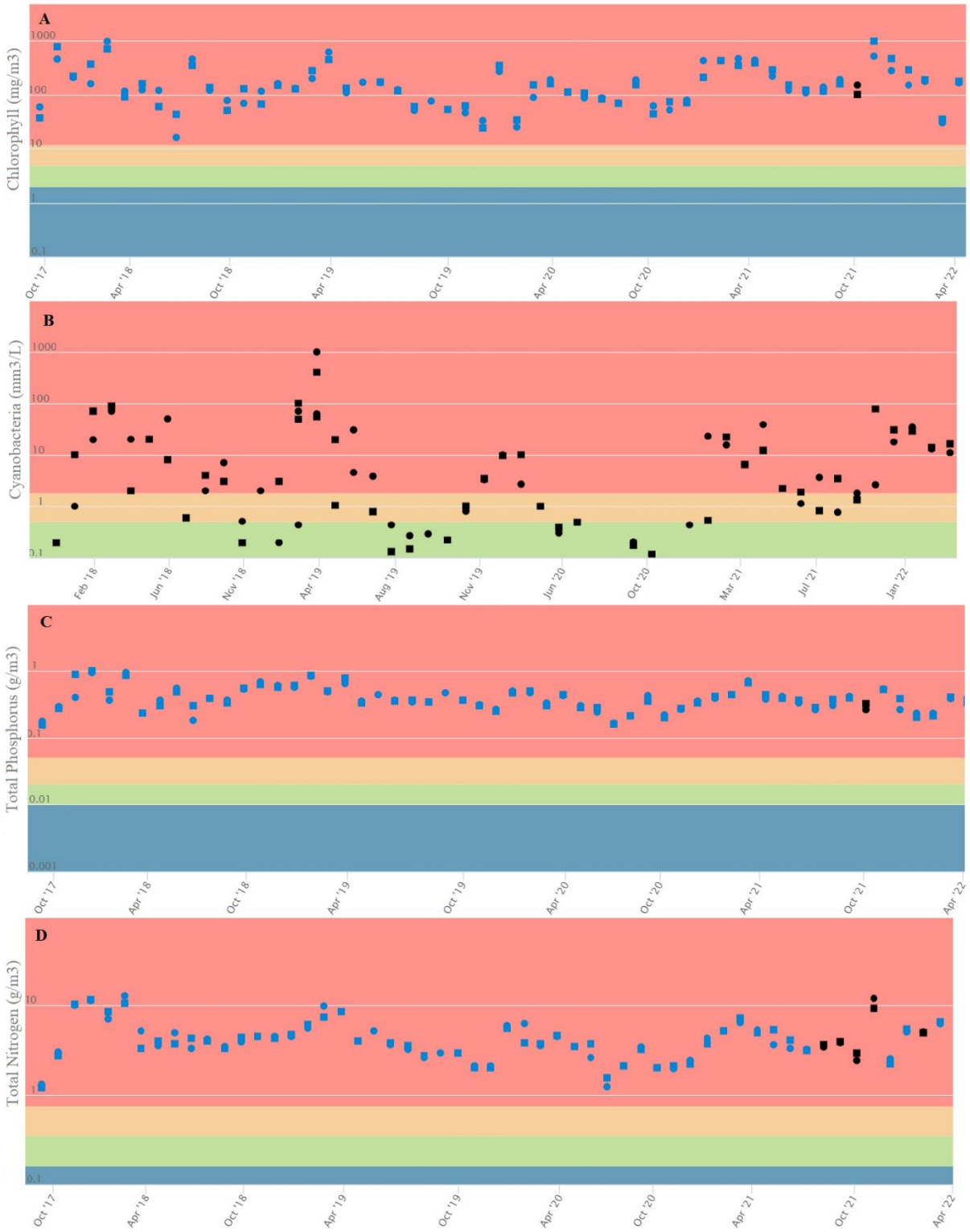

Figure 2. 5 Plotted values of monthly sampling results at Whakakī First and Second Bluff sites over a 5-year period for the main water quality parameters. Note sampling frequency became monthly as of mid-2017. Colour bands on graph indicate the 2020 NPSFM bands ■= 'A' band or 'excellent' ■ = 'B' band or 'good' ■ = 'C' band or 'fair' and ■ = national bottom line or 'D' band for Poor. Blue = checked data Black = unchecked data ■ = Whakakī lake at First Bluff ● = Whakakī lake at Second Bluff

Water clarity is recorded using a Secchi disk monthly. Water clarity values paint a picture of turbid water conditions with little to no visibility (Fig. 2.6). Whakakī Lake rarely had a secchi disk reading greater than 0.3 m, (Fig. 2.6). The persistent poor water clarity is a visual demonstration of the lake's stress from continuous turbid water conditions created by wind-driven resuspended sediment and high algal biomass.

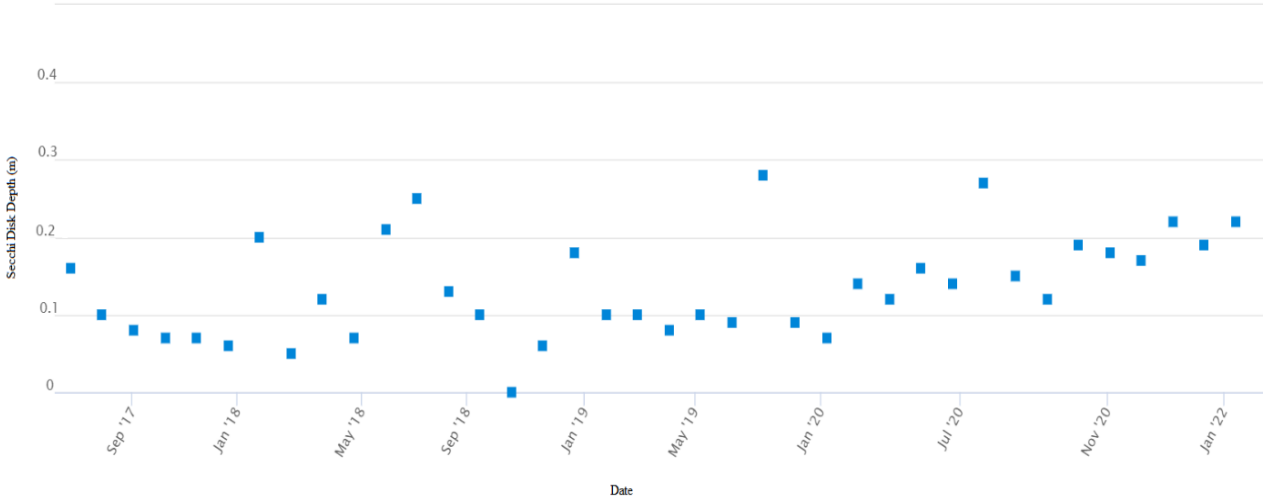

Figure 2. 6 Water clarity data collected by Hawke's Bay Regional Council from 2017-present. Water clarity measured using a Secchi disk. Water clarity values below 0.4m indicate Whakakī lake fails water quality bottom line under NPSFM guidelines.

The periodic opening of the lake to drain it for flood control over autumn and winter has been a contentious point in the lake's history. The draining of the lake occurs throughout the wetter months after or before rainfall events and the lake is not meant to be opened in spring or summer to avoid near-complete drying events. While there is an intention to keep some water in the lake over summer, it can be challenging to predict the

impacts opening the lake may have, as shown below in an image taken on the banks of Whakakī Lake in January of 2018 (Fig 2.8).

Evapotranspiration can cause the lake water level to decrease further than intended and drastically reduce the habitat for fish and macroinvertebrates. Along with the ecological impacts, low water levels in summer can result in high water temperatures, further stressing lake life and allowing algae and cyanobacteria to thrive. When the lake is manually opened over winter, the water level throughout the lake can be as low as 0.4 m deep. Data showing the water depth history of Whakakī Lake over the last year via a continuous water level logger in the middle of the lake can be found in the appendix.

The Hawke's Bay Regional Council are in the stages of building a weir at the Rāhui Channel outlet as of May 2022. This weir will allow for a constant water level to be maintained within the lake year-round, while barriers can be removed from the weir to drain excess water from the lake during rainfall events to ease flooding. If the weir is successful, images such as the one below should be a thing of the past; however, the legacy of poor water quality and drying events will likely persist. The lake water level depicted in Fig 2.7 is a result of an early spring opening and a warm, dry period that followed.

Figure 2. 7 Whakakī Lake January 31st, 2018. Picture taken from the northern bank adjacent to SH2

2.4 Whakakī Lake macrophyte community

Submerged aquatic vegetation plays a vital role in shallow lake systems, trapping and binding sediments by buffering waves and the uptake of nutrients from the lake water (Horppila and Nurminen, 2005). Moreover, macrophytes provide shelter, food and habitat for fish and birds (Bakker *et al.,* 2013). The macrophyte community of Whakakī Lake was described in 1992 (de Winton *et al*., 1992) as being dominated by native aquatic plants, comprising a diverse community of species representative of brackish water conditions. The macrophytes *Ruppia* spp., *Stuckenia pectinatus* (formerly *Potamogeton pectinatus*), *Zannichellia* and *Althenia bilocularis* (formerly *Lepilaena bilocularis*) were present in the 1992 survey, with *Lamprothamnium macropogon* (formerly *Lamprothamnium papulosum*) being the only charophyte species present, and the only true brackish water charophyte in New Zealand (Wood & Mason, 1977) requiring some saline water to grow. de Winton (1992) noted that Whakakī represented the northernmost

record of *Lamprothamnium macropogon.* Plant fragments of the freshwater species such as *Myriophyllum triphyllum* and *Potamogeton crispus* may have been introduced by wildfowl or through hydrological connections with the Wairau Lagoon where such species had been recorded. Similarly, the freshwater species *Chara globularis* and *Zannichellia palustris* were restricted to the area of Whakakī Lake closest to the Tuhara drain inflow, a freshwater inlet on the north-western end of the lake.

Ngā Whenua Rāhui commissioned NIWA (National Institute of Water and Atmospheric Research) of to undertake a follow-up survey of macrophytes in Whakakī in 2007 after concerns over declining submerged plants. A survey was completed over two transects within the lake (Table 2.2).

Table 2. 2 Summary of vegetation recorded in Whakakī Lake in 1992 and 2007 NIWA surveys. Species, sites (number of transects) and maximum cover are shown. Table adapted from de Winton & Champion (2008)

Species	Sites		Maximum cover	
	1992	2007	1992	2007
Azolla filiculoides	1			
Chara globularis	1		1-5%	
Lilaeopsis novae-zelandiae	1	1		-
Lepilaena bilocularis	2	2	2-25%	6-25%
Lamprothamnium macropogon	2		96-100%	
Potamogeton crispus		1		1-5%
Stuckenia pectinatus	2	2	6-25%	26-50%
Ruppia polycarpa	2	1	6-25%	1-5%
Zannichellia palustris	1		1-5%	

Overall, the comparison between the 1992 and 2007 surveys demonstrated a decline in the abundance and diversity of the Whakakī macrophyte community. There was a noticeable absence of the once prominent *Lamprothamnium macropogon* and the restriction of *Ruppia polycarpa* to shallower areas. *Stuckenia pectinatus* is known to survive turbid conditions in shallow lakes (Scheffer *et al.* 1992), with reproduction via tubers allowing quick regeneration after disturbances. *Potamogeton crispus* was the only non-native macrophyte recorded and was restricted to the lake margins. De Winton (2008) noted that the absence of *Lamprothamnium macropogon* may be due to reduced salinity levels or turbid water conditions. Four species germinated from seed bank surveys undertaken by NIWA in conjunction with the 2007 macrophyte survey. *Nitella hyalina, Ruppia polycarpa, Althenia bilocularis* and *Lamprothamnium macropogon* all germinated under different salinity conditions ranging from 0 ppt to 8.5 ppt. *Nitella hyalina* and *Ruppia polycarpa* only germinated in 0 ppt salinity, *Althenia bilocularis* germinated under the complete range of salinities, while *Lamprothamnium macropogon* required low to moderate salinity to germinate (3.5-8 ppt).

LakeSPI is a method that characterizes the ecological health of lakes based on the amount of native and invasive plants. The Native Condition Index characterises the status of native vegetation, the Invasive Impact Index captures the degree of impact from invasive weed species and the LakeSPI Index provides an overall indicator of lake ecological condition. The higher the LakeSPI index, the better the lake's overall health. Whakakī Lake had no vegetation in 2016, having lost its macrophyte community between 2007 and 2016 (Fig. 2.8). As of 2022, there have been no records of macrophytes in Whakakī Lake.

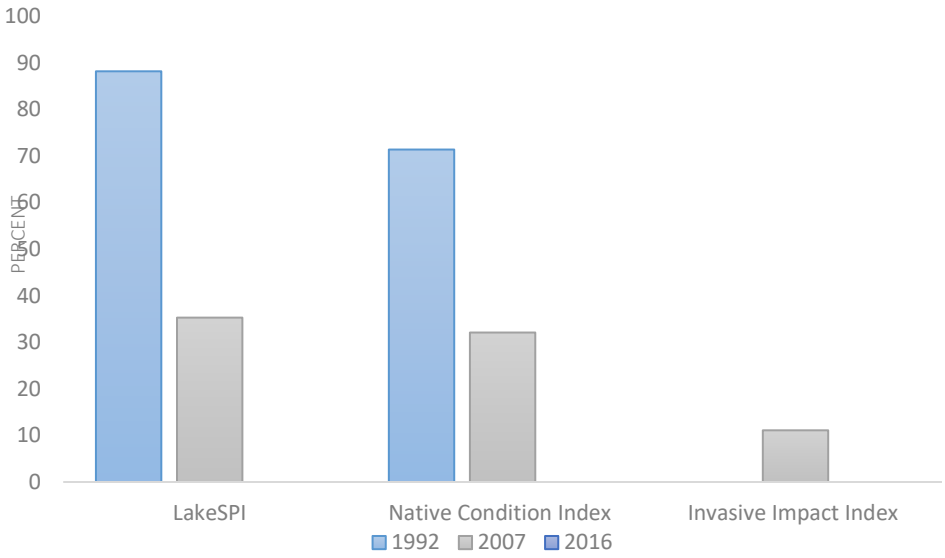

Figure 2. 8 NIWA LakeSPI Report results (2016) depicting reported decline and loss of the macrophyte community from Whakakī lake

Chapter III

Characterising the macrophyte and charophyte seed/oospore bank of Whakakī Lake

3.1 Introduction

Macrophyte recovery may play an essential role in the ecological restoration of Whakakī Lake. A healthy submerged macrophyte community is critical to the ecological health of a shallow lake system (Bakker *et al.,* 2013), and the lack of macrophytes in Whakakī Lake has been a barrier to restoring the lake to a more natural state. If a healthy macrophyte community were able to re-establish, increased nutrient uptake by macrophyte crops could enhance water quality, stabilise the lakebed through root systems, provide food for waterfowl and habitat for fish and macroinvertebrates (Bakker *et al.,* 2013, Horppila and Nurminen, 2005).

Buried, viable seeds play a crucial role in re-establishing macrophyte populations in many lakes, and although there has been extensive research into the longevity and resilience of the seeds of emergent species, relatively little is known about those characteristics of submerged macrophyte seeds. Seed coats of submerged macrophytes tend to be less durable than those of emergent species (Sculthorpe, 1967) and this suggests seed banks are likely to become degraded after an extended period (i.e., ~20 years) of poor germination conditions, such as inadequate water depth or poor water clarity. If attention is not given to the re-establishment of macrophytes in degraded shallow lake systems before this occurs, manual replenishment of the seed bank could be required to achieve successful re-establishment of a macrophyte community. Studies into macrophyte communities from lakes in New Zealand demonstrate the need to understand and protect the seed banks of impacted lake systems, finding that submerged seed banks can conserve seed density and species richness (de Winton *et al.,* 2000). Vegetation recovery and associated improvements in water quality improvement has been achieved

in some places, such as Lake Wolderwijd and shallow lakes in de Wieden, in the Netherlands (Meijer *et al.,*1989, Van Berkum *et al.,* 1995). However, these efforts often fail or are only successful for a short period (Meijer and Hosper, 1997) as long-term success depends on a stable recovery of submerged macrophytes and it may take decades after external nutrient load reduction to achieve stable clear-water conditions with a diverse macrophyte community (Hilt *et al*., 2018).

In this chapter, I investigate the abundance and community composition of the submerged macrophyte seeds in the sediments of Whakakī Lake. It was hypothesised that seeds and oospores of submerged macrophyte and charophytes would be present within the seed bank based on the species last known to be recorded in the lake in 2007. This would suggest restoration efforts may only require improvement in water clarity to facilitate the regeneration of submerged macrophytes in the lake.

3.2 Site Selection

Four sites across Whakakī Lake were selected based on previous macrophyte reports completed by de Winton *et al.* in 1992 and de Winton & Champion in 2008. A transect was selected across the lake's western end at a similar location to those used in 1992 and 2008 (de Winton *et al.* 1992, de Winton & Champion, 2008). Sediment cores were taken from four sites along this transect (Fig. 3.1). The average water depth at the time of coring was 1.1 m, while there is no bathymetry data for Whakakī Lake, site 1 was slightly shallower at approximately 0.95 m due to the natural sloping topography of the lakebed. The transect selected for coring follows a line of old maimai previously used for gamebird shooting.

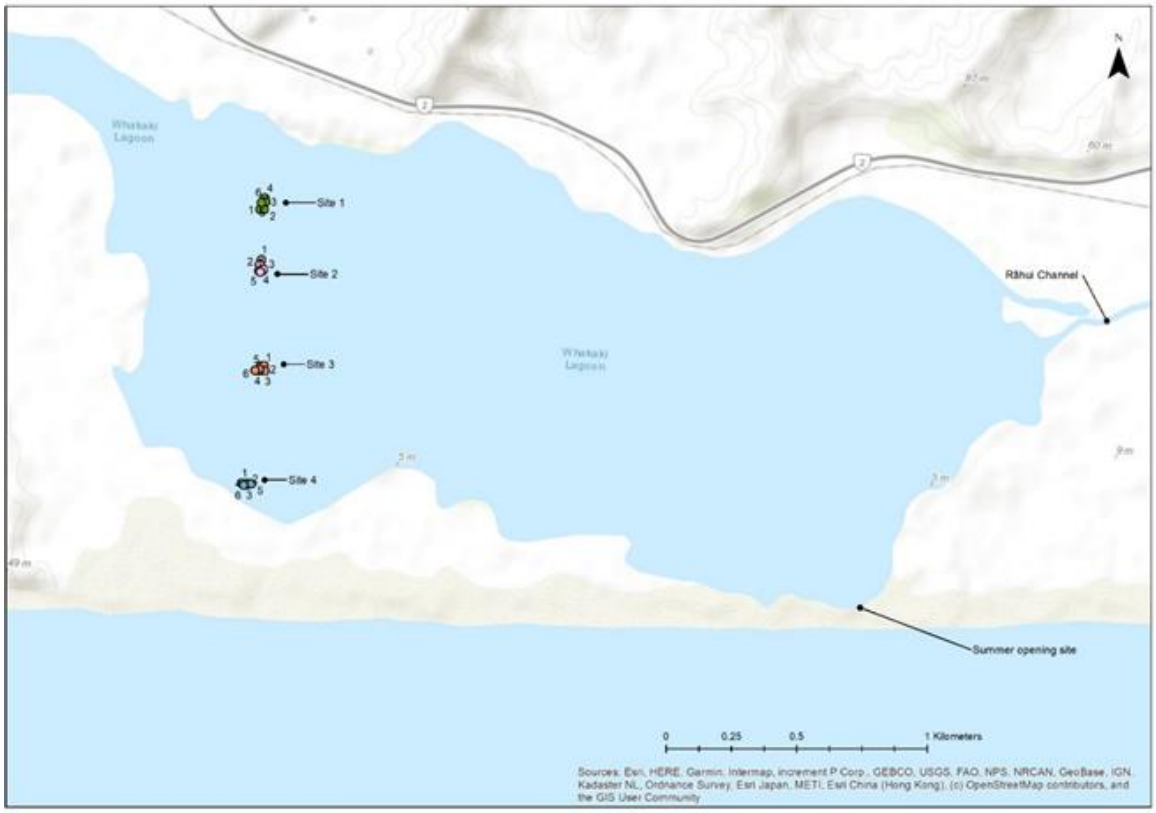

Figure 3. 1 Map of locations of coring at sites 1, 2, 3 and 4.

3.4 Methodology

3.4.1 Sediment core surveys

Sediment cores were collected over three days in January 2021 from the four sites within Whakakī Lake by hammering 2 m sections of 90 mm diameter PVC stormwater pipe into the lakebed, capping the pipe to seal in sediment, and extracting the pipe using ropes. The lake water level was approximately 1.1 m deep at the deepest site at the time

of collection. Six duplicate samples were collected from each coring location. Observations of substrate were recorded from each site, noting whether it was predominantly either, black organic mud, clay, sand, or a mixture, e.g., black mud over clay. Cores were capped at both ends using PVC pipe caps, taped with thread seal tape, duct-taped around caps and kept upright for transportation back to the lab. Using an angle grinder, all cores were split lengthways on two sides of the pipe: splitting the core into two halves. Sediment depth was recorded, and the core sections were photographed (Fig. 3.2). The core depth varied between sites ranging from 190 mm to 510 mm sediment depth; only the top 100 mm was collected for seed bank analysis. The top 100 mm of each core was sectioned off using a clean putty knife and stored in 500 mL sterile containers in a refrigerator to preserve the integrity of the seeds and sediment. Further investigation into the temporal distribution of seeds within 100 mm of lake sediment supports this method, finding seeds and oospores were homogenously distributed within the top 100 mm of sediment. Please refer to appendix for further information on this investigation.

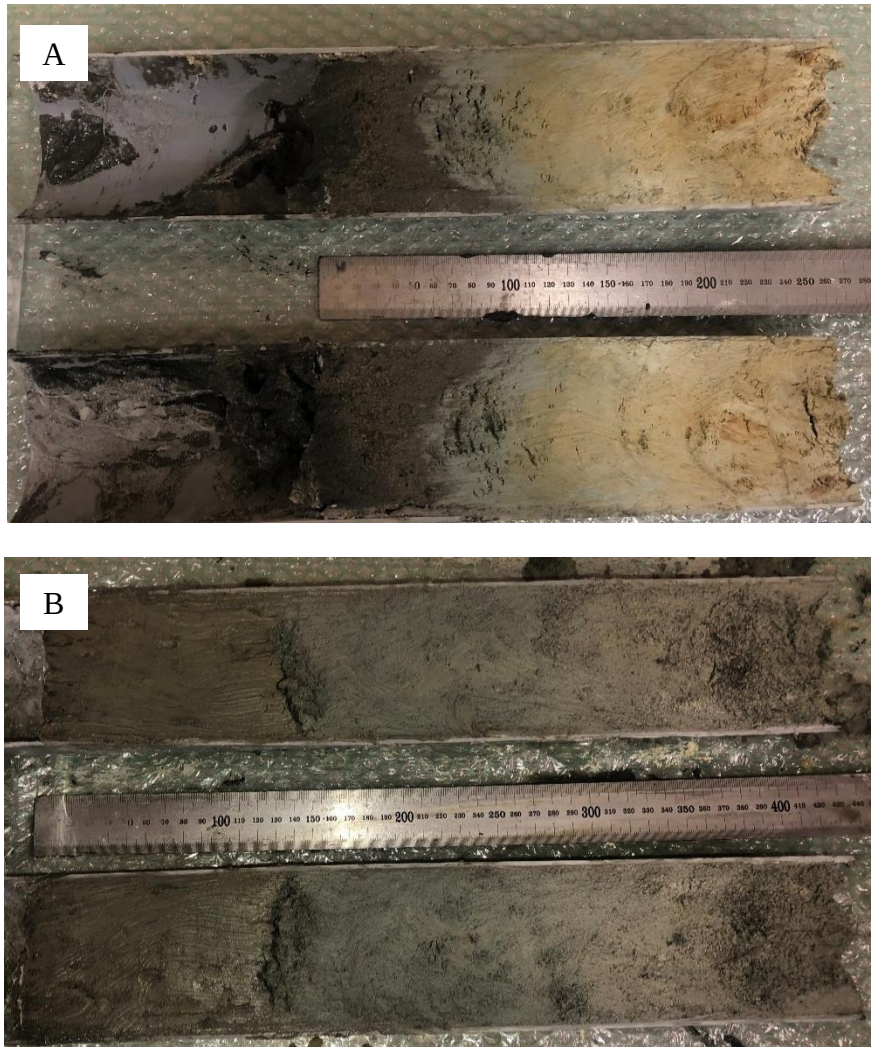

Figure 3. 2 Two cores from Whakakī lake after being split lengthways and depth of sediment measured. A = Site 1 replicate 4, B = Site 4 replicate 6

3.4.2 Seed retrieval

Sediment samples were wet sieved through 200 mm diameter 250 μm stainless steel woven-wire cloth sieves to separate macrophyte seeds and charophyte oospores from fine sediment and debris (Fig. 3.3). A 250 μm sieve size was used as this is the smallest known size for New Zealand macrophyte seeds and charophyte oospores (de

Winton *et al.*, 2007). Samples were further divided into three sizes per sample: 500 µm, 300 µm and 250 µm. This was done to separate organic material and debris to assist in microscope analysis for counting and species identification based on the varying levels of organic material and sandy sediment between core sites. The mesh sieve was rinsed thoroughly between sieving each core segment, and a new pair of gloves was used per core to reduce the possibility of cross-contamination between samples.

Figure 3. 3 The top 100 mm gathered from cores within Whakakī Lake before and after initial sieving with a 250 µm mesh sieve. Left = volume of sediment pre-sieving, Right = volume of seeds/oospores and remaining sediment and debris after sieving.

3.4.3 Seed abundance and species identification

A Leica M60 stereoscopic microscope was used to count and identify all seed species in each sample. Each size class (500μm, 300μm and 250μm) was spread across up to three 90 mm diameter Petri dishes to achieve a single layer of seeds for species identification and counting. Each petri dish was divided into gridded quadrates (10 mm x 10 mm) to assist in viewing and avoid repeated counting. All individual seeds were counted and identified. Seed species identifications were informed by the species found in the de Winton *et al.* (1992) and de Winton and Champion (2008) reports.

Charophyte oospore identifications were made based on the descriptions of Wood & Mason (1977) and the de Winton (2007) key to common charophytes in New Zealand. All seeds of emergent species were identified and counted but were eliminated from this study. Oospores can be distinguished from other plant propagules using their unique sinistral spiral markings (striae) (Fig. 3.4). Charophyte oospores are noted as being resilient to degradation (de Winton *et al.*, 2007), meaning these unique markings and other characteristics such as basal impressions are well preserved. Examples of oospores found and identified in this study are shown below (Fig. 3.4).

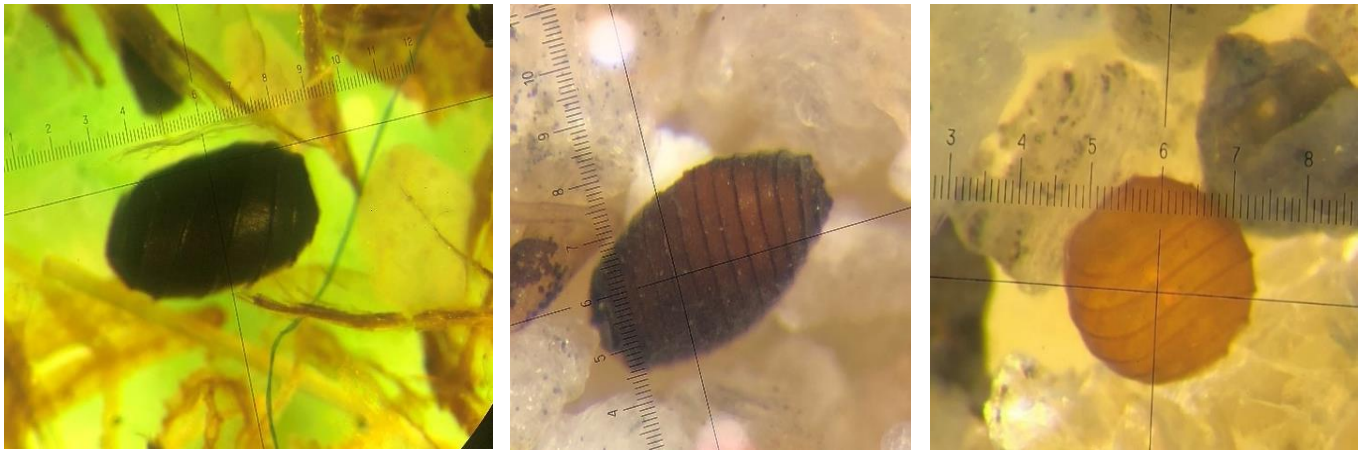

Figure 3. 4 Example oospores identified during the microscope analysis. From left to right: Chara australis, Lamprothamnium macropogon, Nitella hyalina

3.4.4 Statistical analyses

PRIMER v7 (Clarke and Gorley, 2015) with the PERMANOVA+ add on (Anderson *et al.*, 2008) was used to conduct a non-metric multi-dimensional scaling ordination (nMDS) in order to display spatial and temporal patterns in community composition. A resemblance matrix was calculated using the Bray-Curtis similarity index. Seed count data was transformed using log(x+1) to retain information concerning relative abundance, and to ensure that commonly occurring species did not dominate the analysis (Sokal *et al.*, 1995). Goodness-of-fit is measured by 'stress', which measures a rank-order disagreement between observed and fitted distances. A stress value of > 0.24 is a poor result, and interpretation should be reconsidered, whereas stress of 0.05 - 0.1 is good. A PERMANOVA (permutational multivariate ANOVA) was used to examine the differences in community structure between sites. Spatial variations in species compositions were also presented using nMDS with a vector overlay of species. Two-way analysis of variance (Two-way ANOVA) in RStudio version 4.0.5 was used to test

for species differences between sites, with a posteriori Tukey HSD means test. Box plots have been used to summarise and visualise species composition between sites.

3.5 Results

3.5.1 Seed abundance between sites

A total of 12,759 submerged macrophyte seeds were collected from across all four sites in Whakakī Lake. Site 2 had the highest total number of seeds (*n* = 5,463). Site 1 had the second highest abundance (*n*= 4,506). Sites 1 and 2 cumulatively had 7,179 more seeds in total than sites 3 and 4 combined (*n* = 1,609 and *n* = 1,181 respectively) (Fig. 3.5).

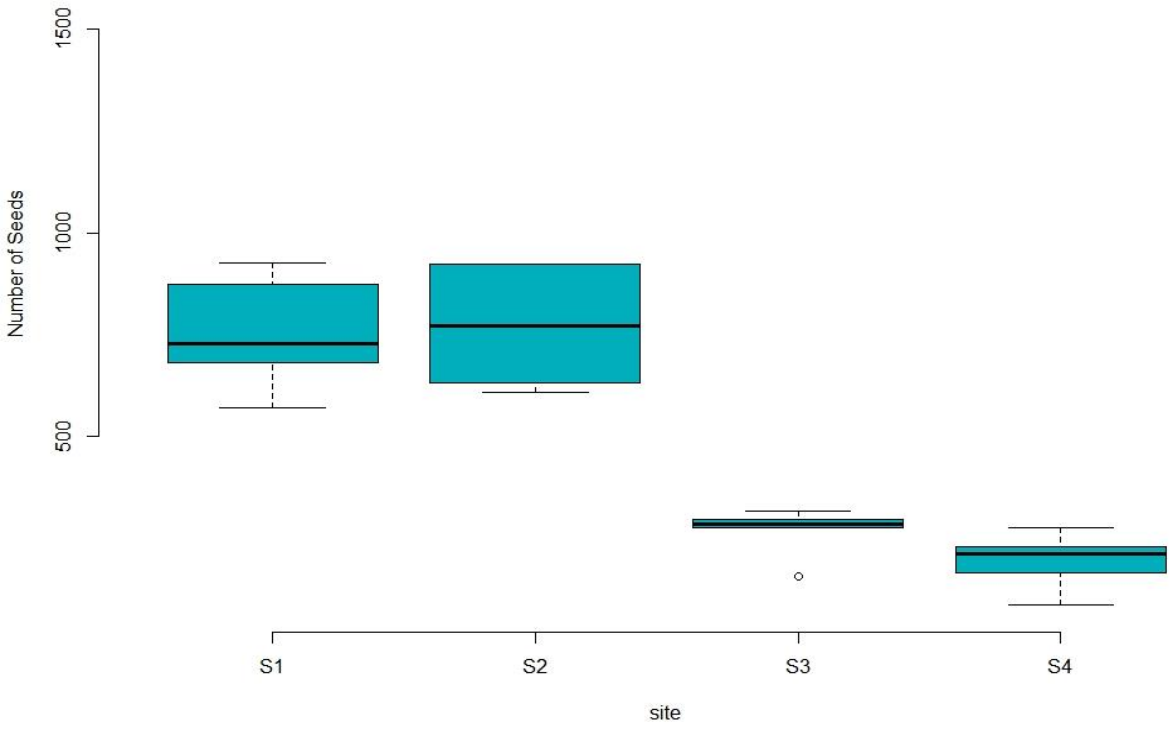

Figure 3. 5 Box and whisker plots illustrating the distribution of seeds collected at the four sites within Whakakī Lake

nMDS plots highlight the visual similarities in community composition between sites, and there was a pattern in community composition between sites (Fig. 3.6). A stress level of 0.09 indicates a good ordination and fit for the model. Groupings of points represent communities that are similar, and points further apart represent communities that differ from each other. The nMDS shows there are two groupings; sites 1 and 2 and sites 3 and 4. These two clusters were visible at the 40% cluster analysis level. PERMANOVA analysis testing results revealed significant differences between all sites (F = 11.99, df = 3, P = 0.0001). Pair-wise testing further supports this finding and results of this can be found in the appendix.

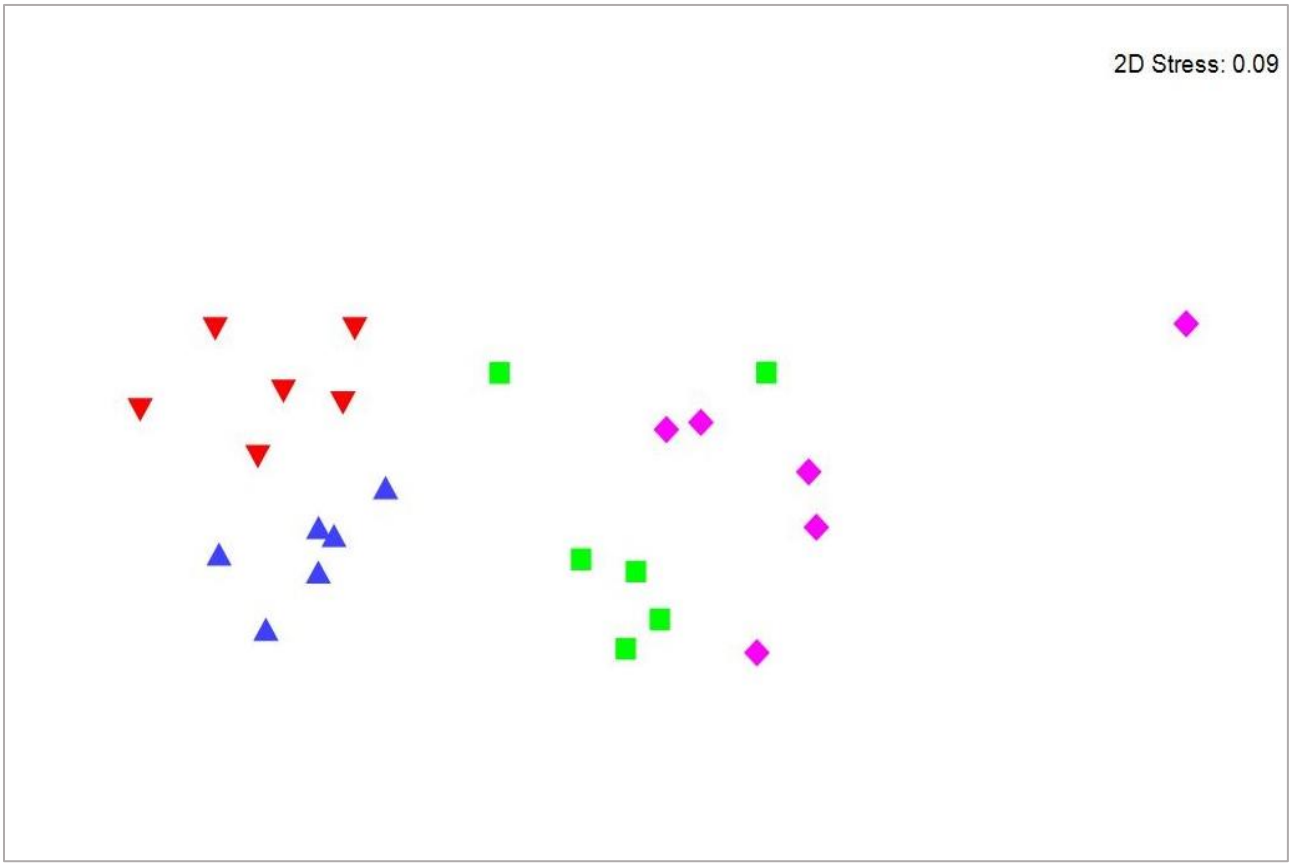

Figure 3. 6 nMDS (non-metric multi-dimensional scaling) ordination of community composition of cores taken at each site within Whakakī Lake (Data transformation: log x+1). ▲ *= Site 1* ▼ *= Site 2* ■ *= Site 3* ◆ *= Site 4.*

3.5.2 Species distributions

Eleven species of submerged macrophyte and charophyte species were found at the four sites in Whakakī Lake (Table 3.1). Five more species were identified from the seed banks in 2021 than were observed in 1992, and four more than were found in 2007. A majority of species that were found in the seed bank in 2021 had been identified as being present in the lake or seed bank in either 1992 and/or 2007, with the exception of *C. australis* and *N. hookeri* which have not been recorded in Whakakī Lake before. Species that were present in 1992 but were not observed growing in 2007 appear to still be present within the seed and oospore banks of the lake, such as *C. globularis*, *L. macropogon* and *Z. palustris.*

Table 3. 1 Table of Charophyte and submerged macrophyte species identified through visual surveys or oospores and seeds from within cores taken from Whakakī Lake. Indicated is what species were present in 1992, 2007 and 2021.

Scientific Name	Family	Common Name	Category	1992	2007	2021
Azolla folliculinids	*Salviniaceae*	*Azolla*	native	✓		
Chara globularis	*Characeae*	Stonewort	native	✓		✓
Nitella hyalina	*Characeae*	Stonewort	native		✓	✓
Lamprothamnium macropogon	*Characeae*	Stonewort	native	✓	✓	✓
Chara australis	*Characeae*	Stonewort	native			✓
Nitella hookeri	*Characeae*	Stonewort	native			✓
Althenia bilocularis	*Potamogetonaceae*		native	✓	✓	✓
Potamogeton crispus	*Potamogetonaceae*	Curly pondweed	non-native		✓	✓
Ruppia polycarpa	*Ruppiaceae*	Horses' mane weed	native	✓	✓	✓
Zannichellia palustris	*Potamogetonaceae*	Horned Pond Weed	native	✓		✓
Stuckenia pectinata	*Potamogetonaceae*	Sago pondweed	native	✓	✓	✓
Lilaeopsis novae-zelandiae	*Apiaceae*		native	✓	✓	
Myriophyllum triphyllum	*Haloragaceae*	Water milfoil	native		✓	✓

Two-way ANOVA results show a significant difference in species between sites (F = 12.245, P = <0.001). An overlay of species to the nMDS plot (Fig. 3.7) displays species as direction lines pointing in the direction in which those species numbers increase, and the length of the line reflecting the strength of the patten in the species counts along that direction. This ordination shows *C. australis* and *M. triphyllum* pull towards the cluster of sites 3 and 4, suggesting these species were prominent features in the community composition of sites towards the southern end of the lake. The cluster comprising of sites 1 and 2, are characterised by the majority of species, however species such as *R. polycarpa* and *A. bilocularis* appear to be most characteristic of site 1, which is the most northern site in the lake. Site 2 has a variety of species contributing to its composition, and *C. Chara globularis* and *N. hookeri* were defining species of this site.

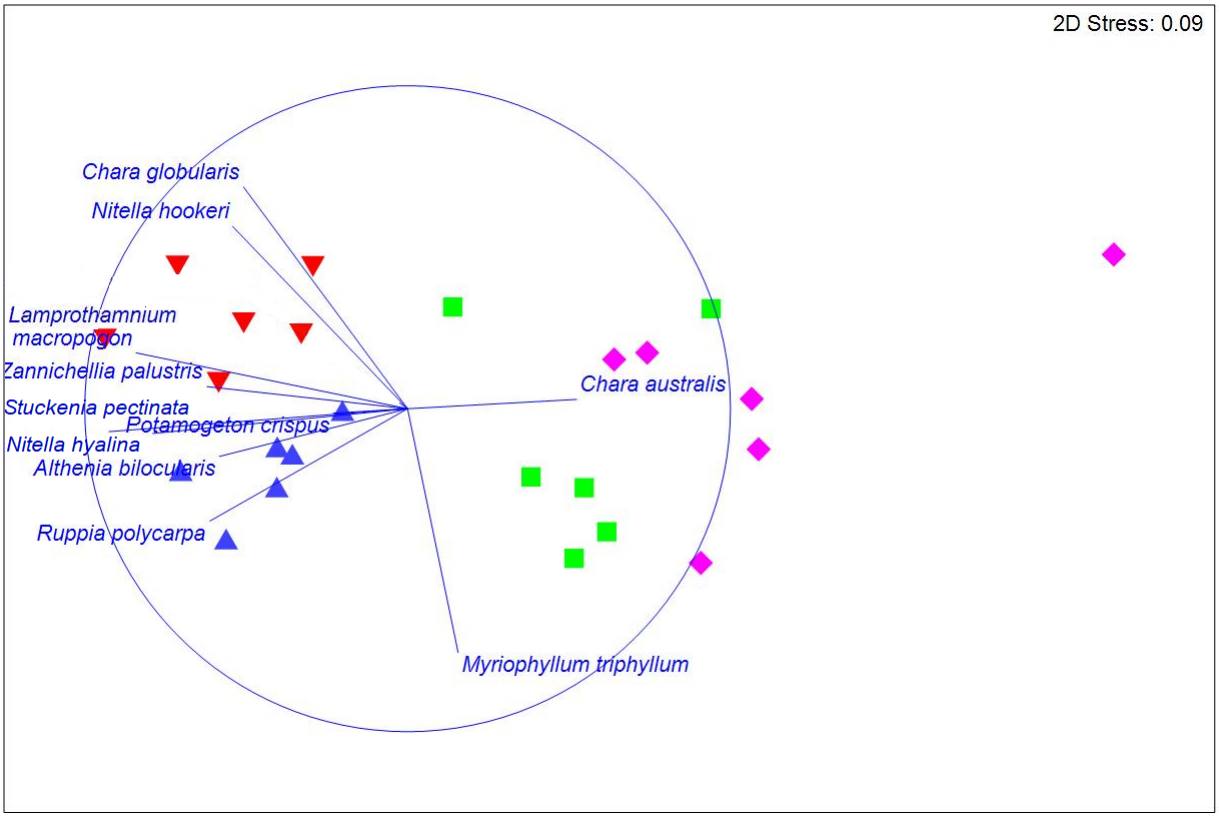

Figure 3. 7 nMDS (non-metric multi-dimensional scaling) ordination of community composition of cores taken at each site within Whakakī Lake (Data transformation: log x+1) with vector overlay of species. ▲ *= Site 1* ▼ *= Site 2* ■ *= Site 3* ◆ *= Site 4.*

Chara globularis

In total, 771 individual *Chara globularis* oospores were identified throughout the lake. Of those, site two had the highest total number of *C. globularis* oospores (Fig. 3.8). *C. globularis* is at the highest abundance at the sites closest to the northern edges of Whakakī Lake. The known preferred habitat of *C. globularis* is slow flowing waters such as watercourses, rocky streams, ditches, shallow lagoons, ponds, and sand-dune lakes (Wood & Mason, 1977). *C. globularis* was identified at one site within the lake in 1992 (de Winton *et al.*, 1992), approximately 100m from the northern shore.

Stuckenia pectinata

A total of 68 *Stuckenia pectinata* seeds were identified throughout the lake and was found at every site in relatively low numbers (Fig. 3.8). *S. pectinata* is known to inhabit brackish water, such as slow-moving tidal streams and lagoons, or shallow lowland ponds (Moore & Edgar, 1970), however may reproduce primarily via tubers rather than seeds.

Ruppia polycarpa

There were 737 *Ruppia polycarpa* seeds collected throughout the lake. *R. polycarpa* seeds are more abundant at site 1 than at any other site within this study (Fig. 3.8), which is the site closest to the northern shore of the lake. *R. polycarpa* was identified as abundant near the lake edge in 1992 (de Winton *et al.*, 1992) but had decreased in per cent cover by 2008. The preferred habitat of *R. polycarpa* is saline ponds, lagoons, brackish streams, and slow flowing freshwater streams (Moore & Edgar, 1970).

Lamprothamnium macropogon

Lamprothamnium macropogon was the second most abundant species of submerged macrophyte seed identified throughout the lake, with 3,545 total oospores. *L. macropogon* was present in high numbers at site 2, one of the closest to the northern shore. The difference in total oospores of *L. macropogon* at site 2 was drastic compared to that found at either site 3 or 4 (Fig. 3.8).

Chara australis

A total of 548 *Chara australis* oospores were found throughout the lake. *C. australis* was the only species identified in the seed/oospore bank analysis that was found in higher abundances in sites 3 and 4, in the centre of the lake and towards the southern shore (Fig. 3.8). The preferred habitat of *C. australis* is lakes or slow flowing waters (Moore & Edgar, 1970) and it is a salt-sensitive species, primarily found in freshwaters (Bisson & Bartholomew, 1984).

Myriophyllum triphyllum

A total of 1,545 *Myriophyllum triphyllum* seeds were identified throughout the lake. *M. triphyllum* seeds were found at all sites, and were in similar abundances at sites 1, 3 and 4. There were slightly less seeds of *M. triphyllum* at site 2 compared to the other sites (Fig. 3.8).

Nitella hyalina

Nitella hyalina was the most abundant species found within the lake, with a total of 4,279 individual oospores. The vast majority *N. hyalina* oospores were found at sites 1 and 2 (Fig. 3.8) and only few (less than 100) oospores identified at either site 3 or 4. *N. hyalina* is generally only found in shallow lakes in New Zealand (Moore & Edgar, 1970)

and cannot tolerate wide fluctuation in salinity (Puche & Rodrigo, 2015). A single replicate from site 2 (Fig. 3.8) contained 1,260 *N. hyalina* oospores, an outlier compared to the abundances in other replicates from that site, and impacts the mean.

Althenia bilocularis

In total 1,074 seeds of *Althenia bilocularis* were identified from within the seed bank and were found in similar numbers across all sites (Fig. 3.8). *A. bilocularis* is usually found in freshwater habitats close to the coast but can tolerate brackish waters and slow flowing rivers (Moore & Edgar, 1970). *A. bilocularis* has been a prominent feature of the submerged macrophyte community of Whakakī Lake, being recorded either growing and/or in the seed bank in 1992, 2007 and 2021.

Other

Potamogeton crispus, *Zannichellia palustris* and *Nitella hookeri* were all identified in the seed bank in low numbers (Fig. 3.8). 11 seeds of *P. crispus* were found between sites 1, 2 and 4. *Z. palustris* was found across all sites, with a total of 90 seeds, 70 of which were found at sites 1 and 2. A total of 91 *N. hookeri* seeds were found across all sites, with half of them found at site 2.

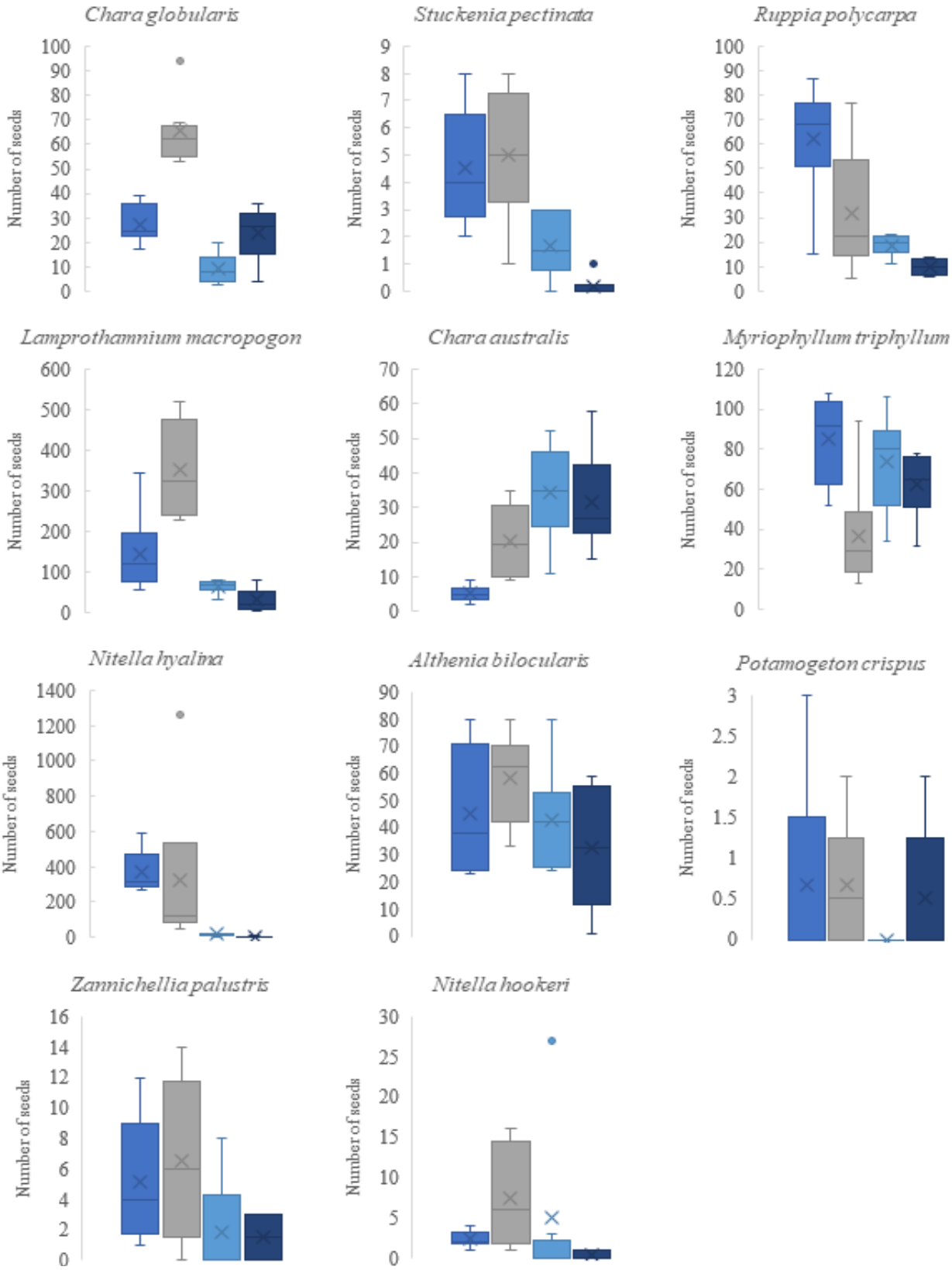

Figure 3. 8 Box plots describing the distribution of species identified within Whakakī Lake seed bank. *= Site 1* *= Site 2* *= Site 3* *= Site 4*

3.6 Discussion

The macrophyte seed bank of Whakakī Lake is abundant and diverse. There are differences in abundance and composition between two regions of the lake (Fig. 3.7). The most abundant seed banks are located on the northern and western edges of the lake, nearest the Tuhara stream inflow (Fig. 3.5). Although seeds were not evenly distributed throughout the lake, the locations in which they were found in the highest abundance are likely the places where macrophytes could regenerate first, based on lake substrate and topography. Sites 1 and 2 were the shallowest sites with the sandiest and firmest substrate near the surface. The lake has a shallow, gently sloping basin, with site 3 the deepest site. Sites nearer the lake's edge will likely benefit first from improving water quality, such as reduced turbidity (de Winton *et al.,* 2004). If macrophytes can establish in these shallower areas of the lake first, the benefits of nutrient uptake and sediment stabilisation could be seen throughout the lake with lower nutrient concentrations and improved water clarity (Bakker *et al.,* 2012). Several studies have shown enhanced water clarity above charophyte vegetation, as they have the ability to trap sediment in high biomass dense stands (Bakker *et al.,* 2012, de Winton *et at.,* 2004). de Winton & Champion (2008) found most species at depths between 0.1-0.6 m, with only *S. pectinatus* found at up to 0.7 m. The 1992 survey (Fig. 3.9) had a similar species composition to the seed bank analysis in this study. The most diverse area of the lake identified in 1992 was the shallower northern edges of the lake, comprised of *S. pectinata, L. macropogon, R. polycarpa, C. globularis and Z. palustris* (de Winton *et al.,* 1992).

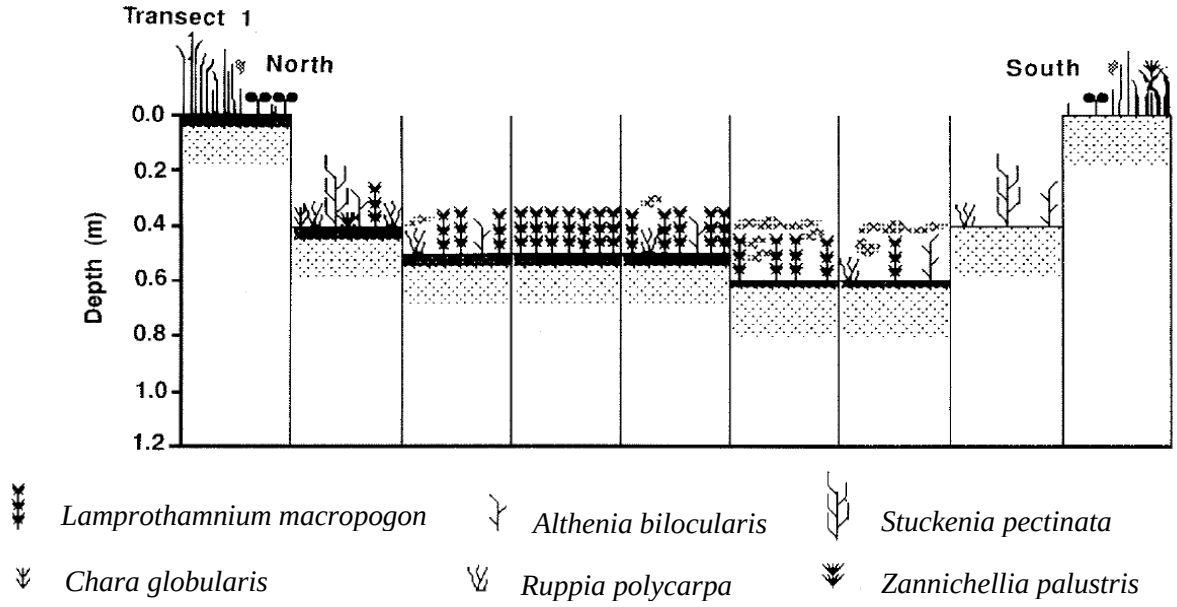

Figure 3. 9 Transect of the macrophyte species distribution within Whakakī Lake in 1992 surveys. Adapted from de Winton et al. (1992)

The similarities between the species distribution in this study and that identified in 1992 and 2008 surveys indicates that the seed bank of submerged macrophytes represents the past communities well. Remnant populations of macrophyte propagules are important to population restoration, as most species of macrophytes and charophytes are clonal (Bakker *et al.*, 2012). A study completed on Lake Fure in Denmark in 2008 (Sand-Jensen *et al.*, 2008) found that macrophyte recovery in favourable water quality conditions was strongly dependent on the presence of clones of species that had originated before the time of eutrophication. The importance of remnant populations of propagules, in this case

seeds and oospores, should be protected from restoration efforts, to maintain the best chances of macrophyte community recovery.

If a species is most prevalent in one site within the lake, it is likely that the seeds produced by this species are still where they originally fell or were released. *N. hyalina* had high numbers of seeds at sites 1 and 2; however, this species was not identified as growing in the lake in either 1992 or 2008, likely due to salinity tolerances. *N. hyalina* may have once been a species characteristic of Whakakī Lake under lower salinity conditions, or the seeds are being deposited from a freshwater source such as the Tuhara stream. The idea that *N. hyalina* seeds are being deposited in the lake from an inflowing stream is supported by the high abundance of species around the Tuhara inlet and low numbers in sites 3 and 4 towards the southern end of the lake. The seed growth pattern of *N. hyalina* shows bunches of tiny seeds that grow in a grape-like fashion, with many seeds forming a single cluster. It could be that clusters of these seeds were collected during coring, resulting in the high number of *N. hyalina* seeds observed from one replicate from site 2 (Fig. 3.8). Alternatively, *N. hyalina* is able to tolerate low levels of salinity and could have been present within the lake on the northern edges, due to the mixing of low salinity water from the inflow of the Tuhara stream.

Species identified within the lake in previous studies but not found in the seed bank were *Stuckenia pectinata, Lilaeopsis novae-zelandiae, Potamogeton crispus* and *Zannichellia palustris. S. pectinata* was observed in relatively low numbers in 2007 compared to a once extensive coverage of the lake (de Winton *et al.*, 2008). This species reproduces primarily via tubers that are produced on the rhizomes. While *S. pectinata* asexually reproduce via seeds, it has been noted that the seeds of this species can float;

this may make them less abundant in the sediment of the seed bank (de Winton *et al.,* 2008). *L. novae-zelandiae* similarly reproduces primarily via runners (Bone *et al.,* 2011). Due to the nature of this study, tubers and runners were not assessed. Some seeds of *P. crispus* and *Z. palustris* were identified in the Whakakī seed bank but were rare and sparsely distributed. The seeds of these species were often torn or incomplete, suggesting either damage during the coring and sieving process or that these species do not preserve well in the seed bank. The fruits of these species are fleshy and store one seed per fruit (Muenscher, 1936). It may be possible that this trait resulted in fewer individuals being gathered during coring.

The location of the Tuhara channel to the sites with the most abundant seeds (sites 1 and 2) may indicate how macrophyte seeds are entering the lake for some species. It was noted in the temporal distribution analysis (appendix) that macrophyte seeds are consistent in count and species throughout a 100 mm gradient of sediment from site 2. This suggests a constant source of seeds to the lake, as there may not have been an internal source for up to 14 years. The Tuhara steam is the only direct inlet to Whakakī Lake and connects the neighbouring Te Paeroa and Wairau Lagoons. Macrophytes have been reported in these lagoons, with Wairau Lagoon often referred to as 'Blue Lagoon' and locals report it to be a clear water lagoon with a healthy macrophyte community. If these lagoons are providing a consistent source of macrophyte seeds to the lake, the benefit to the restoration of Whakakī is great. These neighbouring lagoons may provide a crutch to Whakakī and enable the opportunity for macrophyte regeneration in Whakakī Lake once water quality conditions become suitable via fresh seeds. If this is the case, it is essential not to overlook the need to protect and enhance the Tuhara stream and Wairau

and Te Paeroa lagoons to protect their macrophyte communities and protect the source that Whakakī may rely on.

With the role of draining the neighbouring lagoons and wetlands, the Tuhara stream is a homogenous, artificial channel that carves its way through lowland farmland. The legacy of drainage schemes to drain wetland areas from the late 1800s is seen in the way the banks of the Tuhara stream carry water directly and deliberately into Whakakī Lake. This channelisation of the Tuhara stream has meant that water and flow levels within the stream are driven by the lake water level high up into the channel. When the lake level is high, the stream is stagnant; when the lake level is low, the Tuhara stream is flowing and potentially at its optimum for carrying macrophyte seeds into the lake. Alongside the hydrological challenges, the Tuhara stream has one of the worst water quality states in Hawke's Bay. Draining wetland area, the water is tannin stained brown, with extremely high dissolved reactive phosphorus (DRP) and dissolved inorganic nitrogen (DIN) concentrations (Fig. 3.10). These water quality challenges mean that while the Tuhara stream may be a fresh source of macrophyte seeds to Whakakī Lake, it also provides a continuous source of nutrient-enriched waters.

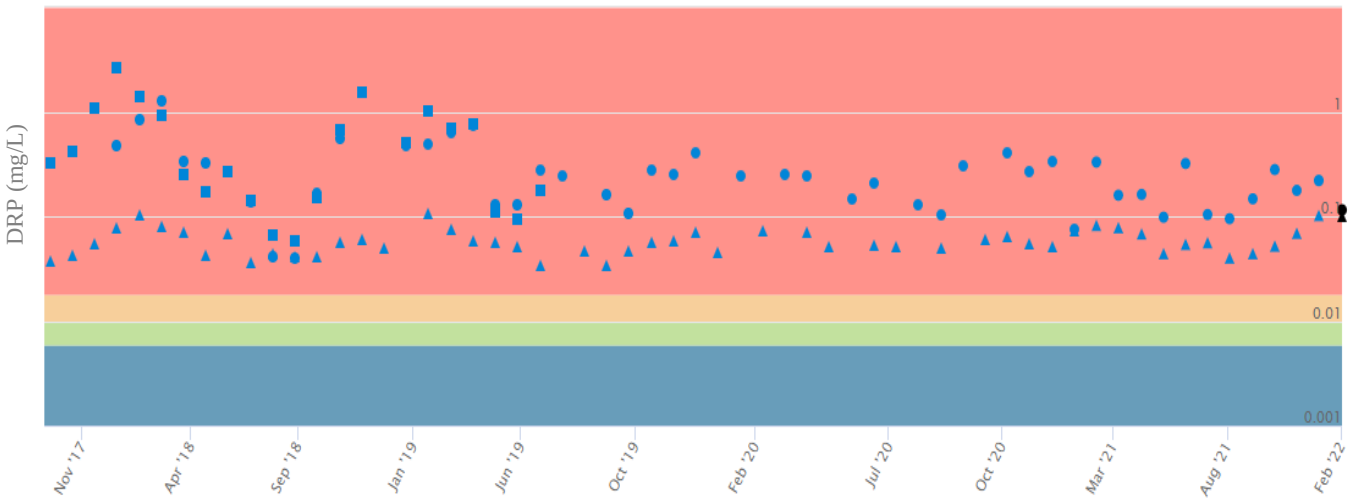

Figure 3. 10 DRP results from tributaries to Whakakī Lake. ● = Tuhara stream DS tributary ■ = Tuhara stream at Iwitea Road △ = Waikatuku stream. Band key (NOF guideline values): Blue = Excellent, Green = Good, Yellow = Fair, Red = Poor

Some degree of saltwater intrusion through the gravel bar separating Whakakī Lake from the sea can be expected. The gradient of salinity within the lake is unknown. If there are high concentrations of saline water closer to the gravel bar on the eastern and southern boundary of the lake this will influence the distribution of macrophyte species. During periods of high-water level, there may be the opportunity for heavier saline water to form a layer near the lakebed. If this is occurring, only species with some salinity tolerance may persist in areas nearer the coast, such as *S. pectinata, R. polycarpa* and *L. macropogon.* This idea is supported by the assessments completed in 1992 and 2008, where the southern sites in the lake comprised predominantly *S. pectinata, R. polycarpa and L. macropogon.* The seed bank somewhat supports this, with the species listed above were present at sites 3 and 4, along with the brackish water species *A. bilocularis*. Seeds

from some freshwater species such as *M. triphyllum* and *C. australis* were found in abundance at sites 3 and 4. *C. australis* was not recorded in either the 1992 or 2008 surveys, and the information available on this species notes it as a freshwater species that inhabits lakes and slow-flowing waters. While it was not recorded as being present in the lake in previous years, the seeds of this species were found in the highest abundances at sites 3 and 4, with fewer at site 2 and nearly none at site 1. It may be that this species is a remnant of a more freshwater past within the lake or that small *C. australis* plants were mistaken for the more common *L. macropogon.* Juveniles of the species may represent *L. macropogon* by its similar simple branchlet structure (de Winton *et al.*, 2007). The wind and waves are likely to play some role in the dispersal of aquatic angiosperm seeds, as seeds on the surface of the lakebed (unburied) are susceptible to secondary dispersal as currents and waves drag them (Koch *et al.*, 2009).

Overall, the submerged macrophyte seed bank of Whakakī Lake is abundant and diverse; this suggests the potential recovery of macrophytes in the lake is bright if water quality improves. The source of the seeds may be less important than the viability of the seed bank itself, if the seeds currently within the lake sediment are lying viable and dormant, the macrophyte seed bank may regenerate successfully regardless of the source(s) of seeds. Suppose a healthy population of macrophytes can germinate and grow from seeds currently available in the seed bank. In that case, these populations may mature, fruit, and produce their own seeds, further replenishing the macrophyte seed stocks within the lake. *P. crispus* was the only non-native macrophyte species found within the seed bank and was present in relatively low numbers. *P. crispus* (curled pondweed) has been identified in the Tuhara stream.

The diversity in species present within the seed bank suggests there is a contingency in macrophyte species for varying water quality conditions. With the installation of a weir to control water levels within Whakakī Lake, the salinity fluctuations the lake has experienced during direct-to-sea openings may change, and the lake may develop slightly lower and/or more stable salinity. If this is the case, there are many species that tolerate lower salinity levels in the seed bank, such as *Nitella hyalina, Chara australis, Nitella hookeri* and *Myriophyllum triphyllum,* which would be able to grow and flourish. On the other hand, species such as *Chara globularis, Lamprothamnium macropogon, Ruppia polycarpa, Althenia bilocularis* and *Stuckenia pectinata* will likely thrive under brackish conditions similar to the current conditions within the lake. This contingency within the seed bank gives hope for species diversity at differing salinity levels, which in turn will support increased diversity of visiting bird species, macroinvertebrates and fish species through increased habitat and food source.

Chapter IV

Seed bank viability of Whakakī Lake

4.1 Introduction

To establish the restoration options for Whakakī Lake, it is important to know what the viability of the seed bank is. There is limited information on how long the seeds of submerged macrophytes can lay dormant in New Zealand. Furthermore, we do not know precisely when the macrophytes in Whakakī Lake disappeared and thus how long the seeds may have been in the sediment. Germination success will be measured over three months (62 days) from October to December. I wanted to assess 1) the overall viability of the Whakakī seed bank 2) whether species germination is influenced by salinity, and 3) whether light availability impacts germination. The salinity conditions in Whakakī Lake have been highly variable since direct-to-sea openings began in the mid-1950s, possibly earlier. We do not know what the natural salinity levels of the lake may have been when the lake was part of a more expansive wetland complex. However, it is important to know how a macrophyte community may respond to salinity levels going forward. Most plant species may not need light to germinate, and a combination of suitable environmental triggers may be needed for germination. It may be that seeds are continuously germinating within Whakakī Lake, but without adequate light or shelter from the wind, are failing to grow. It is hypothesised that the Whakakī Lake seed bank is in general viable and that the species composition of germinates will vary between salinity treatments. It is also hypothesised that seeds will germinate under all light conditions, including total darkness. Tracking of the growth of these species was not possible in this study; however, seedlings will be collected in holding "grow" tanks to aid species identification. Four hypotheses will be tested in the germination trials, 1) Is there a difference in the total germination between salinity treatments? 2) is there a difference

in species germination between salinity levels? 3) Is there a difference in the total germination between light treatments? and 4) is there a difference in species germination between different light treatments?

4.2 Methodology

4.2.1 Germination Trials

Germination trials began in October 2021 and ran for three months (62 days), finishing on the 18th of December 2021. Two categories of treatment were set for the growth trials, salinity, and light treatments. The trials were conducted in the Hawke's Bay Regional Council lab under controlled conditions. Three treatments of each salinity were used, and four light treatments, with six replicates per treatment. In total, 42 replicates would be included in the germination trials. Seeds collected chapter III were used in the germination trials and were not separated by site or species. All seeds were combined in a 5L jug to randomly allocate species per treatment. All contents of the pottles from Chapter III were added to the jug, including the water seeds were stored in. Excess water was drained using the 250 μm sieve once all seeds had been added and prior to the allocation of seeds to a treatment. After all seeds were mixed, 750 mL of seeds and sediment was drained. Each replicate received 17.9 mL of the seed mixture.

Clear, rectangular 1.45 L plastic containers were used for each treatment. A 10 mm layer of sterilised soil was poured into each container. All-purpose potting mix was sterilised in a deep baking pan, covered with foil. The potting mix was then placed in an oven and baked at 90°C for 35 minutes. On top of the layer of sterilised soil, a 10 mm deep layer of fine washed sand was put in each container (Fig. 4.1). The fine washed sand

received the same sterilisation process as above. Each container that was part of the light treatment was wrapped in four layers of shade cloth around all four sides, so the light was only available to the seeds from the top of the container. All containers in the salinity treatments were left unwrapped, with the light available to access through all sides of the clear containers.

Figure 4. 1 Example of the containers used for the light treatment in the germination trials. Image shows replicate with seed layer added. Shade cloth wrapped around outside of container.

Light, Photosynthetic Active Radiation (PAR) was replicated under lab conditions using a 1200m Hortitek Growsaber 40W LED 6500k grow light, targeted to propagation/vegetative (6500k) light wavelengths. The grow light has an 80% CRI (colour rendering index) and produces a more natural light source compared to common full-spectrum LED lights. The grow light was suspended within the hydroponic grow tent, approximately 15 cm from the tent's roof and 40 cm from the seed pottles. Seeds were subjected to a 15-hr photoperiod from 6 am – 9 pm NZST, with light controlled by an automatic timer. The grow light had a beam angle of 120°, ensuring all seed bottles were reached by light, with some added reflection from the aluminium foil tent interior.

The grow tent used during the germination trails was a Certa Hydroponic Indoor Grow Tent, dimensions 60 x 60 x 140 cm (Fig. 4.2). A hydroponic grow tent was chosen to house the germination trials to best manage humidity, light and temperature. The interior of the tent has a highly reflective aluminium lining and is light and airtight. Access to the tent was achieved through zipper doors, and access was reduced only to opening for necessary checks of the germination progress. The tent was laid on its side to house all 42 replicates, with the light bar suspended as outlined above.

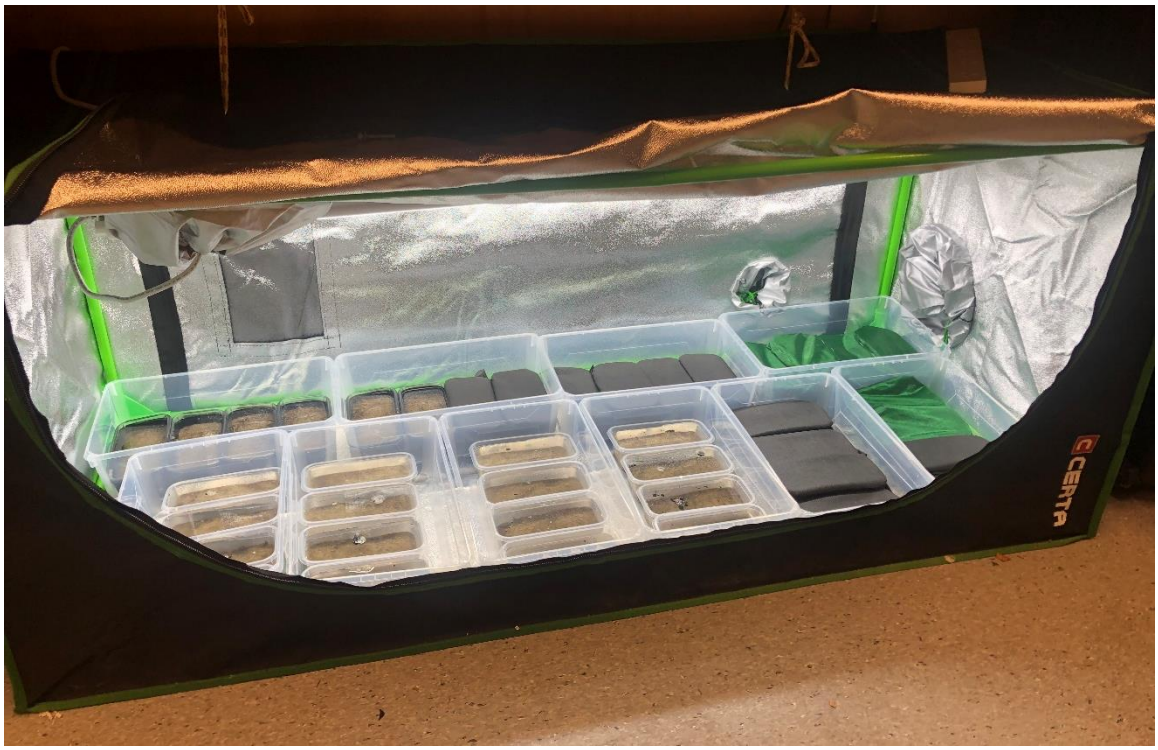

Figure 4. 2 Germination trials set-up, 42 replicates housed within hydroponic grow tent and grow light suspended above.

The temperature within the tent was monitored throughout the growth trials using a HOBO Tidbit v2 temperature data logger with ±0.2 °C accuracy. The Tidbit recorded temperature readings from within the tent every 15 minutes. No additional heating or cooling was added to the tent. The maximum temperature within the tent over the duration of the germination trials was 24.3°C and a minimum of 17.1°C. Full records of temperature inside the grow tent can be found in the appendix. Germination trials were begun in spring to replicate natural environmental conditions best.

4.2.2 Phase 1 – Salinity

Three salinity treatments were chosen for the germination trials based on the fluctuations in salinity Whakakī Lake experiences and previous work done by de Winton, (2008). In the de Winton (2008) investigation into the seed bank of Whakakī Lake, three salinity treatments were used to access germination: 0 ppt (zero), 3.5 – 4.5 ppt (low) and 6.5 – 8.5 ppt (moderate). Whakakī Lake is generally referred to as a brackish coastal lake. Hawke's Bay Regional Council has an in-situ monitoring platform at the centre of Whakakī Lake, which continuously monitors water quality parameters including salinity using a YSI Exo Sonde. Salinity data over a year in 2020 shows Whakakī Lake can experience salinity up to 9.12 ppt and as low as 1.54 ppt depending on the lake water level. For this study, the salinity treatments were 0 ppt, 2 ppt and 8 ppt.

Salinity mixtures were created by slowly dissolving API® aquarium salt into distilled water. This aquarium salt is made from evaporated sea salt. A YSI Exo Sonde was used in the tank to monitor real-time conductivity as the salt was added and dissolved. For the 0 ppt treatment, pure distilled water was used. The different salinity mixtures were then poured into the treatment containers, with six replicates per salinity treatment. Salinity was maintained throughout the germination trials by frequently remaking the salinity mixtures and topping up containers with water when required. All containers were kept at a constant water level up to the brim of the container (Fig. 4.1).

4.2.3 Phase 2 – Light

Three different treatments of light were set up to simulate germination scenarios under reduced light conditions. All light replicates were kept in water with 2 ppt salinity. Plants require photosynthetically available radiation as a source of energy to grow and

undergo photosynthesis, which is received by plants (terrestrial and aquatic) in the natural environment through solar radiation (sunlight). Of the three bands of solar radiation, PAR, or visible light (400-700nm), is the best wavelength range for photosynthesis to occur; plants respond to different wavelengths of PAR based on their growth habits. It was hypothesized that seeds would germinate best under 100% light availability, but also under both reduced and no light.

Whakakī Lake PAR

Photosynthetically available radiation (PAR) was assessed to see how much, if any, was reaching the bed of Whakakī Lake. Routine secchi disk records indicate water clarity within the lake is poor, and continuous monitoring of turbidity (FNU) from within the lake shows continuously high turbidity. Two Odyssey® Photosynthetic Active Radiation (PAR) loggers were deployed in Whakakī Lake in early November 2021. Loggers were deployed on two waratahs approximately 5 m apart. Loggers were deployed when the lake level was approximately 40 cm deep, allowing for one logger to be deployed on the lake bottom 15 cm above the lakebed. The waratah used for deployment was short to avoid shading. The second logger was deployed on a 1.8 m waratah to capture daylight PAR. This logger sat at 1.1 m above the lakebed and once again was deployed to have the sensor face sitting higher than the waratah to avoid any shading.

Odyssey® PAR Integrating PAR sensor is a self-contained, submersible, cylindrical (4 cm diameter x 16 cm long) logger with a planar cosine-corrected sensor. This sensor provides high-resolution data within the 400-700 nm wavelength range. PAR

changes seasonally and varies depending on latitude and time of day. PAR levels are greatest during midday in summer, on clear and cloud-free days. These loggers were deployed over four months from mid November 2021 to late March 2022. This range captured the highest likely values for PAR levels for the year and a period of stable high/moderate water levels within the lake, as high water levels are maintained within the lake over the dry summer months. An important consideration for the use of Odyssey® PAR loggers is that they do not come factory calibrated. The loggers used in this experiment could not be calibrated to a reference meter. Thus, caution should be placed on the specific values of µmol recorded, as each logger is only relative to itself.

PAR: germination trials

The same Odyssey® PAR loggers, described above, were used to determine the PAR available under the artificial light within the tent used for the germination trials. The two loggers were deployed within the tank directly under the artificial grow bar, with all tent flaps closed. Loggers were sat directly upright so sensor faces were pointed directly towards the grow light, with no objects shading the view. The results from this deployment were considered to be maximum PAR availability produced by the artificial grow bar. The same loggers were deployed repeatedly with 'shading' measures in place to simulate restricted light conditions. Artificial light restriction was achieved by layering 50 gsm, black, spunbonded weed mat and calculating the percentage difference from full light. Light availability was set at three low-light conditions 1) 90% PAR reduction 2) 95% PAR reduction, and 3). 100% light reduction. Both 90% and 100% PAR reduction were achieved using the weed mat method, requiring two and four layers, respectively.

To achieve 95% light reduction layers of a thinner and less tightly woven green fabric were used to reduce light by 95%. PAR was limited by 95% by using four layers of green fabric.

4.2.3 Assessing seed viability

The emergence of seedlings was monitored approximately every three days from 18th October 2021 – to 18th December 2021. Emergence was defined as the development of a germinated seedling that could be detected by eye. At each check, all emerged seedlings were removed so that developing plant density effects would not alter germination of other seedlings. Seedlings were identified under a Leica M60 stereoscopic microscope by reference to vegetative and seed characteristics. Some seedlings were able to be identified by their seed characteristics, as the seeds were still intact and attached to the germinant (Fig. 4.3).

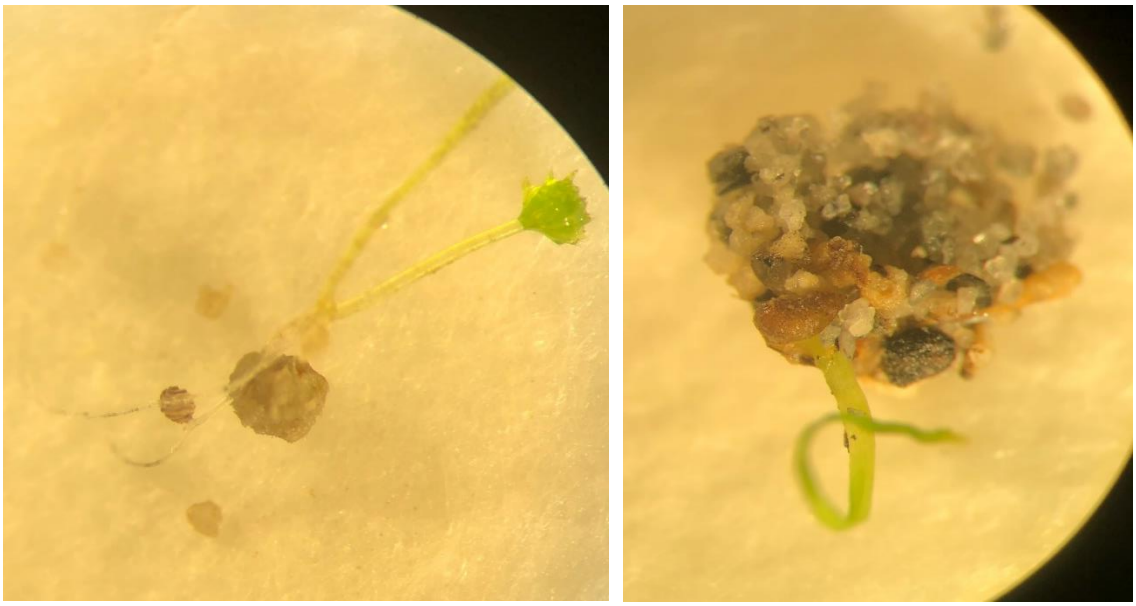

Figure 4. 3 Germinated seeds of Nitella hyalina (left) and Ruppia polycarpa (right) with seeds still intact

Species identifications were recorded under the date of observation. Species that were unable to be identified upon germination were noted and added to the incubation tanks to continue to grow to a size at which they could be identified. There were three incubation tanks, each filled with either 0 ppt, 2 ppt or 8 ppt water. The tanks were then further separated into six quadrates to monitor which replicate a germinant had come from (Fig. 4.4). An aquarium aeration bubbler line was run to each incubation tank to ensure adequate oxygenation of the water. An additional Hortitek Growsaber 30W LED 6500k 900mm grow light was suspended above the incubation tanks. These tanks were not kept in a hydroponic tent but were adjacent to the germination tent inside the HBRC lab.

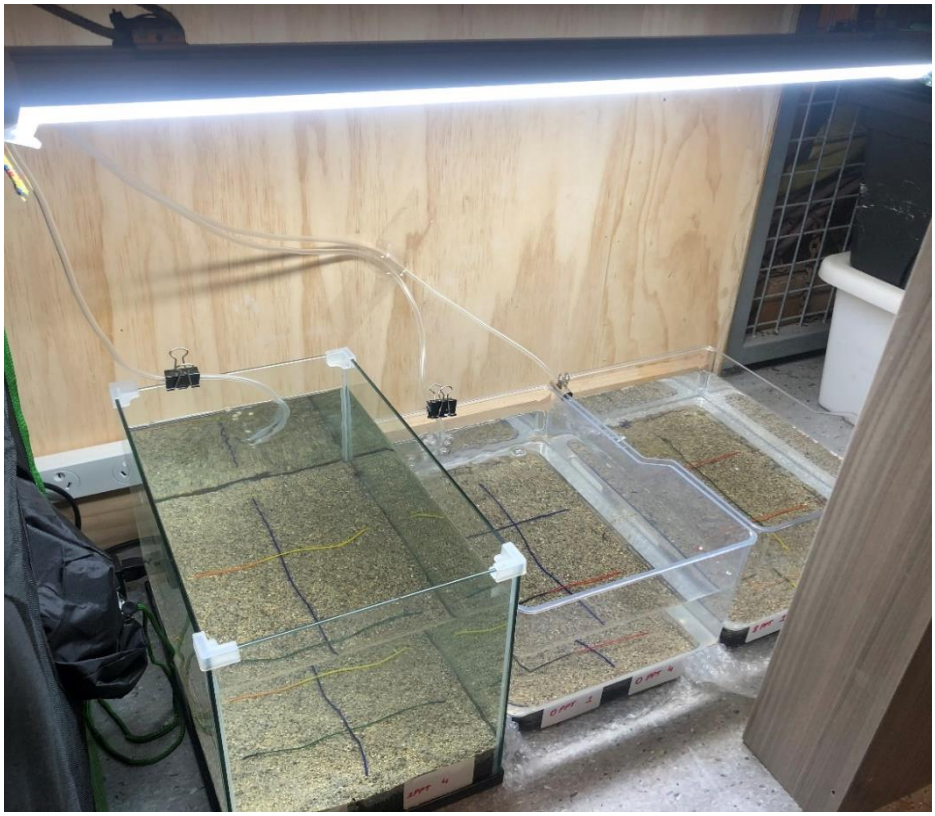

Figure 4. 4 Set-up of incubation tanks, each with different salinity water. Sting quadrates indicate replicates from within the germination trials. From left to right: 2ppt, 0ppt, 8ppt. Grow light suspended above tanks approximately 500 mm with aeration bubbler line running to each tank

4.2.4 Statistical analyses

PRIMER v7 (Clarke and Gorley, 2015) with the PERMANOVA+ add on (Anderson *et al.,* 2008) was used to conduct a non-metric multi-dimensional scaling ordination (nMDS) in order to display spatial and temporal patterns in community composition. A resemblance matrix was calculated using the Bray-Curtis similarity index. Due to the Poisson distribution of the data, germinate count data was transformed using the fourth root to meet the assumptions of ANOVA (Sokal *et al.,* 1995). Goodness-of-fit is measured by 'stress', which measures a rank-order disagreement between observed and fitted distances. A stress value of > 0.24 is a poor result, and interpretation should be reconsidered, whereas stress of 0.05 - 0.1 is good. A PERMANOVA (permutational multivariate ANOVA) was used to examine the differences in germination between treatments. Spatial variations in species germination were also assessed using nMDS. Two-way analysis of variance (Two-way ANOVA) in RStudio version 4.0.5 was used to test for differences between treatments, with a posteriori Tukey HSD means test. Box plots have been used to summarise and visualise species germination between treatments for each macrophyte species germinated in either light or salinity trials.

4.2.5 eDNA for species identification

Seedling samples from known and unknown species were collected for eDNA analysis. Samples were clipped from plants growing in the incubation tanks, and the plant fragments placed in vials with DNA/RNA shield preservative. Two fragments were taken from each species and placed in separate vials, 14 vials in total for seven species (Fig.4.5). The seven species sent for eDNA identification were *Ruppia polycarpa*, *Nitella hyalina (2ppt), Lamprothamnium macropogon, Nitella hyalina (0ppt), unknown 1,*

unknown 2 and unknown 3. eDNA was compared to plant species sequences in GenBank® (Benson *et al.* 2008). There are limitations to eDNA sampling, such as only sequences in the genetic library of known species can be compared, and taxonomic level identification is unknown (e.g., family, genus, species).

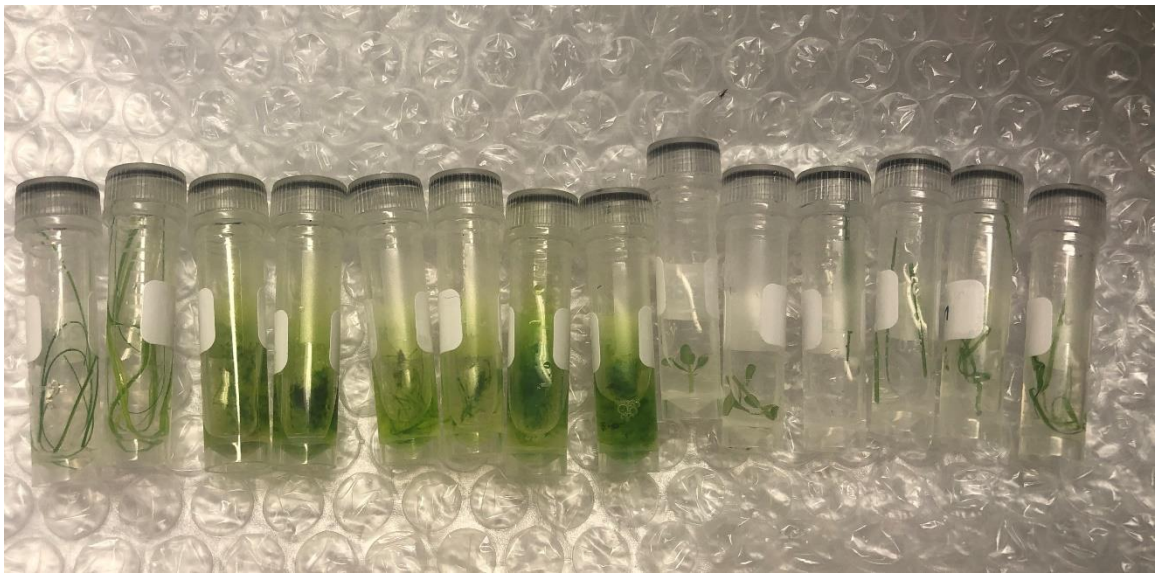

Figure 4. 5 Vials of plant fragments to be sent to Wilderlab NZ for eDNA identification. From left: Ruppia polycarpa, Nitella hyalina (2ppt), Lamprothamnium macropogon, Nitella hyalina (0ppt), Unknown 1, Unknown 2, Unknown 3

4.3 Results

4.3.1 Phase 1 - Salinity

In total, 187 seeds germinated across all salinity treatments. Of the species identified in the seed bank, six germinated during the salinity trials, including *N. hyalina, L. macropogon, A. bilocularis, R. polycarpa, Bolboschoenus sp*. and *Lythrum sp*. *Bolboschoenus* and *Lythrum* (commonly loosestrife) seedlings were identified in the eDNA. Both species were found in the seed bank but were removed from the study and

excluded from statistical analysis as both are emergent plants. The low salinity treatment, 2 ppt had the highest number of germinates, with 79 across all species. 0 ppt and 8 ppt salinity treatments had similar total germination (Fig. 4.6).

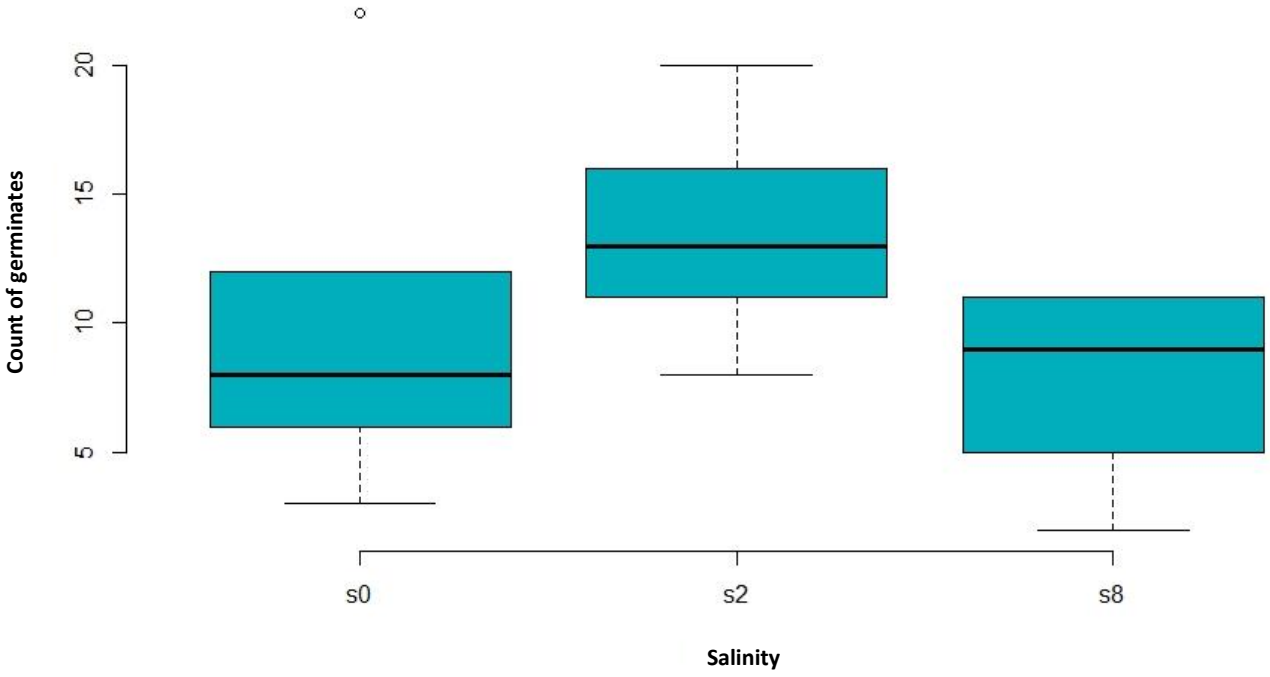

Figure 4. 6 Box plots describing the distribution of germinants across three salinity treatments

nMDS plots highlight the visual similarities in community composition between salinity treatments, and there was a pattern in community composition between salinity treatments (Fig. 4.7), a stress level of 0.05 indicates a good ordination and fit for the model. PERMANOVA results show a significant difference in community composition between salinities (df = 2, F = 6.016, P = 0.006). Pair-wise testing further elaborates that

the significant differences lie between salinity 0 ppt and 8 ppt (t = 3.433, P = 0.002) and between 2 ppt and 8 ppt (t = 0.0399, P = 0.041). The addition of the species vector overlay shows *L. macropogon* was a characteristic of the higher ppt treatments (Fig. 4.7).

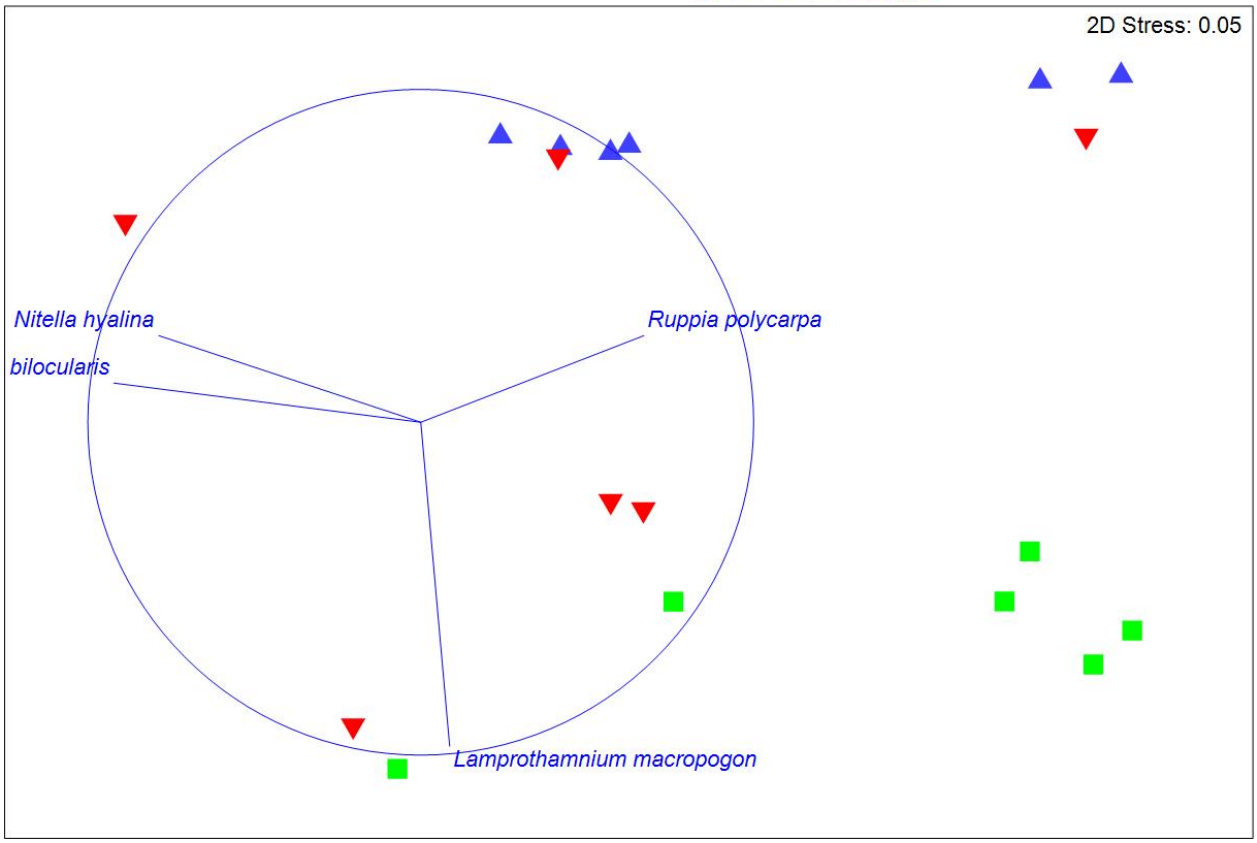

Figure 4. 7 nMDS (non-metric multi-dimensional scaling) ordination of community composition of replicates at each salinity treatment from the germination trials (Data transformation: fourth root) with vector overlay of species ▲ *= Salinity 0ppt* ▼ *= Salinity 2ppt* ■ *= Salinity 8ppt*

Species distributions - Salinity

Not all species germinated under every salinity treatment, with 2 ppt and 8 ppt having the highest diversity in germination success (Fig. 4.8). *Nitella hyalina* germinated

the most out of all species and across all salinity treatments (Fig. 4.8). Two-way ANOVA results show a statistical difference between species germination ($F = 29.898$, $P = <0.001$) and a relationship between species and salinity treatment ($F = 3.667$, $P = 0.004$) on germination.

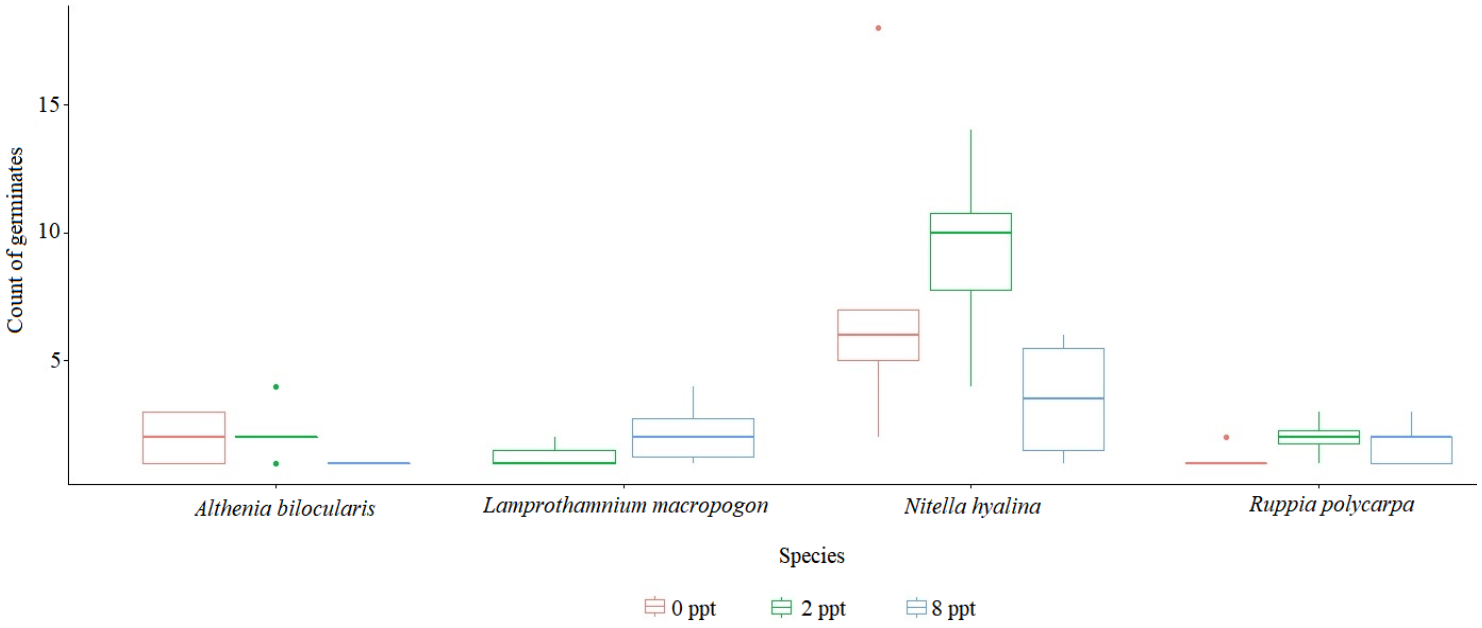

Figure 4. 8 Box plots describing species germiation between salinity treatments

4.3.2 Phase 2 - Light

In total, 64 individuals germinated during the light trials. The highest abundance of species germinated under 100% light availability out of the four light treatments, with a total of 49 germinates. Four species germinated during the light trials under 100% light availability, *N. hyalina, L. macropogon, A. bilocularis* and *R. polycarpa*. Of that, only

two (*N. hyalina* and *R. polycarpa*) germinated under 10% light availability (Fig. 4.9). *R. polycarpa* germinated twice under 5% light availability. There was no germination under complete darkness (0% light availability).

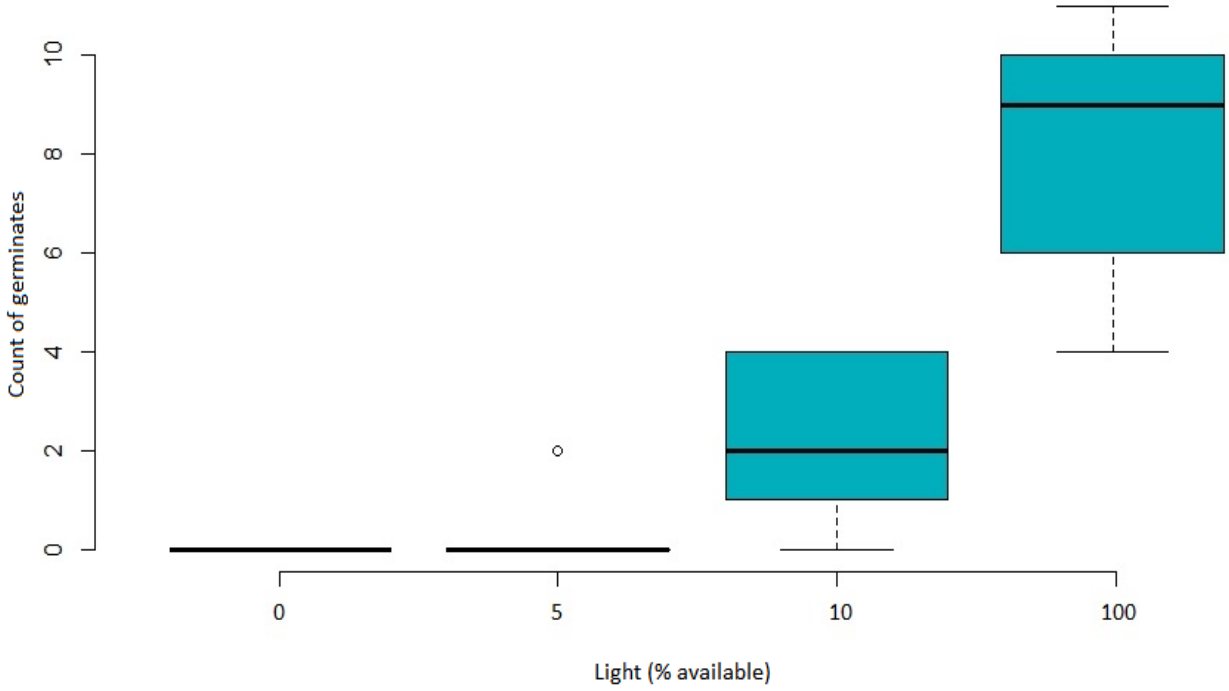

Figure 4. 9 Box plots describing the distribution of germinants across four light treatments

nMDS plots highlight the visual similarities in community composition between light treatments, and there was a pattern in community composition between light treatments (Fig. 4.10), a stress level of 0.01 indicates a good ordination and fit for the model. The nMDS shows a single grouping comprising 100% and 10% light treatments indicating their composition is similar. 100% and 10% light treatments were

characterised by all four species, *N. hyalina*, *L. macropogon*, *A. bilocularis* and *R. polycarpa*.

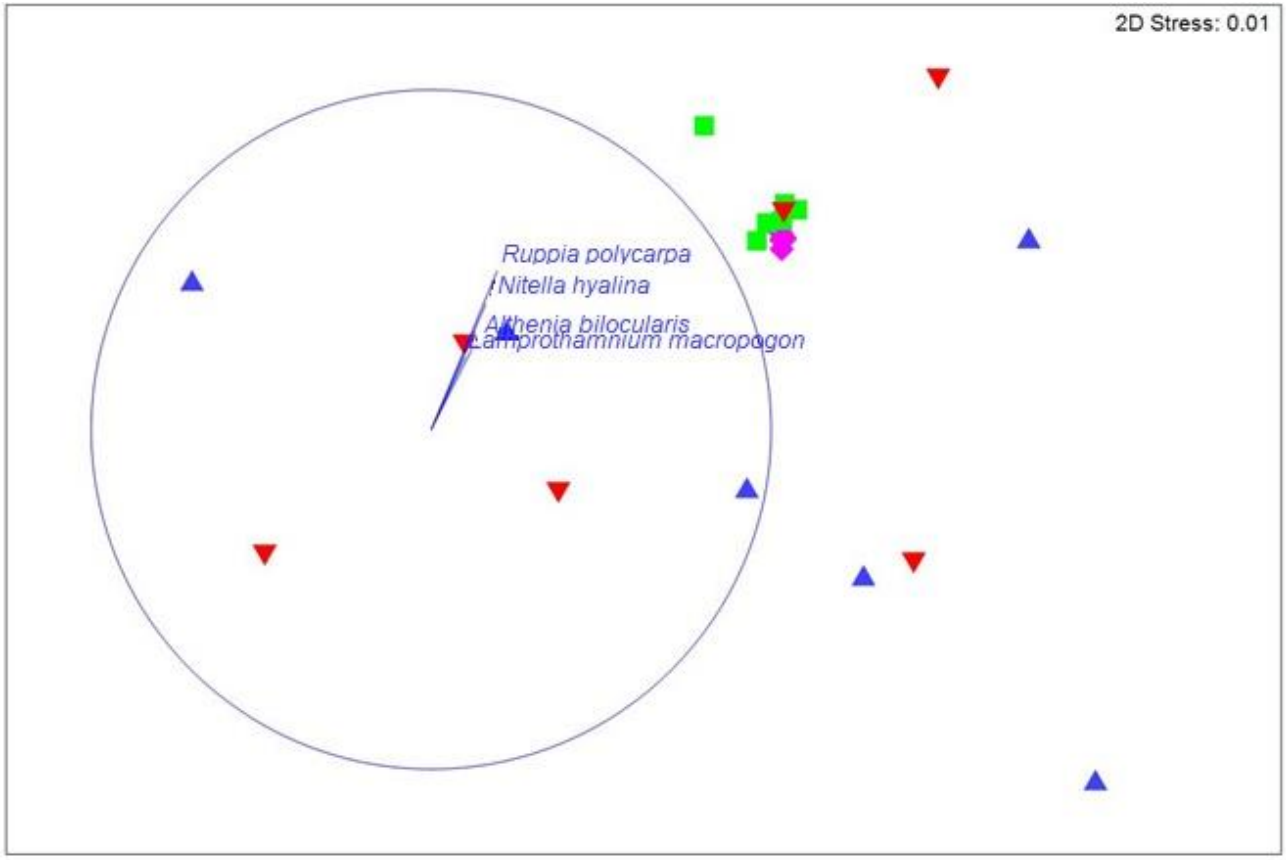

Figure 4. 10 nMDS (non-metric multi-dimensional scaling) ordination of community composition of replicates at each light treatment from the germination trials (Data transformation: fourth root). with vector overlay of species. ▲ = 0% light ▼ = 5% light availability ■ = 10% light availability ◆ = 100% light availability.

Species distributions – light availability

The 100% light treatment had the highest diversity in species germinating (Fig. 4.11) compared to the other light treatments. Two-way ANOVA results show a relationship between light and species (F = 11.69, P = <0.001). While the nMDS plot

shows a clustering of similar community compositions between 100% and 10% light, a posteriori Tukey HSD means test shows a significant difference between the 100% light treatment and all other treatments. Results for this test can be found in the appendix. *Nitella hyalina* germinated the most frequently compared to the other species (Fig. 4.11), and *Ruppia polycarpa* was the only species to germinate at all under 5% light availability.

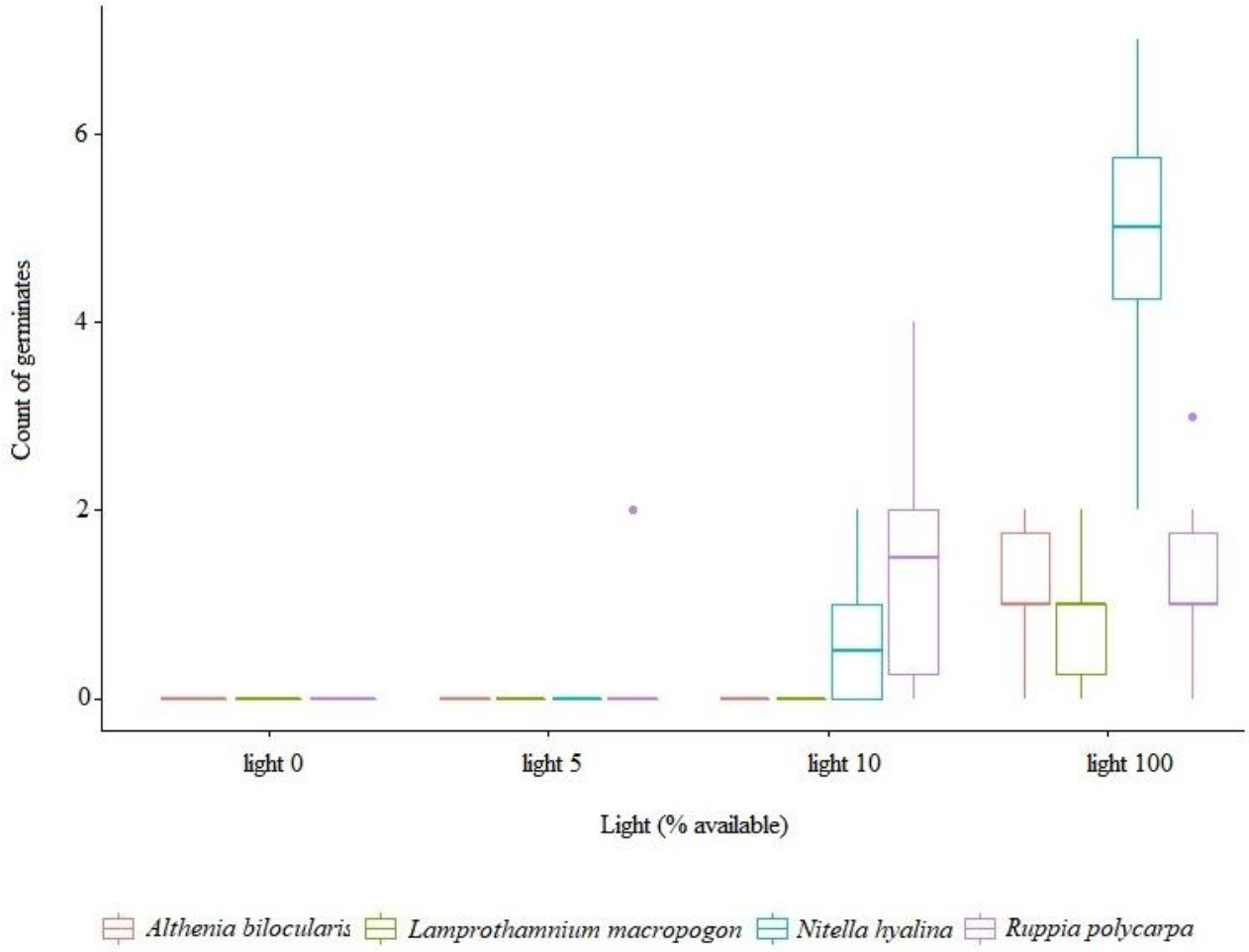

Figure 4. 11 Box plots describing species germination between light treatments

4.3.3 Whakakī Lake PAR

Data from the PAR light logger deployed at the bottom of Whakakī Lake show that there were periods when the bottom of the lake was getting some PAR between the hours of 5 am – 7 pm (Fig. 4.12). The maximum PAR reached at the bottom of the lake was 7020 µmol $m^{-2}s^{-1,}$ with the average throughout deployment 121.94 µmol $m^{-2}s^{-1}$ and the minimum 0 µmol $m^{-2}s^{-1}$. Periods of higher PAR availability to the lakebed are likely to be during high wind conditions when waves across the lake surface can alter the water above the logger. The water level in the lake filled to a stable water level of 1 m after the loggers were deployed. Water level data from the time of deployment in the lake's center can be found in the appendix.

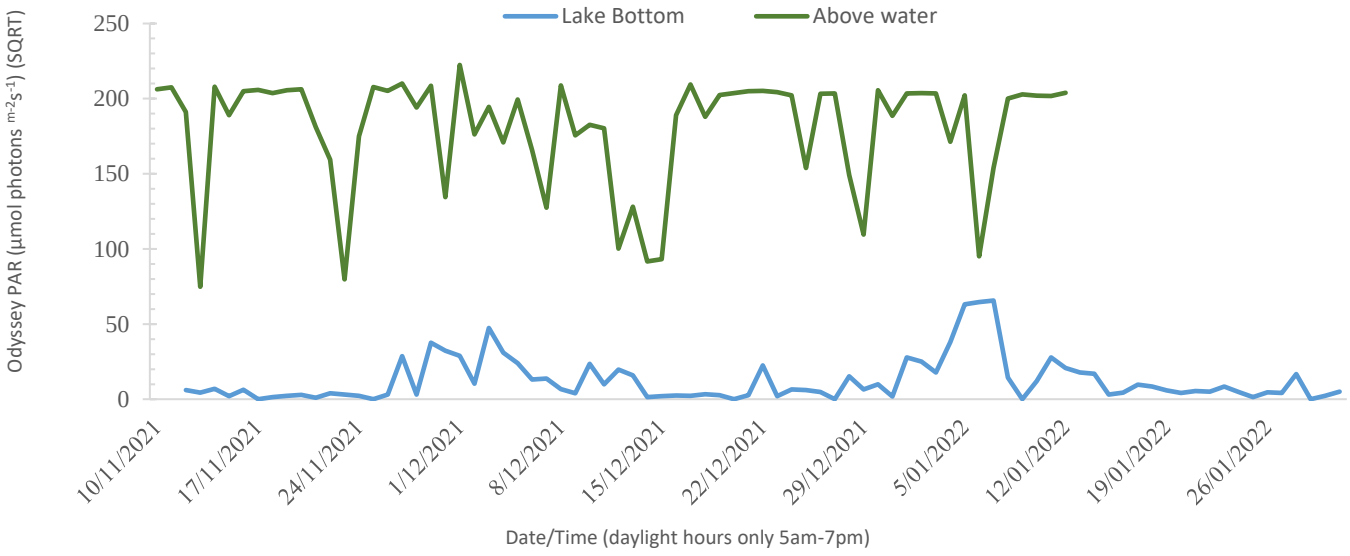

Figure 4. 12 Odyssey PAR (µmol photons m-2s-1) output from loggers deployed at the bottom of the lake and above the water. Data transformation: square root. Only daylight hours included between 5am - 7pm. Series for 'Above water' cuts out at approximately 12/01/2022 due to logger malfunction

4.3.4 eDNA

eDNA identification indicated the three unknown species were *Althenia bilocularis*, a species from the Bolboschoenus genus and a species from the *Lythrum* genus. Bolboschoenus is a genus of plants in the sedge family, with some species, such as *Bolboschoenus fluviatilis*, a native to New Zealand coastal lowland marshes and saline areas, commonly known as kukuraho or pūrua grass. Bolboschoenus seeds were found in the seed bank analysis described in chapter III. Bolboschoenus seeds were discounted from further analysis as this research aimed to focus on the submerged macrophyte community. Similarly, Lythrum is a genus of species of flowering plants commonly referred to as loosestrife. Species such as *Lythrum salicaria* are known to inhabit lake margin and other wetlands in New Zealand; these seeds were similarly discounted from this study.

The remaining samples sent for eDNA identification were confirmed to be the species identified as *Nitella hyalina, Lamprothamnium sp.* and *Ruppia polycarpa.* It was noted in the results that the sample 'unknown 3' was identified as *Althenia*, being 100% identical to *Althenia* (*Lipilaena*) *australis* as there was no reference sequence for the species *Althenia bilocularis*, a threatened species within New Zealand. Although this sample came back as a 100% hit for *Althenia*, the sequence is also 99% similar to several *Potamogetons* and *Stuckenias.* From the eDNA, there was a possibility that sample 'unknown 3' could have been *Stuckenia pectinata*, a species identified within the lake in previous macrophyte reports (de Winton, 1992 and de Winton, 2008) or *Althenia bilocularis* (formally named *Lepilaena bilocularis* - Potamogetonaceae) could be confused for *Zannichellia palustris*. However, by allowing the seedlings to continue to

grow in the incubation tanks, this species has grown into mature fruiting plants within the 2 ppt tank and can be confirmed to be *Althenia bilocularis.*

4.4 Conclusion

4.4.1 Phase 1 – Salinity

The salinity trials found the seed bank within Whakakī Lake had a diverse range of viable species able to germinate and thrive under a range of salinity conditions. The fact that seeds germinated under all salinity conditions is promising for the future outlook of the lake, where salinity levels remain uncertain . If a salinity level of under 8 ppt can be maintained within Whakakī Lake, species such as *R. polycarpa, L. macropogon, A. bilocularis* and *N. hyalina* could germinate given suitable light, clarity, and water quality conditions. It is possible that other species identified within the seed bank that did not germinate during these trials could germinate within the lake, such as *Stuckenia pectinata* which reproduces best via tubers. If the tubers of *S. pectinata* and *L. novae-zealandia* remain intact within the sediment, their presence at Whakakī Lake could be re-established. There was notable absence of some freshwater species such as *C. australis, N. hookeri* and *M. triphyllum* which did not germinate. It is possible that the germination trials did not continue long enough to allow their germination or some other key environmental cue was missing. The salinity germination trials lasted for 8.5 weeks or 62 days. de Winton (2004) outlines a timeframe of between 13-20 weeks as an appropriate time frame for a substantial germination response from charophyte species and for plants under high light conditions to approach adult size. While successful germination of two charophyte species occurred during this study, other species such as *C. australis, C.*

globularis and *N. hookeri* may not have had adequate time to germinate. *C. globularis* and *C. australis* were both prevalent in relatively high numbers within the seed bank. Further investigation with longer germination trials under full light conditions may be required to assess the viability of these species.

The same species which germinated from seeds bank surveys conducted in 2008 (de Winton, 2008) germinated in these salinity trials (Fig. 4.13). Thus the seed bank represents a species composition characteristic of the last known macrophyte community in the lake (de Winton *et al.*, 2008). Missing from both germination studies but recorded in the lake in 2007 is *S. pectinatus, L. novae-zelandiae* and *P. crispus.* It is likely these species were missed from both germination trials for reproductive reasons stated above.

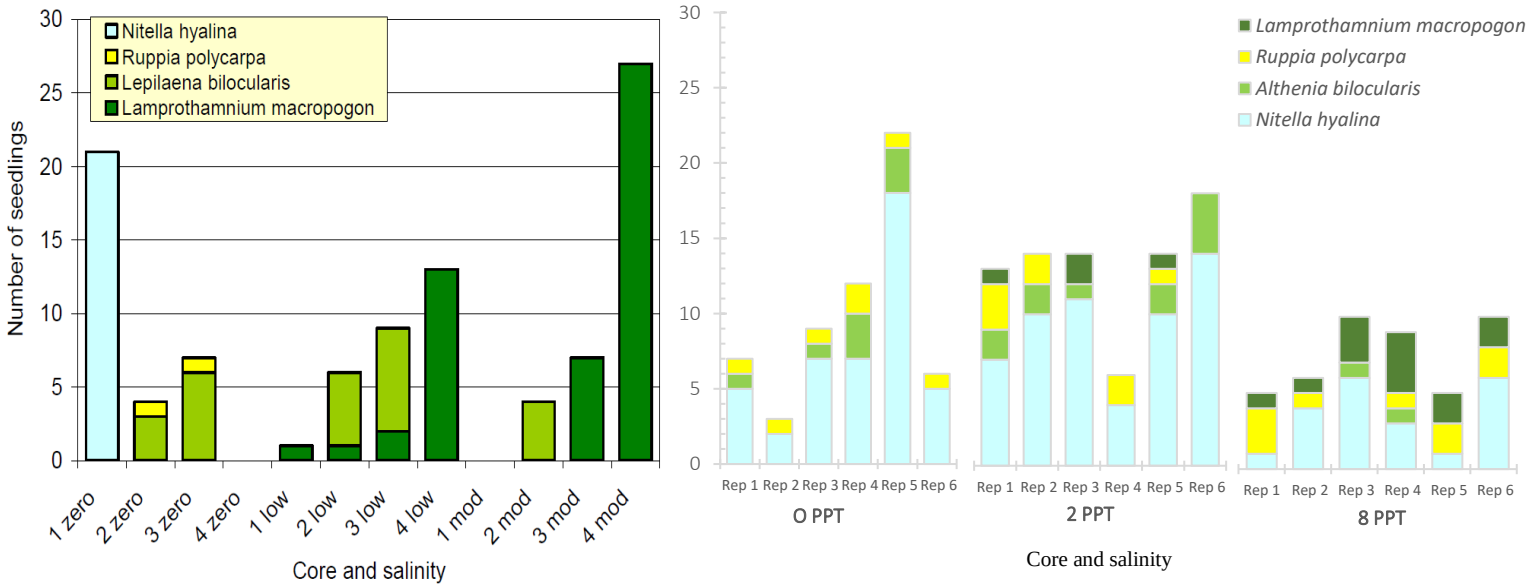

Figure 4. 13 Number of seeds germinated under different salinity conditions during the 2007 (right) seed bank survey (de Winton, 2008) compared with species germinated during this research (left). Note Althenia bilocularis was formerly Lepilaena bilcoualris.

During the 2007 seed bank survey, *N. hyalina* only germinated in zero salinity (0 ppt) from a core taken from the northern shore of the transect. *N. hyalina* was found in the seed bank predominantly at sites 1 and 2 in this study, with site 1 being closest to the site outlined in the 2007 seed bank survey. Contrary to the findings of 2007, *N. hyalina* germinated in all salinity conditions during this research. Of all species to germinate over the salinity trials, *N. hyalina* was the most prevalent, with the highest number of germlings of all plant and charophyte species. *N. hyalina* is known to inhabit shallow zones of lakes, swamps, and slow-flowing waters however it is considered a freshwater species (de Winton, 2008). While *N. hyalina* had the highest rate of germination in the salinity trials, it may be that salinity does not act as a primary environmental cue for germination of *N. hyalina* oospores. *N. hyalina* began to germinate at approximately 35 days (5 weeks) into the germination trials, and germlings continued to germinate from then until the end of the trials. Upon successful germination in the trials, individual germlings of N. *hyalina* were moved to the incubation tanks, being placed in the incubation tank with salinity from which it germinated (Fig. 4.14). Growth was not measured once individuals were placed in the incubation tanks, however, it was observed that *N. hyalina* did not persist in 8 ppt salinity. *N. hyalina* was able to establish and thrive in both zero, and low (2 ppt) salinity conditions in the grow tanks and became one of the dominant species within both tanks. As outlined in chapter 3.6, *N. hyalina* oospores were more abundant at sites near the northern edges of the lake near the Tuhara stream. The results of these germination trials support this notion and suggest *N. hyalina* could only successfully progress beyond germination in areas of the lake with freshwater influence, or if the salinity within the lake remained consistently low around 2 ppt (Fig. 4.14).

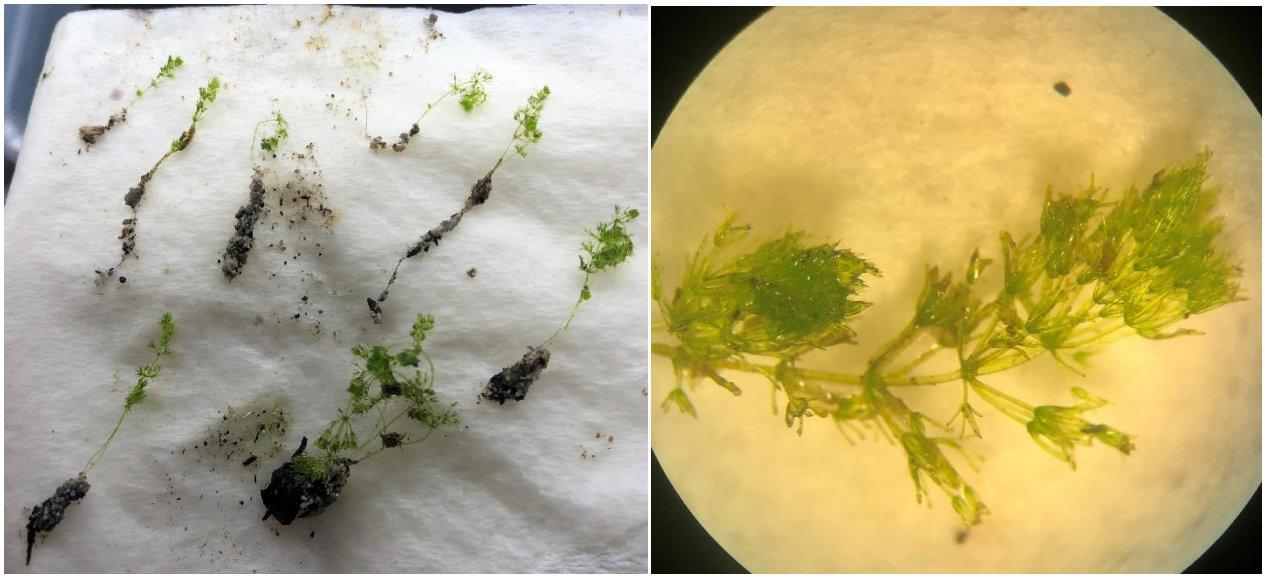

Figure 4. 14 Nitella hyalina germlings from 2 ppt (low) salinity trial before being placed in the incubation tank

Ruppia polycarpa germinated across all salinity levels in this experiment, whereas it was only found to germinate under zero salinity conditions in 2007. *R. polycarpa* was germinated from cores taken near the centre of the lake in 2007, similar to sites 2 and 3 in this study. It was found that *R. polycarpa* seeds were at the highest densities towards the northern shores of Whakakī Lake at sites 1 and 2 but were present at all sites. *R. polycarpa* is noted to inhabit saline ponds, lagoons, and brackish and freshwater streams (Mason, 1967). Based on *R. polycarpa* life-history traits, it seems most plausible that it should germinate and thrive under all salinity conditions trialled in this study. *R. polycarpa* was the first species to germinate within the salinity trials, with the first germinates appearing on day 9 of the trials. *R. polycarpa* continued to be the only species to germinate for the first 35 days before *N. hyalina* also began to germinate. It was observed that the germination of *R. polycarpa* began to slow, with fewer seedlings germinating as the trials went on. *R. polycarpa*, or commonly horse's mane weed, is

likely a good pioneering species that can germinate and establish quickly given suitable water quality conditions (Mason, 1967). In some places in New Zealand, horse's mane weed is regarded as a pest species as it can grow to spread across the water's surface and form dense mats. Research into the characteristics of other *Ruppia* species indicate a reliance on large annual reproductive events, and large clusters of *Ruppia* can produce numerous seeds if not nutrient limited during "windows of opportunity" (Strazisar *et al.,* 2016). The complete mortality of vegetative population within the lake of *R. polycarpa* could be having significant long-term impacts on the potential regeneration of the species if the seed bank is not being replenished. Salinity thresholds will also play a key role in the ability for *R. polycarpa* to re-establish in Whakakī Lake, with an upper germination tolerance of 40-50 ppt, and growth significantly impacted at salinities above 45 ppt (Sim *et al.,* 2006). This salinity threshold was far beyond this scope of salinities trialled in this study, however with salinity fluctuations in the lake and the unknown future of salinity post weir, ongoing monitoring of salinity level is suggested to ensure best chance of *R. polycarpa* recovery in the future.

Lamprothamnium macropogon was found in 2007 to require low to moderate salinity to germinate. The findings from this study echo this sentiment, with *L. macropogon* only germinating under 2 ppt (low) and 8 ppt (moderate) salinities. This species is known to inhabit brackish water and coastal lakes and lagoons in New Zealand (Wood & Mason, 1977). As Whakakī Lake is unlikely to become a freshwater lake and likely never was, it is possible that *L. macropogon* could become a prominent species within the lake if a charophyte community could re-establish. *L. macropogon* began germinating around day 44 (6 weeks) into the germination trials. Upon successful

germination, each germling was moved to the corresponding incubation tank, where *L. macropogon* was observed to establish and continue growing in both low and moderate salinity tanks. The upper limit for germination of *L. macropogon* has been found to be 30-40 ppt, and a suggested optimum for adult plants between 10 ppt – 20 ppt (Sim *et al.*, 2006).

Althenia bilocularis was the only other species to be germinated under the 2007 seed bank analysis and the salinity trials in this study. *A. bilocularis* was the only species in 2007 to germinate under all salinity conditions tested, corresponding to the results found during this study. Germinates were added to incubation tanks as outlined above (Fig. 4.15). It was observed that *A. bilocularis* established and continued to grow in tanks of zero and low salinity. Two seeds germinated under 8 ppt conditions and were added to the 8 ppt incubation tank. It was observed that the seedlings were able to persist in the moderate salinity conditions for some time, however failed to thrive and remained small and stunted before dying after approximately three weeks. There has been less research into the life history traits of *A. bilocularis* compared to the other species in this study. Moore and Edgar (1970) note this species as inhabiting lakes, brackish water and slow flowing rivers in both New Zealand and Australia. This research supports this notion and the presence of *A. bilocularis* in both 1992 and 2007 indicate the ability for mature populations to withstand salinity fluctuations similar to current conditions.

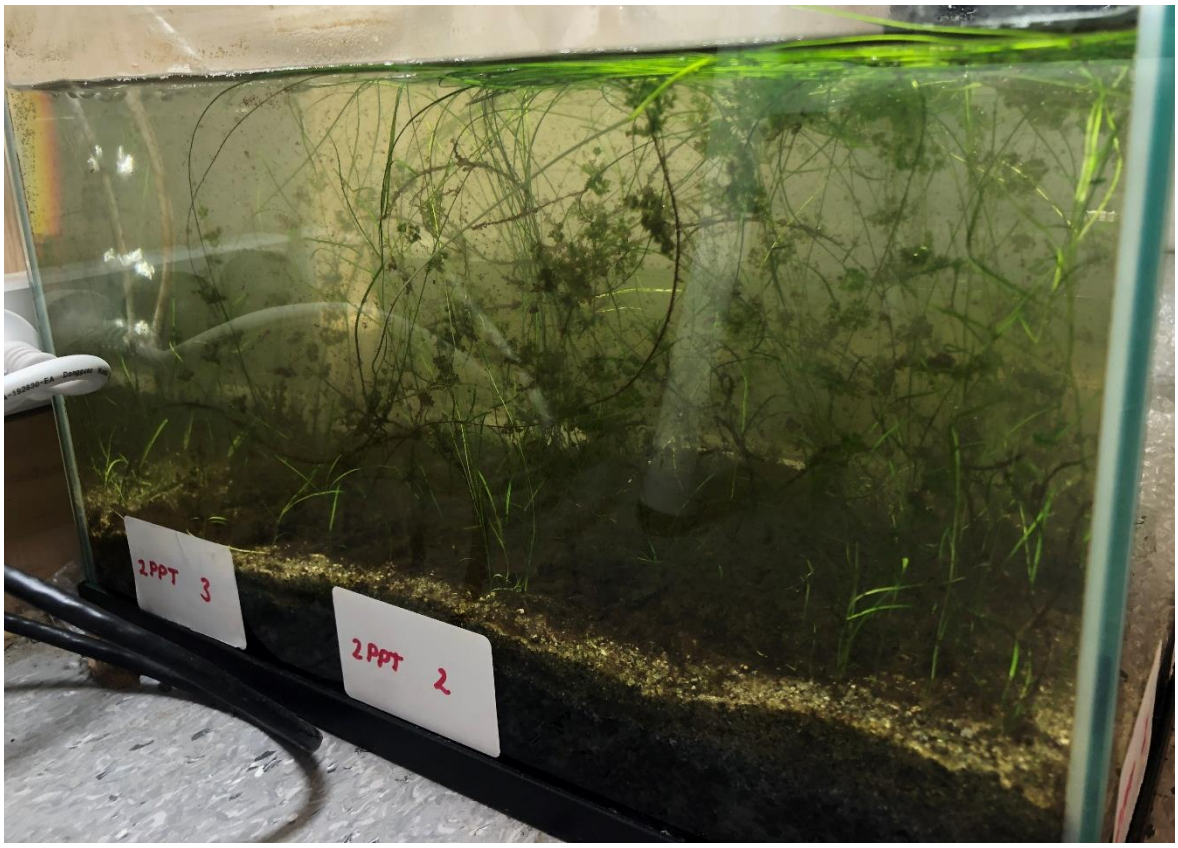

Figure 4. 15 2 ppt (low) salinity incubation tank pictured at the conclusion of the germination trials. Image depicts established R. polycarpa, N. hyalina, A. bilocularis and L. macropogon

Managing salinity conditions in Whakakī Lake would be challenging and there is limited scope for this. Salinity levels within the lake are influenced by stream inflow, evapotranspiration, rain, sea spray, weather events that can cause waves to overtop the dunes and the artificial opening of the Rahui channel (de Winton & Champion, 2008). Salinity fluctuations were likely a natural feature of the Whakakī wetland system and could promote diversity and development of the submerged vegetation. Lower salinity conditions e.g., less than 8 ppt could be beneficial within the lake during spring to support seedling germination and growth (de Winton & Champion, 2008).

4.4.2 Phase 2 – Light

PAR data from the bottom of Whakakī Lake shows that there is inconsistent and minimal light reaching the bed of the lake for seeds to germinate. While there were short periods of moderate PAR (~7020 µmol $m^{-2}s^{-1}$), these periods of light were short-lived and inconsistent, resulting in a much lower average amount of PAR available seeds with frequent durations of zero PAR availability (Fig. 4.12). It is theorized that these inconsistent light levels within the lake result in seeds and oospores within the seed bank not germinating. Germination of two species was found during the light trials where light was reduced to 10% light availability, *Ruppia polycarpa* and *Nitella hyalina.* There was significantly more germination of seeds and oospores under full PAR light availability than when PAR was reduced.

It was hypothesised that seeds and oospores would germinate under reduced light and full dark conditions, however, would not continue to grow due to unsuitable PAR levels for photosynthesis. It was found that this was not true for 5 % and 0% light levels as no seeds or oospores were found to be in any stage of germination. This suggests that within Whakakī Lake if zero PAR is reaching the lakebed, as observed in the Odyssey PAR data, it is doubtful any seeds or oospores are germinating. It has been recorded that some charophyte oospores do not require light cues to germinate, such as *Chara australis* and *Chara globularis* (de Winton, 2004), and have been recorded germinating under very low/no light conditions. Both *C. australis* and *C. globularis* were identified as being present within the oospore bank of Whakakī Lake; however, they failed to germinate under salinity or light treatments. Taking into consideration the findings of others, it is possible that for some charophyte species, including those identified in this research, light

is required for establishment rather than gemination (de Winton, 2004). It may be possible that given a longer duration of time, some species such as *C. australis* and *C. Chara globularis* may have germinated under 0% PAR availability.

Although germination under low/no PAR light availability is interesting, it is likely of no use to the regeneration of charophytes and macrophytes to Whakakī Lake if there is not enough energy available for them to grow. *Ruppia polycarpa* was able to germinate twice under 5% PAR availability. While growth was not measured as part of this study, it was noted that the two seedlings that germinated did not grow and died shortly after germination. This further supports the idea that even with some PAR light reaching the lakebed in Whakakī periodically, it is not enough to support the growth and establishment of a population of any macrophyte or charophyte species included in this study. *R. polycarpa* has the ability to tolerate and adapt to some low light conditions, noted to have much longer stems when under low light conditions (Mason, 1967).

Germination success in 10% PAR reduction was better than any other treatment where the light was manipulated. However, only 13 individuals across two species were able to germinate. More importantly perhaps, than the process of germination itself, was the ability of a handful of these individuals to persist under 90% reduced light for the duration of the light trials. Unlike in the salinity trials above, seedlings and germlings were not removed from the pottles under shade cloth but were counted, identified, and left to continue receiving the treatment. Although growth after germination was not measured during these trials, it was observed that 9 out of the 13 individuals were able to survive as small seedlings of *R. polycarpa* (n = 7) and *N. hyalina* (n = 2). This further supports the requirement of PAR for seedlings and germlings to continue to grow. While

a majority of individuals that germinated under 10% light availability were able to survive, it is unknown if they would have been able to grow further beyond their stunted seedling size, and further research would be required to investigate this. Other work on the germination of oospores under low irradiance notes that insufficient light availability to sustain germling growth may be an important loss for oospore banks under unfavourable light conditions (de Winton *et al.*, 2004).

Unsurprisingly, seeds and oospores germinated best under full light conditions and were able to establish and mature, given time. While this result was expected, it is further encouraging support for the general viability of the Whakakī Lake seed bank and supports the results found under low (2 ppt) salinity during the light trial phase of this experiment. It would be interesting to delve into the relationship between light availability in Whakakī Lake and macrophyte and charophyte growth after installing the weir and if persistent, managed water levels effects PAR availability. Wave action will likely continue to impact the shallow coastal lake and cause variations in light availability as high winds move across the lake's surface and continue to cause sediment resuspension. Further research into the light availability within the lake could delve into light availability spatially across different areas of the lake. Once a minimum water level has been established, there may be sheltered pockets within the lake that are less exposed to wave action and may have (if only slightly) higher or more consistent levels of PAR reaching the lakebed. Identification of these areas would be useful to help inform potential restoration or macrophyte regeneration projects in the future.

Chapter V

General Discussion

5.1 Introduction

The aim of this research was to build on previous work around the macrophyte communities that were once present in Whakakī Lake and to contribute to ongoing efforts for lake restoration. This was achieved by characterising and quantifying the submerged seed and oospore bank of Whakakī Lake and assessing the viability of the seed bank under optimal and experimental conditions. Results from sediment coring within the lake show that a diverse and abundant seed and oospore bank still exists within Whakakī Lake, with differences in species compositions between sites. Overall, the abundance of seeds and oospores was significantly higher at sites closest to the northern shoreline, located in the shallowest areas cored in this research and nearest the Tuhara inlet stream. The number of different species identified in the seed bank gives confidence to a robust and resilient seed bank that could promote macrophyte re-establishment under an array of salinity conditions. Germination trials revealed a viable seed bank, with germination of four of the eleven species identified in the lake sediment. This work supports the idea that if water quality and clarity in Whakakī Lake improved, the macrophyte community might be able to re-establish, which is an essential step to restoring Whakakī Lake to its pre-eutrophic state.

5.2 Whakakī Lake Seed Bank

The picture painted by the seed bank analysis of Whakakī Lake is of a once distinctly brackish aquatic flora. Five of the eleven species identified in the seed bank were charophyte species. Charophytes play an essential role in shallow lakes, and the re-establishment of charophytes is a desired outcome during efforts in shallow lake restoration. Charophytes are able to bind sediment against wave disturbance without

impacting recreation or water circulation, thanks to their low growing life-forms (de Winton *et al.,* 2004). Additionally, their sediment stabilising properties make them helpful species in mitigating turbidity in shallow lakes. Charophytes can reproduce either via vegetative fragments or oospores. Oospores can persist in lake sediment and exhibit dormancy characteristics (de Winton and Clayton, 1996; de Winton *et al.,* 2000). The ability of oospores to persist in lake sediment make seed banks the best mechanism for charophyte recovery in shallow lakes systems where vegetation has been lost. The presence of a variety of oospores from charophyte species within Whakakī Lake suggests with decreased turbidity, some species could re-establish in the lake. Charophytes are sensitive to turbidity, and light plays an essential role in the germination and growth of charophytes (de Winton *et al.,* 2004). *N. hyalina* was the most abundant species of charophyte in the seed bank, followed by *L macropogon,* the only true brackish water charophyte in New Zealand (Wood & Mason, 1977). *C. globularis* was a prominent feature in the 1992 records of the lake; however, it had vanished by 2007. Two species of charophytes, *C. australis* and *N. hookeri,* were not recorded in previous macrophyte records however were present in the seed bank, with *C. australis* significantly more abundant at the central and southern sites. Both species are noted as being found in shallow lagoon and coastal lagoons habitats (Wood & Mason, 1977).

The vertical distribution of seeds and oospores would be the next step in further investigating the Whakakī Lake seed bank. This aspect of the seed bank was not adequately investigated in this research and may affect the way in which the seed bank responds over time. Research has found a relationship between seed burial depth and germination response, showing a significant difference in germination of unburied seeds

(up to 98% success) compared to seeds buried at 2 to 5cm depth (Bonis & Lepart, 1993). Seeds that are buried within the sediment of Whakakī Lake may be brought back to the surface via resuspension of lake sediment through wind-driven resuspension and in turn become available for germination. The age of these seeds will likely impact their germination success (Bonis & Lepart, 1993), and restoration efforts should consider this potentially delicate reserve.

5.3 Germination Trials

Seeds and oospores germinated significantly better under full light conditions when compared with reduced light availability. However, there was a complete lack of germination under complete darkness. These findings contradict those of others (de Winton *et al.*, 2004; Takatori and Imahori, 1971; Carr and Ross, 1963; van den Berg *et al*., 1999) who found germination of other charophyte species could occur under darkness. Seeds and oospores likely have some germination tolerance to darkness to enable germination when seeds are buried beneath sediment (de Winton *et al.*, 2004). The limitation to these experiments was that growth post germination under reduced light conditions was not recorded. Future research into the growth after germination for species in this study would determine if the reduced light levels were sufficient to support growth beyond germination. Based on the PAR levels recorded in Whakakī Lake and the inconsistency of PAR to the lakebed, it is unlikely there would be sufficient light to support further growth and that germlings would die. These germination trials reveal Whakakī has a viable and diverse seed bank that can germinate under a range of salinities. The species that germinated during the salinity trials mimic those that germinated from the seed bank in 2007 (de Winton & Champion, 2008), indicating the

viable seed bank has not changed in 17 years. The presence of species that favour a range of salinity levels builds resilience to the Whakakī Lake seed bank and will encourage diversity if the macrophyte community can be re-established.

5.4 Recommendations and future research

The problem of restoring shallow lakes impacted by eutrophication is complex. A history of failed restoration attempts (Gross & Hagy, 2017; Sondergaard *et al.*, 2007) highlights the considerable challenges environmental managers face to achieve successful restoration, with 'trial and error' techniques being favoured historically. Fig. 5.1 highlights the global challenges of shallow lake restoration, all of which apply to Whakakī Lake.

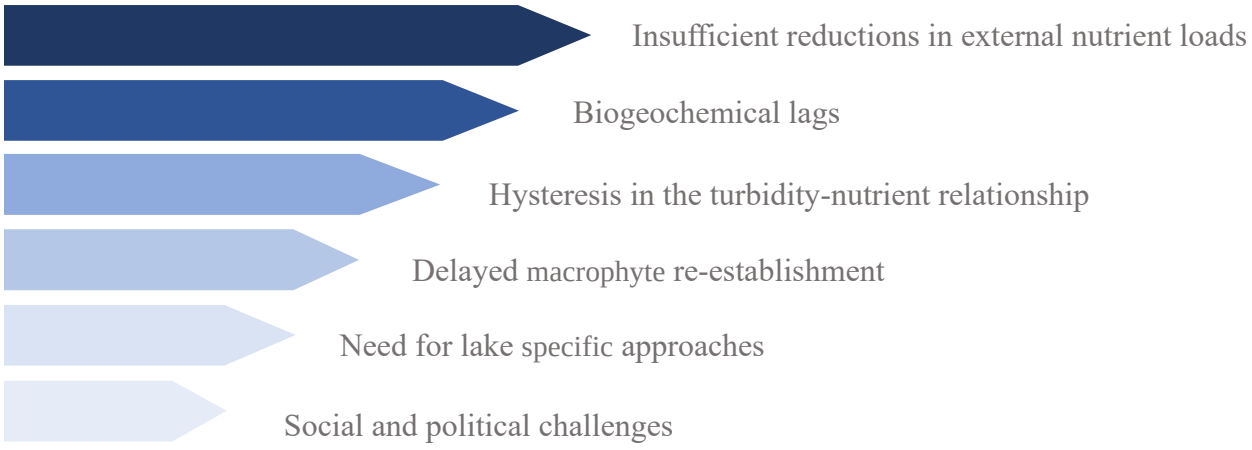

Figure 5. 1 Infographic overview of the challenges faced in restoration of shallow lakes adapted from Abell et al. (2020).

Various methods have been trialled globally to mitigate shallow lake eutrophication, including the reduction of external nutrient loads. Methods to address lake eutrophication include effective land management, internal load reduction via sediment capping, dredging or flocculation, biomanipulation (e.g., floating wetland, macrophyte harvesting, algicides), hydrologic alterations and water level management (Fig. 5.2) (Abel et al., 2020, Bakker et al., 2012).The best course of action for Whakakī Lake will be a combination of these methods. The restoration of macrophytes will need to be preceded by a reduction of external and internal nutrient loads and efforts to reduced sediment re-suspension to achieve improvements to water clarity and reach a critical turbidity threshold for macrophytes to re-establish (Abell *et al.,* 2020). Changes to land management practices, fencing, and planting waterways to create riparian buffers may help reduce external nutrient loads entering the lake. Further investigations into how to reduce the internal nutrient load specific to Whakakī Lake need to be investigated. Dredging or flushing the lakebed may negatively impact the seed stocks by removing viable propagules and hinder the re-establishment of macrophytes from the seed bank. Without an internal source of propagules to initiate regeneration, a substantial amount of work and money would be needed to reintroduce macrophytes.

Floating wetlands may be a good option for Whakakī Lake in areas most sheltered from the wind. Floating wetlands remove nutrients from lake water via the uptake of nutrients into plant tissues, with some nitrogen removal through denitrification in root mats (Abell *et al.,* 2020). By thoughtful placement of floating wetlands, some reduction in sediment resuspension could be achieved as the wetland creates some buffer from the

wind. Floating wetlands can provide temporary bird habitats and provide a visual and engaging representation of lake restoration.

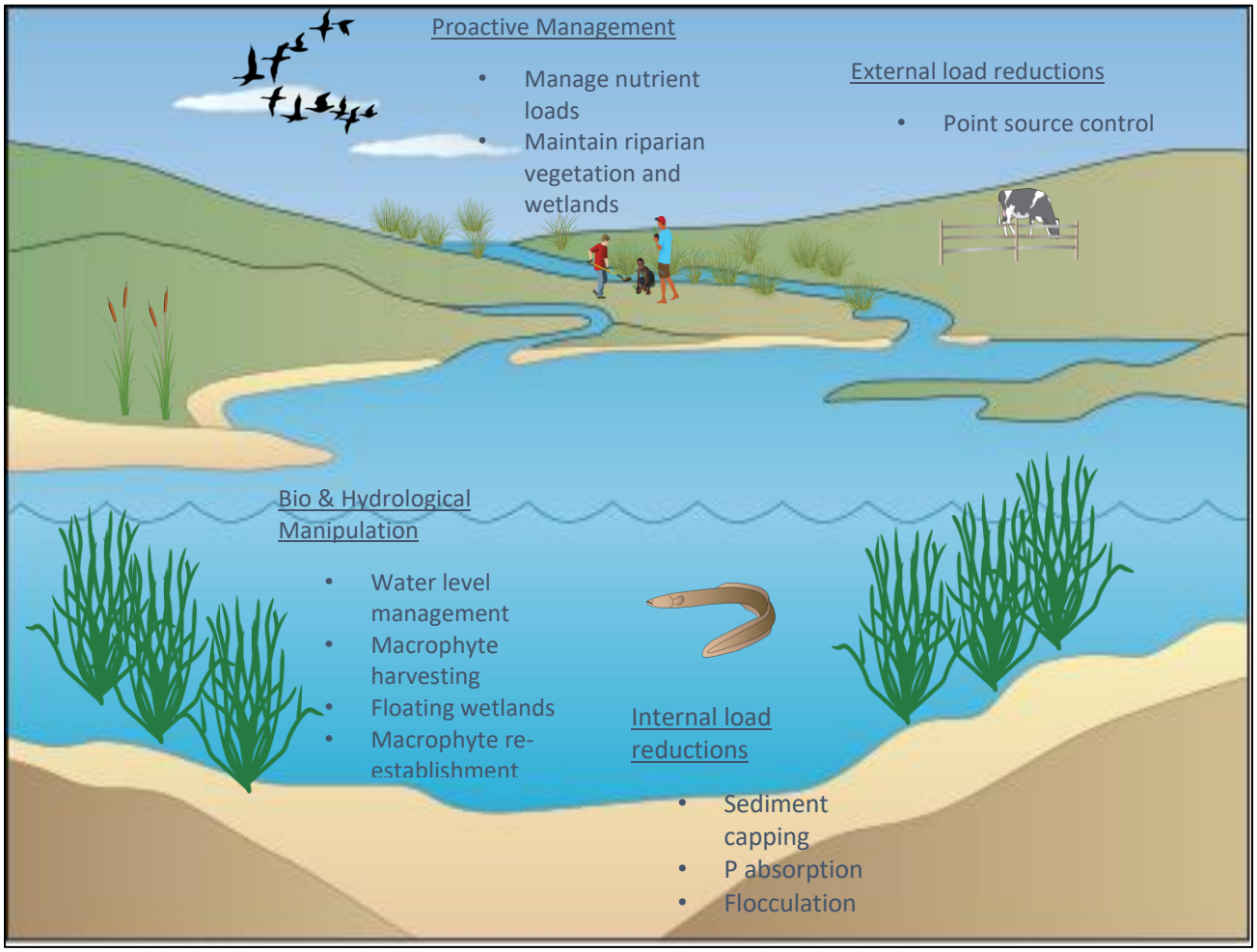

Figure 5. 2 Conceptual overview of the possible approaches to control eutrophication in shallow lakes, adapted from Abell et al. (2020). Image attribution: Saxby (2005)

If water clarity in the lake improves, steps could be taken to establish nursery crops of macrophytes that are buffered from wind, waves and benthivorous fish. Charophyte species are known to be effective at stabilising sediment and creating clear water conditions around them. (Casanova *et al.*, 2003). Nursery crops of charophytes

could be an exciting way to kick off macrophyte re-establishment in some areas of the lake. Nutrient sequestration in the sediment of Whakakī Lake is likely fuelling internal loading and would create a lag in the response of the lake to water quality and restoration actions (Abell *et al.*, 2020; Cooke *et al.*, 2005). With sufficient reduction in external nutrient loads, internal loads should decline and result in water quality improvements over decades. In the case of Whakakī Lake, which is in a severely degraded hypertrophic state, the lag in seeing meaningful improvements may be longer.

The road to macrophyte recovery in Whakakī Lake is long, and action to reduce internal and external nutrients available to the lake is the first step in this complex issue. The results from this study give hope that if the correct action is taken to manage and implement lake restoration properly, with improvements to water quality, the macrophyte and charophyte community of Whakakī could re-establish from the abundant internal seed and oospore bank.

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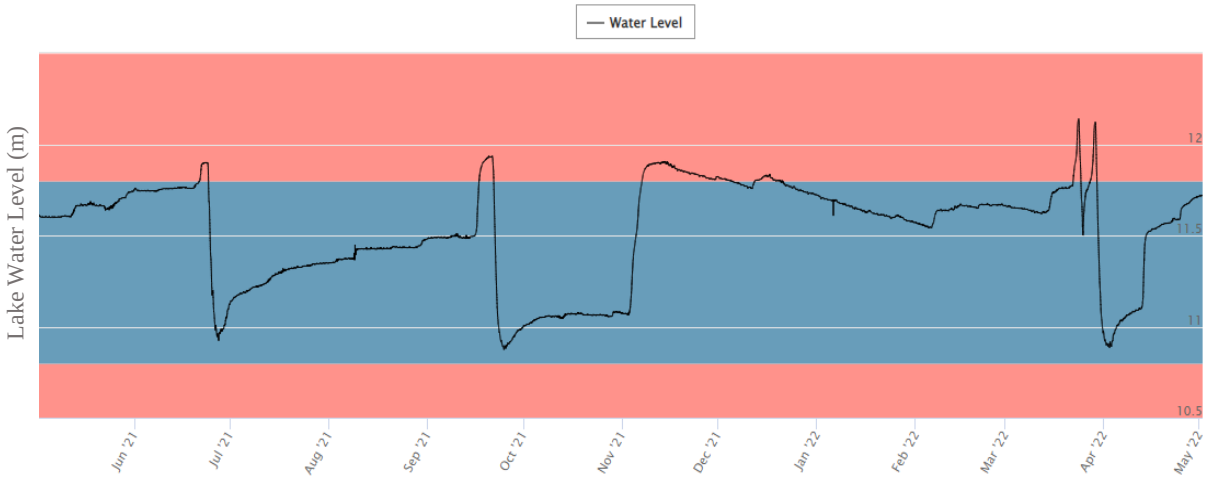
Appendix

Whakakī Lake Water Level Data

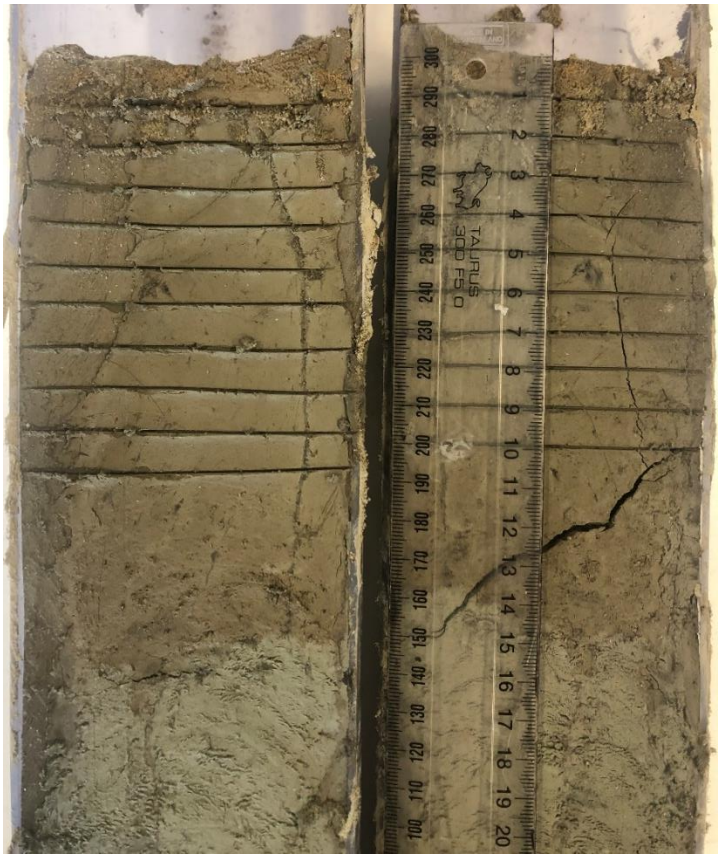


Water level data collected by Hawke's Bay Regional Council at the continuous monitoring site in the middle of Whakakī lake over a one-year period. ■ *= lake water level threshold for flooding breached* ■ *= lake water level is within the threshold*

Temporal distribution analysis

A temporal distribution analysis was completed to access the distribution of seeds and oospores within the sediment gradient. One additional core was taken from Whakakī Lake in September 2021 to access the depth distribution of seeds within the first 100 mm of sediment. Site S2 was selected and cored using the coring method outlined in 3.4.1 and transported to the lab. Site 2 was selected as it is close to sites 1 and 3 and should be representative of most samples collected. The core was then cut and split horizontally, and the top 100 mm separated into 10x 10 mm subsamples. Subsamples were each sieved individually using a 250 μm stainless steel woven-wire cloth sieve. Each subsample was

analysed under the microscope with species identification and enumeration as outlined in 3.4.3. Fig. 3.6 shows the core taken from site S2 for this analysis and how the core, once split horizontally, was sub-sampled. Each side of the split core has been sectioned; the corresponding sides were mixed for storage and analysis upon sectioning the subsamples. A linear model was run on the total seed count between depths and found no significant relationship between depth and seed count (R^2 = 0.031, F = 0.26, P = 0.624) and seeds neither increase nor decrease with depth.



Core from site S2 split horizontally and sectioned off into 10mm subsamples

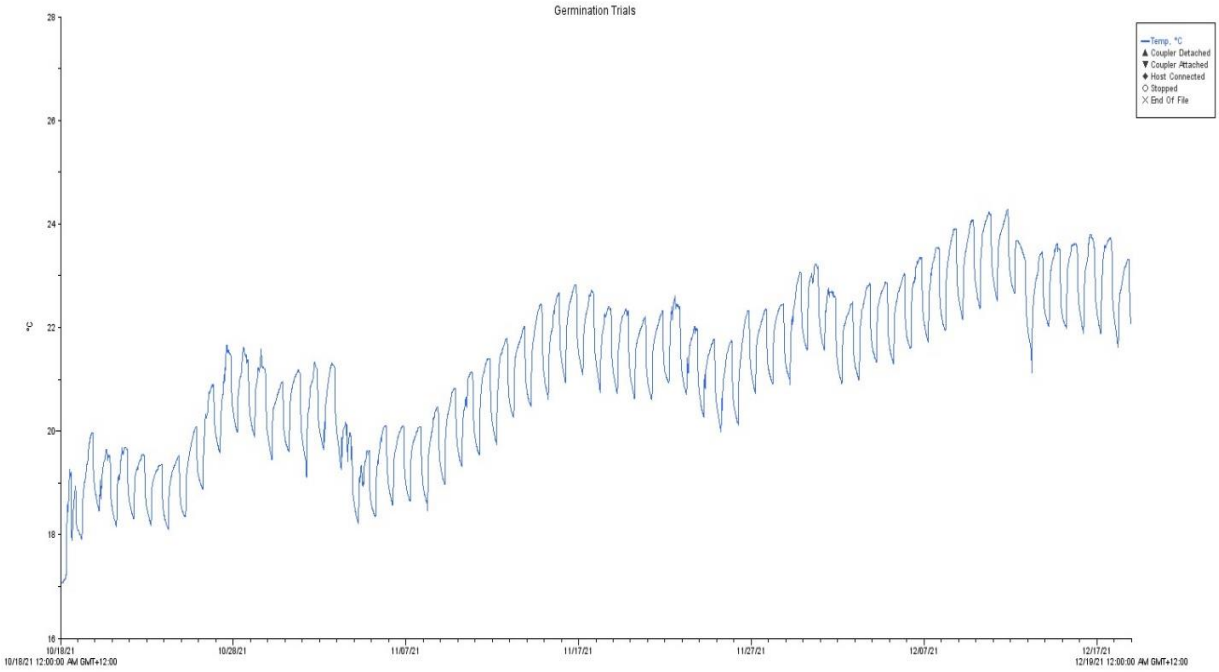
R output: depth analysis

```
Residuals:
    Min      1Q  Median      3Q     Max
-4.0412 -2.5700  0.7043  2.2085  3.7315

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  7.77905    4.58046   1.698    0.128
Count       -0.01888    0.03703  -0.510    0.624

Residual standard error: 3.16 on 8 degrees of freedom
Multiple R-squared:  0.03147,    Adjusted R-squared:  -0.0896
F-statistic: 0.2599 on 1 and 8 DF,  p-value: 0.6239
```

Grow tent temperature



Plot of HOBO Tidbit temperature readings from within the grow tent over the duration of the germination trials.

Statistical results and outputs – Chapter III

PERMANOVA

PERMANOVA table of results

source	df	ss	MS	Pseudo-F	P(perm)	unique perms	P(MC)
si	3	3691.9	1230.6	11.999	**0.0001**	9937	**0.0001**
Res	20	2051.1	102.56				
Total	23	5743					

Details of the expected mean squares (EMS) for the model

source	EMS
si	1*V(Res) + 6*5(Si)
Res	1ev(Res)

Construction of Pseudo-F ratio(s) from mean squares

Source	Numerator	Denominator	Num.df	Den. df
si	1*si	1*Res	3	20

Estimates of components of variation

Source	Estimate	Sq.root
S(si)	188.01	13.712
V(Res)	102.56	10.127

PAIR-WISE TEST

Term 'Si"

Groups	t	P(perm)	Unique perms	P(MC)
1, 2	2.5339	**0.0024**	462	**0.0008**
1, 3	3.7634	**0.0019**	462	**0.0002**
1, 4	4.329	**0.0023**	462	**0.0002**
2, 3	3.6085	**0.0024**	462	**0.0002**
2, 4	4.048	**0.0017**	462	**0.0001**
3, 4	1.8492	**0.015**	462	**0.0305**

Denominators

Groups	Denominator	De.df
1, 2	1*Res	10
1, 3	1*Res	10
1, 4	1*Res	10
2, 3	1*Res	10
2, 4	1*Res	10
3, 4	1*Res	10

Average Similarity between/within groups

	1	2	3	4
1	91.269			
2	84.384	86.901		
3	78.691	76.328	86.935	
4	71.576	69.966	81.296	82.81

Statistical results and outputs – Chapter IV

Salinity between treatments

```
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)    9.833      2.042   4.816 0.000227 ***
salinitys2     3.667      2.887   1.270 0.223471
salinitys8    -2.000      2.887  -0.693 0.499103
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 5.001 on 15 degrees of freedom
Multiple R-squared:  0.209,      Adjusted R-squared:  0.1035
F-statistic: 1.981 on 2 and 15 DF,  p-value: 0.1724
> summary(saloverall.aov)
            Df Sum Sq Mean Sq F value Pr(>F)
salinity     2   99.1   49.56   1.981  0.172
Residuals   15  375.2   25.01
> TukeyHSD(saloverall.aov)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = seeds ~ salinity, data = salinity_df)

$salinity
           diff        lwr       upr     p adj
s2-s0  3.666667  -3.833250 11.166583 0.4327794
s8-s0 -2.000000  -9.499917  5.499917 0.7712977
s8-s2 -5.666667 -13.166583  1.833250 0.1559158
```

Salinity/Species:

```
> summary(sal0.aov)
                 Df Sum Sq Mean Sq F value  Pr(>F)
Species           3  420.8  140.27  29.898   6e-12 ***
salinity          2   24.3   12.17   2.593 0.08314 .
Species:salinity  6  103.2   17.20   3.667 0.00362 **
Residuals        60  281.5    4.69
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’
```

Light overall

```
Residuals:
    Min      1Q  Median      3Q     Max
-4.1667 -0.3333 -0.0833  0.4167  2.8333

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   8.1667     0.6646  12.288 8.92e-11 ***
lightfive    -7.8333     0.9399  -8.335 6.15e-08 ***
lightten     -6.0000     0.9399  -6.384 3.15e-06 ***
lightzero    -8.1667     0.9399  -8.689 3.18e-08 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1.628 on 20 degrees of freedom
Multiple R-squared:  0.8298,     Adjusted R-squared:  0.8042
F-statistic: 32.49 on 3 and 20 DF,  p-value: 6.953e-08

> summary(lightoverall.aov)
            Df Sum Sq Mean Sq F value   Pr(>F)
light        3  258.3   86.11   32.49 6.95e-08 ***
Residuals   20   53.0    2.65
---
```

```
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> TukeyHSD(lightoverall.aov)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = seeds ~ light, data = light_df)

$light
                diff         lwr        upr     p adj
five-all  -7.8333333 -10.4639363 -5.2027304 0.0000003
ten-all   -6.0000000  -8.6306029 -3.3693971 0.0000175
zero-all  -8.1666667 -10.7972696 -5.5360637 0.0000002
ten-five   1.8333333  -0.7972696  4.4639363 0.2395589
zero-five -0.3333333  -2.9639363  2.2972696 0.9842543
zero-ten  -2.1666667  -4.7972696  0.4639363 0.1303137
```

Two-way ANOVA Light

```
> summary(light2)
             Df Sum Sq Mean Sq F value   Pr(>F)
light         3  64.55  21.517   39.61 7.37e-16 ***
Species       3  21.99   7.331   13.50 3.18e-07 ***
light:Species 8  50.79   6.349   11.69 6.64e-11 ***
Residuals    81  44.00   0.543
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’
> TukeyHSD(light.aov)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = count ~ light * Species, data = light2_df)

$light
                         diff         lwr        upr     p adj
light_10-light_0    0.54166667 -0.06116224  1.1444956 0.0938603
```

light_100-light_0	2.04166667	1.43883776	2.6444956	0.0000000
light_5-light_0	0.06666667	-0.50974802	0.6430814	0.9902317
light_100-light_10	1.50000000	0.94188888	2.0581111	0.0000000
light_5-light_10	-0.47500000	-1.00447070	0.0544707	0.0946468
light_5-light_100	-1.97500000	-2.50447070	-1.4455293	0.0000000

Supplementary material

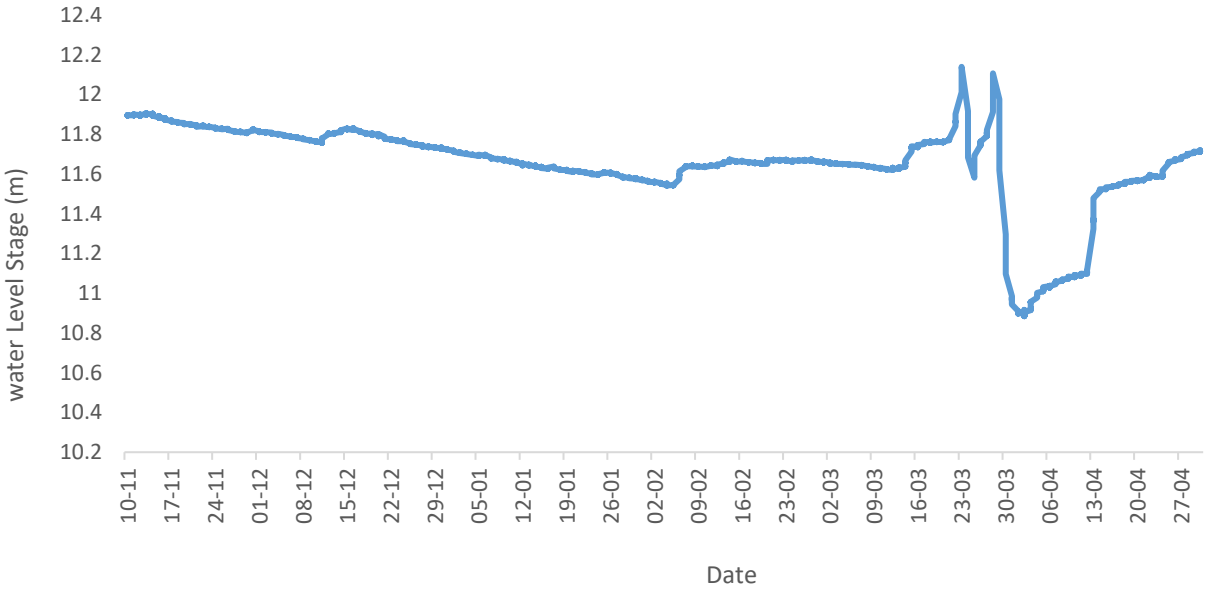


Figure 4. 16 Water level trace taken over the course of PAR logger deployment. Water level measured via a OTT CBS compact bubbler sensor at the centre of the lake