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Investigating drivers of cyanobacterial blooms in Aotearoa – New Zealand lakes using sedimentary ancient DNA

A thesis

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Abstract

Healthy lake ecosystems support biodiversity and human populations. They provide many ecosystem services such as water, food and energy. Lakes can be impacted by natural disturbances, but they are increasingly threatened by human-induced disturbances. Studies have shown that eutrophication and climate change often enhance cyanobacteria over other photosynthetic taxa. As cyanobacterial blooms are becoming more frequent and intense throughout the world, more lake systems are being investigated. In some cases there is not a clear link between eutrophication and cyanobacterial blooms. One such example is Lake Pounui (Wairarapa, New Zealand), which has little intensive agriculture in its catchment but water quality has degraded markedly in the last decade. The lake now experiences heavy cyanobacterial blooms every summer. This could be due to the presence of a non-native fish population, the European perch (*Perca fluviatilis*). This thesis examined the relationship between cyanobacterial blooms and perch introduction in New Zealand lakes, including a multi-trophic study in Lake Pounui. Perch were introduced c. 1870 in New Zealand but introduction records are patchy and sometimes non-existent. Moreover, most lake systems are not studied until they are already degraded. This thesis used a combination of traditional proxies (pollen, charcoal, pigments) and modern proxies (sedimentary ancient DNA, XRF scanning) from lake sediment cores to reconstruct lake ecology in pre-human times, after Māori settlement between the 13th to 15th century, and after European settlement from 1840 AD. Timelines and intensity of human impact were reconstructed with pollen, charcoal analysis, and sediment dating when possible.

Cyanobacterial communities in six lakes were reconstructed through their sed-

imentary ancient DNA (sedaDNA) using metabarcoding and droplet digital PCR (ddPCR) in Chapter 2. Bloom-forming species were present in all lakes prior to human arrival; however their overall abundance was low. Total cyanobacteria abundance and richness increased in all lakes after European settlement but was very pronounced in four lakes, where bloom-forming taxa became dominant. The trends in cyanobacterial abundance from ddPCR were then compared to cyanobacterial pigments (canthaxanthin, echinenone, myxoxanthophyll and zeaxanthin) using high-performance liquid chromatography in Chapter 3, to assess the likelihood of the historical increase observed. Pigments / sedaDNA relationships were more consistent when all pigments were summed, which is likely due to differences in species composition across lakes. The positive correlations confirmed an increase in cyanobacterial biomass since European arrival.

Due to patchy records for fish introduction, fish sedimentary DNA was compared to environmental DNA (eDNA) from water samples as a methodological check (Chapter 4) before applying this method to the sediment cores. This study was undertaken in three small and shallow lowland lakes by targeting perch and rudd (*Scardinius erythrophthalmus*). Fish DNA was evenly distributed across the whole lake except when the fish population was low. Samples collected from the sediment contained fish DNA more often than water samples in two out of the three small shallow lakes (including Lake Pounui). Sediment geochemistry probably impeded detection in the third lake. Perch sedaDNA was therefore used as an indication of fish presence in Lake Pounui for Chapter 5, which explored multitrophic changes in Lake Pounui over the last c. 1,000 years. In addition to pollen, charcoal, and 14C dating, XRF scanning was used to reconstruct mineralogic shifts from the catchment (Ti/inc, K/inc) and within the lake (inc/coh). Biological trends were reconstructed by targeting the sedaDNA of bacteria (16S rRNA), microeukaryotes (18S rRNA), metazoans (CO1), and macrophytes (*rbc*L, *trn*L). Complemented by historical records and studies, the data produced in this thesis indicated that the biggest changes in Lake Pounui happened after European settlement (c. 1845), with land clearance, perch introduction, climate change, and probable fertiliser application

driving the degradation of the water quality in c. 180 years. This study revealed shifts in native communities (macrophytes, bacteria, oligochaete worms) and the appearance of new species (perch, macrophytes, freshwater nematodes) previously undocumented using sedaDNA. The results highlight just how complex yet fragile lake ecosystems can be and how little we still know about them. Sedimentary ancient DNA is a useful tool to study the insidious and long-lasting impact of non-native species on freshwater ecosystems because it widens the range of species that can be studied. However, it needs to be complemented with other proxies. This thesis provides a framework to study fish DNA in small shallow lakes (Chapter 4). It can also inform future management and restoration strategies in lakes, especially in Lake Pounui, by retracing historical water quality (Chapter 2) and identifying taxa present prior to, during, and after lake degradation (Chapter 5).

Résumé

Lorsqu'ils sont en bonne santé, les écosystèmes lacustres peuvent supporter une biodiversité riche, ainsi que les populations humaines. Les lacs fournissent de multiples services écosystémiques, tels que l'accès à l'eau, à la nourriture, et en tant que sources d'énergie. Ils peuvent être affectés par les catastrophes naturelles, mais ils sont surtout de plus en plus menacés par les forçages humains. Les études montrent que l'eutrophisation and le changement climatique mènent souvent à la prolifération de cyanobactéries au détriment des autres espèces photosynthétiques. A mesure que de nouveaux lacs sont étudiés, ils révélent que ces proliférations deviennent plus fréquentes et plus intenses à travers le monde. Dans certains cas, le lien de cause à effet entre l'eutrophisation et la prolifération de cyanobactéries n'est pas clair. Lake Pounui (Wairarapa, Nouvelle-Zélande) est un bon exemple, car malgré le peu d'activité dans son bassin versant, la qualité de l'eau s'est dégradée dramatiquement au cours des dix dernières années et les cyanobactéries y prolifèrent chaque été. Une cause majeure pourrait être la présence d'un poisson non-natif à la Nouvelle-Zélande, la perche commune (*Perca fluviatilis*). Cette thèse examine donc la relation entre les proliférations de cyanobactéries et l'introduction de perches communes dans les lacs de Nouvelle-Zélande, notamment avec une étude multi-trophique de Lake Pounui. La perche commune fut introduite vers 1870 en Nouvelle-Zélande mais les archives documentant les introductions sont incomplètes, parfois même manquantes. De plus, la plupart des lacs ne sont étudiés que lorsque la qualité de leur eau est déjà dégradée. Cette thèse utilise donc une combinaison d'approches paléo-limnologiques traditionnelles (pollen, particules de charbon, pigments) et modernes (ADN sédimentaire ancien, scanneur de minerai) issues de

carottes sédimentaires pour reconstruire l'écologie de quelques lacs avant la présence de population humaines en Nouvelle Zélande, après l'arrivée des Māori entre 1250 et 1350, et après l'arrivée des Européens à partir de 1840. La chronologie et l'intensité de l'impact humain sont reconstruites en utilisant l'apparition ou disparition de certains pollens, l'augmentation de particules de charbon, et en datant le sédiment dans la mesure du possible.

Les communautés cyanobactériennes de six lacs sont reconstruites grâce à leur ADN sédimentaire ancien (ADNseda) en utilisant le métabarcoding et la *droplet digital PCR* (ddPCR) (Chapitre 2). Les espèces formant des proliférations étaient présentes avant l'arrivée des Māori, cependant l'abondance totale de la communauté cyanobactérienne était faible. L'abondance et la richesse des cyanobactéries augmentent dans tous les lacs après l'arrivée des Européens, mais cette tendance est surtout prononcée dans quatre lacs où les espèces proliférantes sont devenues dominantes. La quantité d'ADNseda de cyanobactéries obtenue par ddPCR fut ensuite comparée à la quantité de quatre pigments produits par les cyanobactéries (cantaxantine, échinenone, myxoxanthophylle et zéaxantine) obtenus par chromatographie en phase liquide à haute performance (Chapitre 3), pour déterminer la vraisemblance de l'augmentation en ADNseda observé. La relation pigments / ADNseda est plus cohérente lorsque tous les pigments sont additionnés, ce qui est probablement dû à des espèces différentes d'un lac à l'autre. Les corrélations positives confirment cependant une augmentation de la biomasse cyanobactérienne depuis l'arrivée des Européens.

Dû aux lacunes présentes dans les archives historiques, la fiabilité de l'ADN sédimentaire pour indiquer la présence de poissons exotiques est explorée dans le chapitre 4, pour pouvoir ensuite appliquer cette méthode aux carottes sédimentaires. La présence de perches communes et rotengles (*Scardinius erythrophthalmus*) est analysée en comparant leur ADN sédimentaire (sédiment de surface) à leur ADN environnemental présent dans l'eau. Cette étude se base sur trois lacs de basse altitude, de petite taille et faible profondeur. L'ADN des deux espèces était réparti uniformément à travers les lacs, sauf lorsque la population était de petite taille.

Dans deux des trois lacs (dont Lake Pounui), les échantillons provenant du sédiment contenaient plus souvent l'ADN de l'espèce cible comparé à l'eau. Il est probable que la composition géochimique du sédiment soit responsable des faibles détections dans le troisième lac. Par conséquent, l'ADNseda fut utilisé comme indicateur de la présence de perches communes pour reconstruire la chronologie de Lake Pounui, et Chapitre 5 explore les changements multi-trophiques qui sont survenus dans ce lac au cours du dernier millénaire. Outre les analyses de pollen, charbon, et datation au carbone 14, un scanneur à minerai est utilisé pour reconstruire les changements minéralogiques provenant du bassin versant (Ti/inc, K/inc) and provenant du lac lui-même (inc/coh). Les communautés biologiques sont reconstruites en amplifiant l'ADNseda de bactéries (16S rRNA), micro-eucaryotes (18S rRNA)), métazoaires (CO1), et de plantes aquatiques (*rbcL, trnL*). En complément avec des études et archives historiques, les données produites dans cette thèse indiquent que les plus gros changements subit par Lake Pounui surviennent après l'arrivée des Européens (vers 1845). La déforestation du bassin versant, l'introduction des perches, le changement climatique, et l'application probable d'engrais sont les principaux facteurs qui mènent à la dégradation de la qualité de l'eau en 180 ans. Cette étude révèle, grâce à l'ADNseda, des modifications dans la composition des communautés natives (plantes aquatiques, bactéries, vers oligochètes) et l'apparition de nouvelles espèces (perche, plantes aquatiques, nématodes aquatiques) jusqu'alors inconnues. Ces travaux soulignent à quel point les écosystèmes lacustres sont à la fois complexes et fragiles. L'ADN sédimentaire ancien est une approche utile pour étudier l'impact insidieux et durable des espèces exotiques sur les écosystèmes d'eau douce, puisqu'il permet d'étudier un éventail plus large d'espèces. Cependant, il doit être complémenté avec d'autres approches. Les travaux de cette thèse développent un cadre pour concevoir des études d'ADN de poissons dans de petits lacs (Chapitre 4). Ils peuvent aussi informer les stratégies futures de gestion et restauration des lacs, surtout dans Lake Pounui, en retraçant un historique de la qualité de l'eau (Chapitre 2) and en identifiant les espèces présentes avant, pendant, et après la dégradation (Chapitre 5).

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Statement of Authorship

This thesis has been written as a series of manuscripts including three published and one to be submitted. Therefore, there are inevitable repetitions between the introductions and discussions of these chapters. This thesis is the intellectual and analytical work of the author and Prof. Ian Hawes, and Dr Susie Wood, Ass. Prof. Xavier Pochon, and Dr Marcus Vandergoes who provided advice and support throughout. The contributions of all co-authors and the publications for each chapter are outlined in each chapter and below:

Chapter 2 was published in the journal Scientific Reports in June 2022 and is presented in near identical form. The citation for the original publication is: Maïlys Picard, Xavier Pochon, Javier Atalah, John K. Pearman, Andrew Rees, Jamie D. Howarth, Christopher M. Moy, Marcus J. Vandergoes, Ian Hawes, Samiullah Khan, and Susanna A. Wood (July 27, 2022a). "Using metabarcoding and droplet digital PCR to investigate drivers of historical shifts in cyanobacteria from six contrasting lakes". In: *Scientific Reports* 12.1. Number: 1 Publisher: Nature Publishing Group, p. 12810. ISSN: 2045-2322. DOI: 10.1038/s41598-022-14216-8

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Chapter 1

General introduction

1.1 The importance of healthy lakes

Water is an essential element of life on planet Earth, and fresh water is critical for all organisms not adapted to deal with salted water. Unfortunately, only 4% of all water on Earth is fresh water and most of it is frozen at the poles (Shiklomanov, 1993). Amongst the remaining fresh water, lakes act as reservoirs all year round, are accessible from the land and the air, and as such they support a great diversity of organisms that have evolved in this environment. They also serve as drinking supply for terrestrial organisms, provide habitat and food for migrating species. Humans have gone as far as creating lakes or modifying existing ones to ensure they have a constant water supply for them, their animals, and their crops. Local communities often rely on native aquatic communities (fish, mussels) as a food supply. In very remote locations, lakes are used for navigation and transportation in the place of roads. Finally, lakes are a place of enjoyment, for example swimming, boating, diving, or simply as a beautification factor. Lakes have therefore an important place in ecosystems and human societies, but they are increasingly being polluted by human activities, and water suitable for sustaining life is becoming scarce. The notion of ecosystem services has been developed so that users could realise and quantify the use and the value of lakes, and the cost (ecological and economical) of degrading ecosystems. Interest has risen in what lives in and around lakes, what

leads to their degradation, and which measures need to be taken to protect or restore lakes (Schallenberg et al., 2013; Reynaud et al., 2017). While studies have been reporting the degradation of freshwater ecosystems since the 20th century (Hasler, 1947; Banks et al., 1953; Kiss et al., 1969; Byron et al., 1989), identifying drivers of degradation and proposing solutions remain challenging. This is due partly to the diversity of drivers that can lead to water quality degradation and our lack of knowledge on their impacts when they are combined.

1.2 Main drivers and consequences of degradation

The degradation of a lake ecosystem is associated with measurable, often visible changes compared to a reference condition, leading to negative outcomes. Degradation reduces biodiversity, which reduces the resistance (the ability to stay in the same state) and resilience (the ability to bounce back to a stable state) of the ecosystem following disturbance events (Côté et al., 2010; Downing et al., 2012; Ponce-Campos et al., 2013; Isbell et al., 2015).

1.2.1 Abiotic drivers

Natural catastrophes and anthropogenic actions can affect the physico-chemical characteristics of lakes, such as nutrient levels, lake depth, pH and water temperature, in turn affecting the ability of a lake to support biodiversity.

Many studies have reported that the main driver of water quality degradation in lakes is nutrient enrichment due to human activities (Hasler, 1947; Scheffer et al., 2001; Lathrop, 2007; Qin et al., 2013). This phenomenon is called lake eutrophication. Healthy lakes are generally considered to have clear waters with low nutrient concentrations, partly due to the terrestrial vegetation around the lake acting as nutrient buffers (preventing nutrients from getting in the lake) and due to macrophyte communities creating positive feedback loops (Hasler, 1947; Harper, 1992). Upon human settlement around lakes, the vegetation is cleared to make way for buildings, crops and pastures for animals. Erosion rates increase due to the disap-

pearance of the vegetation cover, and nutrient levels in the lake increase due to the disappearance of the riparian plants acting as buffer (Bennike et al., 2021). Lake nutrient levels can further escalate from nutrient run-offs coming from pastures and fields improved with fertiliser, as well as untreated sewage from urban and industrial landscapes. The main nutrients involved in these fluxes are nitrogen (N) and phosphorus (P). They play a major role in the growth of photosynthetic organisms, therefore a change in their concentration or relative ratios can lead to a change in aquatic plants (macrophytes) and microalgae (phytoplankton) communities (Scheffer et al., 2001).

Higher nutrient levels will affect the lake biota until a tipping point is passed, changing the ecosystem until a new stable state is reached (Scheffer et al., 2001). Shallow healthy lakes are typically characterised by clear waters and an extensive macrophyte cover (Figure 1.1). This state is stable due to macrophytes using dissolved nutrients for their own growth, releasing allelopathic compounds to prevent phytoplankton growth, and, depending on the macrophyte species, also preventing sediment resuspension or providing shade by floating at the surface (Mulderij et al., 2007; Mohamed, 2017; Seto et al., 2013). When a tipping point is passed due to increased nutrient levels, pelagic phytoplankton increase until they become the dominant photosynthetic community (Figure 1.1), resulting in the shading of macrophytes and their eventual collapse (Scheffer et al., 2001; Qin et al., 2013; Phillips et al., 2016). This is due to a succession of factors benefiting phytoplankton over macrophytes. (1) High N and P levels benefit phytoplankton and epiphytes, which multiply exponentially as long as the conditions (temperature, light, nutrients) are favourable for them (Zhang et al., 2015; Phillips et al., 2016). (2) When phytoplankton bloom in high amounts, these microscopic cells can block sunlight from reaching macrophytes and they deplete the upper layers (epilimnion) of nutrients (nitrogen, phosphorus, silica for diatoms, dissolved CO_2). Some phytoplankton species can also produce allelopathic compounds to compete with macrophyte communities (Mohamed, 2017). Over time, this leads to the disappearance of macrophyte communities, thus promoting phytoplankton blooms whenever the conditions are

favourable. (3) When blooms die, the cells sink to the bottom of the lake, where they are digested by bacteria which consume oxygen. If there are no macrophytes, the oxygen is not renewed, creating a bottom layer (hypolimnion) depleted in oxygen but rich in nutrients. This affects benthic communities, which have to survive in an anoxic or oxygen-poor environment until the water column mixes and dissolved oxygen becomes available again. Heavy phytoplankton blooms can lead to mass-mortality events, most evident when dead fish come floating at the water surface. These can be due to toxins secreted by phytoplankton or prolonged anoxia after the blooms die off (Mhlanga et al., 2006; Mancini et al., 2010; Lindholm et al., 1999; Ernst, 2008). Lake eutrophication also leads to shifts in grazers, with zooplankton unable to control by grazing the amount and types of phytoplankton that proliferate in eutrophic waters (Phillips et al., 2016). Studies have also found that lake eutrophication can lead to shifts in the fish community, benefiting cyprinids (carps and similar) over whitefish (e.g., trout and coregonid) (Minder, 1926; Hasler, 1947). This would further destabilise remaining macrophyte communities and increase water turbidity, since cyprinids feed by foraging in the sediment, thus uprooting macrophytes and resuspending particulate matter and nutrients (Akhurst et al., 2017). In cases of extreme nutrient enrichment, the dominating communities become bacteria specialised in high nutrient conditions, and protozoan feeding on these bacteria (Qin et al., 2013).

A few other human-driven factors can contribute to lake health degradation. Land-use change is generally accompanied with hydrological modifications to ensure a constant water supply, reduce floods and reclaim land (e.g., by draining wetlands). These changes affect the variability of lake levels and their propensity to flood, their water retention time, and the amount of suspended matter (e.g., Lake Mendota, U.S.A., Lathrop, 2007; Lake Wairarapa, NZ, Waters et al., 2018). Pollution from fecal contaminants arises from pasture run-offs or untreated sewage. This can lead to diseases (in humans and aquatic species) and thus require the closure of a drinking / entertainment area (So et al., 2016; Hatvani et al., 2018). Plastic pollution is also a growing field of interest, though studies have mostly been focused on the

marine environment and less on freshwater systems. On a more global scale, studies focusing on climate change are also starting to highlight the rate of warming waters and their impact on phytoplankton growth (Paerl et al., 2008; Paerl et al., 2009; Paerl et al., 2012). All of these stressors contribute to destabilising lake ecosystems (Jenny et al., 2020).

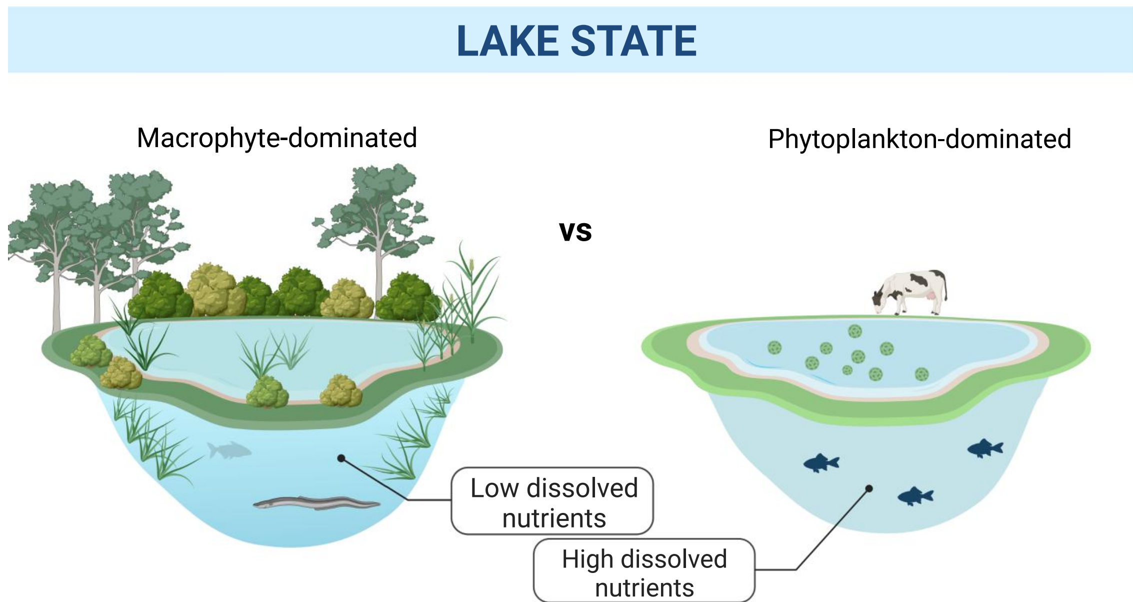

Figure 1.1: Comparison of healthy (left) and degraded (right) alternative states. The healthy state is characterised by clear waters and low dissolved nutrient concentrations in the water due to macrophytes and catchment vegetation filtering nutrients. The degraded state is dominated by phytoplankton species (represented by green rounds in the water) which multiply exponentially when the conditions are right. Lake eutrophication (nutrient enrichment) can have multiple causes, here represented by the clearance of native vegetation and nutrient enrichment from unrestrained agricultural practices.

Finally, some ecoregions are more impacted by natural disasters than others. Lakes can be affected by earthquakes, tsunamis, volcanic eruptions, which act as pulse disturbances, sometimes at regular intervals. For example, the Alpine fault (South Island, New Zealand) ruptures every c. 300 years, creating an earthquake of magnitude 8 or similar. Lakes near the fault would experience massive sediment influxes at regular intervals (Howarth et al., 2012), which impacts the organisms living in the lake (Brasell et al., 2021). Tsunamis create a sudden input of salt water as well as sediment in coastal lakes (Bondevik et al., 1997) and may cause long-

term salinity variations with consequences on zooplankton and benthic assemblages amongst others (Ruiz et al., 2010). Finally, volcanism leads to very different physico-chemical conditions in affected lakes. In Chile, a mega-eruption of the Puyehue volcanic complex led to increase in phosphorus, suspended solids and light scattering due to ash and pumice in the nearby oligotrophic lakes (Modenutti et al., 2013).

1.2.2 Biotic drivers

A change in species composition in a lake ecosystem can lead to water quality degradation. The introduction of new species due natural dispersal is possible, but studies report an exponential rate of introduction over the last decades, due to anthropogenic activities. Countries from around the globe are more connected to each other than ever before, and therefore more goods are being exchanged (c. 4100% growth since 1950; wto.org). Living organisms can also be transferred away from their native range - either through trade (e.g. trout for angling, goldfish for aquaria), because they live on the biological material being transferred (e.g. plants being a vector for small insects and eggs laid on them), or because they are illicit voyagers on the vessel used for trading (e.g. rats on ships, organisms attached to the hull or present in ballast waters).

These exchanges have knowingly or unknowingly contributed to the distribution of thousands of species around the world. Some managed to adapt to their new environment, becoming naturalised over time, and in some cases having negative impacts on the native environment (thus labelled 'invasives'). Their impacts depend on the environment and species introduced, but it usually involves impacting the local food-web (new predation pressure) or outcompeting native species (for preys, space, sunlight, etc). If no other species can keep the newly-introduced one in check (e.g. by predation), then exotic organisms can often reproduce exponentially and spread out of control. The impact of non-native species is now recognised world-wide, affecting native ecosystems and the services that humans derive from them. Management practices are focused on preventing the introduction of known noxious

species as much as possible, and if they are already present, limiting their spread.

Studies have started to apply molecular methods to the field of biosecurity for faster, cost-effective results. The importance of biosecurity in freshwater differs depending on the country / ecoregion, for example the region of the Great Lakes (North America) has been greatly affected by a number of non-native species (zebra mussel, quagga mussel, bighead and black carp, etc.) with heavy economical consequences (Horan et al., 2005). Another example is the introduction of an aquatic primrose (*Ludwigia peploides*) in France, originating from Oceania, which creates dense mats and outcompete any native species. It is considered the most invasive non-native aquatic plant in France, and is banned from trade in France, Belgium, the Netherlands, Portugal, and the UK. In New Zealand, the spread of non-native aquatic species ranging from the water flea *Daphnia pulex* (Ye et al., 2021) to many fish species, one of the most threatening being the brown-bullhead catfish (*Ameiurus nebulosus*), is closely monitored and stopped where possible. The importance of identifying quickly the presence of non-native species and stopping them before they spread and cause greater damage is therefore paramount. Accurate detection methods are needed, and in some cases identifying the introduction date of a given species would be helpful to link its impact to changes in the local ecosystem.

1.2.3 Conservation and restoration attempts

Healthy lakes can provide many ecosystem services which are threatened by the factors mentioned above. Many communities and governments are now investing significant efforts into the conservation or restoration of lakes to ensure their sustainable future, with various degrees of success. Because most lakes are impacted by multiple stressors (Jenny et al., 2020), identifying which variables are most important and should be prioritised for mitigation actions is challenging. This is sometimes more straight forward when there are long monitoring records, and when eutrophication has been documented. For example, successful lake management led to the de-eutrophication of Lake Konstanze (Germany) in the early 2000s by re-

ducing its total phosphorus levels to pre-eutrophication times (1950s) (Güde et al., 2000). However, lake management has failed to control the eutrophication of Lake Erie (Canada) to date (western basin from c. 1990), where nitrogen runoff from the surrounding catchment plays a critical role. The size of the catchment and the diffuse nature of the nutrient inputs make mitigation very challenging (Paerl et al., 2016). In New Zealand, several lake restoration projects have been attempted, success is mitigated by the lack of realistic targets and some small but important ecological differences compared to the well-studied lakes in the Northern Hemisphere.

1.3 Cyanobacteria blooms indicating lake health degradation

Studies have reported increases in the frequency, magnitude, and duration of cyanobacterial blooms in freshwater environments over the last few decades across the globe (Kosten et al., 2012; Taranu et al., 2015; Huisman et al., 2018). Scientists, governments, lake managers, and members of the public alike have become more aware of this recent issue, which is threatening the use of lakes and rivers for drinking, irrigation, recreational activities and the harvesting of food (Chorus et al., 1999).

1.3.1 Cyanobacteria overview

Cyanobacteria are a type of gram-negative bacteria which can live in many habitats: marine, freshwater, terrestrial, even animal guts for some species. Geological records show that cyanobacteria were one of the earliest life forms on Earth and the first known oxygenic photosynthetic organisms, creating communities as early as the Archean era (c. 2.8 giga years ago; Magnabosco et al., 2018). They contributed to the rapid oxidisation of our planet (the Great Oxygenation Event) some 2.33 billion years ago (Kranendonk et al., 2012) by evolving the capacity to undertake oxygenic photosynthesis, while led to an increase in oxygen atmospheric concentrations from approx. 0% to 20.95% around 500 millions years ago (Lyons et al., 2014). Fossils

of these unicellular organisms have been found in layered microbial communities, forming stromatolites which resemble mille-feuille (multi-layered) sedimentary rocks. Recent molecular analyses discovered a non-photosynthetic cyanobacteria class - Vampirovibrionia (syn. Melainabacteria), which fixes nitrogen through obligatory fermentation in anaerobic environments (Di Rienzi et al., 2013; Grettenberger et al., 2020). The ecology of this clade is not well understood and it appears that they are mainly found in animal guts (Utami et al., 2018; Kohl et al., 2018; Baldo et al., 2018), and some of them may be predators of other unicellular organisms (Soo et al., 2015). Therefore, the mention *cyanobacteria* throughout this thesis refers to the photosynthetic class, unless otherwise stated.

Cyanobacteria vary in shapes and sizes: from less than 3 µm (picocyanobacteria) to up to 60 µm (Figure 1.2). They can be single cells or form colonies, be pelagic (in the water column) or benthic (attached to a substrate). In lakes with high water clarity and low nutrients, cyanobacteria cells are generally benthic (e.g. *Nostoc, Microcoleus*) since sunlight reaches down to the lakebed, and they have to cope with low nutrient levels (Whitton, 2012). Studies have also shown picocyanobacteria dominating the cyanobacterial phytoplankton in oligotrophic lakes (Callieri, 2008), however, these are difficult to observe and identify under the microscope (Figure 1.2). When lakes become eutrophic, there is usually a shift to pelagic bloom-forming species (>3 µm). These cells multiply in the water column (Figure 1.2), until they deplete the waters of nutrients, die and descend to the lakebed, where they are degraded by bacteria. This cycle can be repeated multiple times a year, thus threatening native communities which need sunlight, nutrients, and/or oxygen.

Some cyanobacteria can also produce natural toxins, commonly known as cyanotoxins. Over fifty different species are known to produce cyanotoxins, including some of the common bloom-forming species such as; *Dolichospermum, Aphanizomenon, Microcystis*, *Oscillatoria* (*Planktothrix*), and *Lyngbya* (Carmichael, 2001). Based on their chemical structure, there are three types of cyanotoxins: alkaloids, cyclic peptides, and lipopolysaccharides (Ferrão-Filho et al., 2011). Amongst alka-

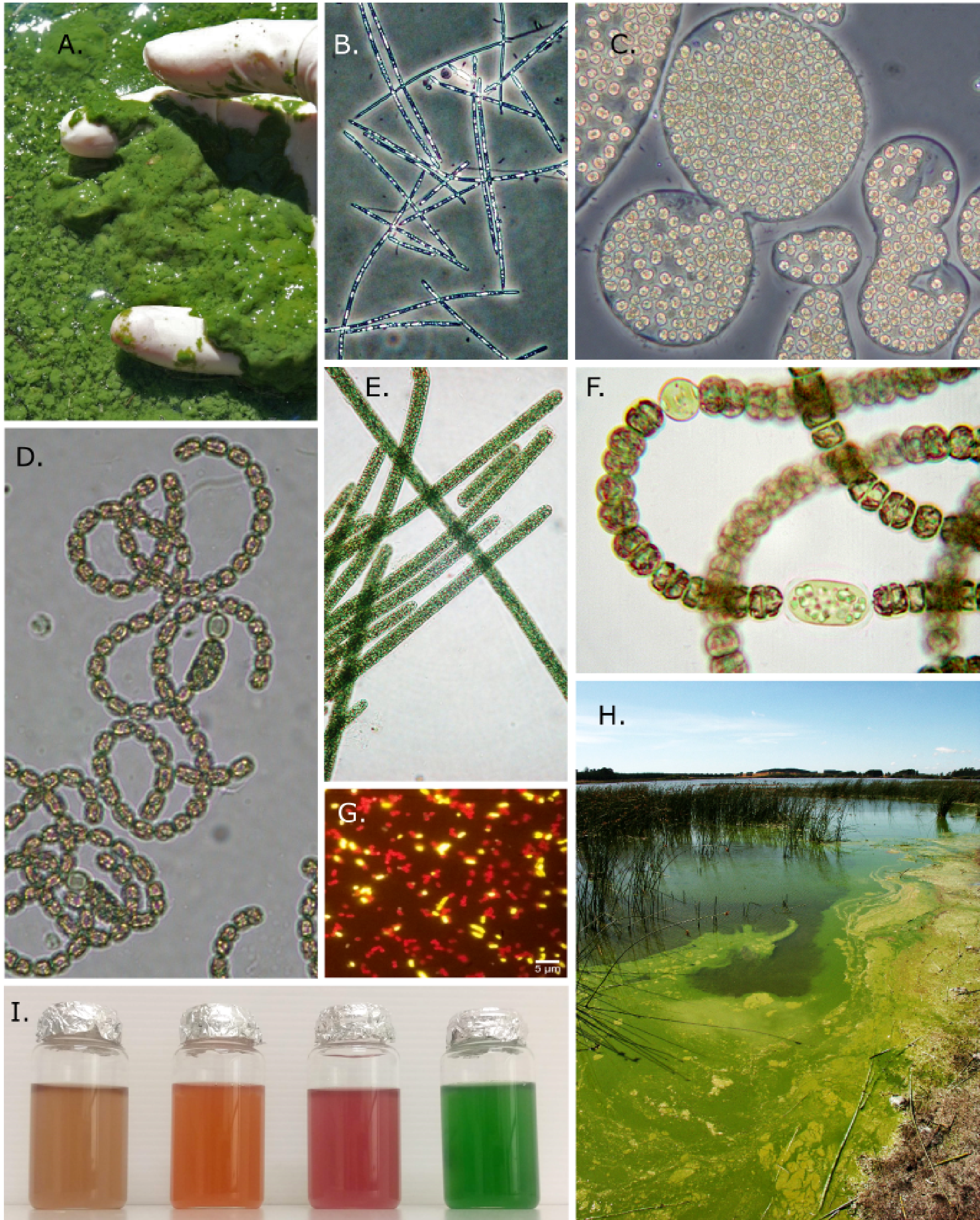

Figure 1.2: Cyanobacteria photos from the naked eye and through microscopy. A) Dense cyanobacteria scum, Lake Rotoehu (Rotorua, NZ), B) *Raphidiopsis raciborskii*, C) *Microcystis wesenbergii*, D) *Dolichospermum lemmermannii*, E) *Planktothrix* sp., F) *Dolichospermum* sp., G) phycoerythrin-rich (yellow) and phycocyanin-rich (red) picocyanobacteria, H) dense cyanobacterial bloom, Lake Horowhenua (Levin, Manawatū-Whanganui region, NZ), and I) picocyanobacteria cultures rich in phycoerythrin (brown, orange and red) and rich in phycocyanin (green). Photos from Dr. Lena Schallenberg and Dr. Susie Wood.

loids, anatoxin-a, anatoxin-a(S), and saxitoxin are neurotoxic, cylindrospermopsin is hepatotoxic, lyngbyatoxin-a and aplysiatoxin are dermatotoxic. The cyclic peptide group is comprised of two hepatotoxic cyanotoxins: microcystin and nodularin. Last, lipopolysaccharides are the least dangerous, only causing rashes (dermatotoxic) (Bláha et al., 2009). The range in toxicity is quite variable, from the acutely neurotoxic anatoxin-a to the potentially irritant lipopolysaccharides. Each cyanotoxin can be produced by multiple different species. The conditions promoting cyanotoxin production are still not completely understood. Within a species, both toxic and non-toxic strains can exist, therefore, identifying a potentially toxin-producing species does not guarantee that toxins are been produced.

1.3.2 Shifts in cyanobacterial communities

1.3.2.1 Abiotic factors

Climate change and human-induced eutrophication are increasingly being recognised as the main drivers of cyanobacterial blooms (Beardall et al., 2004; Ma et al., 2019; Paerl et al., 2009; Paerl et al., 2012). However, because there are limited monitoring records, their impact over longer periods of time is unknown. Toxic cyanobacterial blooms were identified in the 19th century in Australia (Francis, 1878), thus providing evidence that they are not a new occurrence. It is possible for lentic systems to be naturally eutrophic (Ashley, 1983; Burnison et al., 1974; Murphy et al., 1983; Cochrane, 2017) therefore cyanobacterial blooms could have always been present in a lake due to naturally high nutrient levels. Some lakes in Africa have long been eutrophic, and taxa from the genus *Arthrospira* have been a traditional food source for centuries (Vonshak et al., 2002).

As mentioned in section 1.2.1, high nutrient levels give cyanobacteria a competitive advantage over macrophytes, leading to their exponential multiplication until they deplete the water column of N and P. Furthermore, some cyanobacterial taxa contain specialised cells known as heterocysts which can transform atmospheric nitrogen into bio-available nitrogen (diazotrophic). These cells eliminate oxygen,

allowing the anaerobic nitrogenase enzyme to transform ammonia for subsequent metabolism processes. There are other diazotrophic mechanisms, but these are less common. This attribute can give a competitive advantage to diazotrophic species over other phytoplankton taxa, especially when nitrogen is limited (Whitton, 2012).

Temperature increases due to climate change may also benefit cyanobacteria (Paerl et al., 2008; O'Neil et al., 2012). Compared to other phytoplankton taxa, many bloom-forming cyanobacteria generally prefer waters above 25 °C (Reynolds, 2006; Jöhnk et al., 2008). An increase in air temperature leads to stronger and potentially longer water stratification, thus reducing mixing and benefiting buoyant cyanobacteria which can migrate vertically in the water column accessing nutrient rich waters. Droughts may also increase water residence time, and floods may increase nutrient inputs into lakes (Paerl et al., 2020; Meerhoff et al., 2022).

1.3.2.2 Biotic factors

Another possibility leading to cyanobacterial blooms is a top-down process such as the action of a predator (or absence thereof). Studies in the Northern Hemisphere have found several trends, including the fact that the dominating size class of zooplankton could influence the dominating cyanobacteria species due to their grazing preferences (Figure 1.3. For example, *Daphnia* species, the biggest zooplankton (Cladoceran class), can graze particles up to 50 µm (Gliwicz, 1969; Nadin-Hurley et al., 1976). This includes single-celled cyanobacteria, however, when they form filaments they may be able to resist to grazing pressures. This may lead to top-down shifts in phytoplankton communities, for example *Daphnia pulex* and *Daphnia pulicaria* have been observed as unable to graze the filamentous *Aphanizomenon flos-aquae*, therefore their grazing impact on other competitive phytoplankton species led to blooms of *Aphanizomenon* (Losos et al., 1973; Fott et al., 1974; Lynch et al., 1981).

These findings apply well to the Northern Hemisphere where lake communities are more varied, however in the Southern Hemisphere, and particularly Oceania, aquatic food-webs are more simple. Already at the end of the 20th century,

Boon et al., 1994 detailed the inadequacy of native Australian zooplankton to control cyanobacterial blooms, due to their small class size (mostly calanoid copepods and rotifers). Studies found similar trends in New Zealand lakes, observing the dominance of small cladocerans (*Ceriodaphnia dubia, Bosmina meridionalis*) and calanoid copepods (*Boeckella dilatata, Calamoecia lucasi*; Chapman et al., 1985; Burns et al., 1980; Greenwood et al., 1999). Native *Daphnia* species are present, but seemingly unable to graze efficiently on cyanobacteria (Burns, 1987).

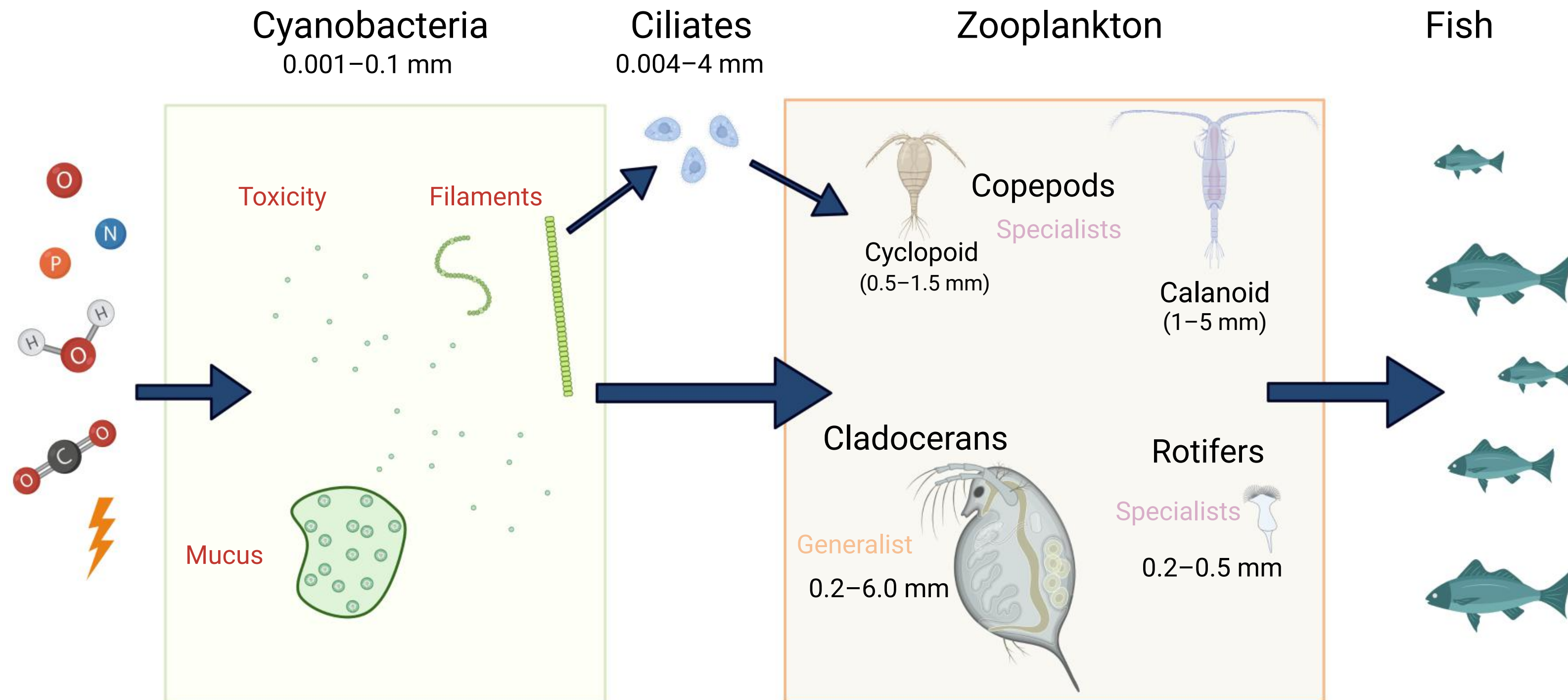

Figure 1.3: General overview of a lake food web centered on cyanobacteria. From left to right, cyanobacteria need basic molecules (phosphorus, nitrogen, oxygen, carbon dioxide, water) and light to grow, can exhibit several adaptations to resist to grazing from bigger eukaryotes (adaptations in red). Zooplankton are the biggest predators of cyanobacteria, though other micro-eukaryotes such as ciliates also prey on cyanobacteria. Cladocerans can prey on a range of cyanobacteria due to their size, but smaller zooplankton (copepods, rotifers) can only graze on specific species. Finally, different class sizes of fish will prey on different class sizes of zooplankton, ultimately affecting cyanobacteria grazing.

Changes in the native food-web could lead to a change in fish or zooplankton species, and therefore changes of grazing pressure on phytoplankton. Studies in New Zealand have shown that fish communities have a strong impact on the zooplankton community (Jeppesen et al., 2000; Jeppesen et al., 1997). Furthermore, non-native introduced fish were found to have a negative impact on the water clarity of New Zealand lakes (Rowe, 2007), but no specific fish species could be linked to this effect due to most lakes having more than one introduced species. Finally, several studies and records have found correlations between the introduction of the European perch (*Perca fluviatilis*) and cyanobacterial blooms in three New Zealand lakes, without any obvious role of land-use in the catchment (Smith et al., 2006; Smith et al., 2007; Cochrane, 2017; Hicks et al., 2013).

Enclosure experiments and *in-situ* observations in Roto Kawau (reservoir, Wellington) found that perch were mainly juveniles (6 - 11 cm) predating on cladocerans, which decreased the grazing pressure on phytoplankton, therefore leading to blooms of *Dolichospermum lermermanii*. The impact of nutrients was also investigated and did not seem to promote cyanobacterial blooms as much as juvenile perch predation on grazing zooplankton (Smith et al., 2006). Natural lakes in New Zealand can also undergo heavy cyanobacterial blooms, concomitant with perch presence but without heavy land-use impact in their catchment. Lake Rotokare (Taranaki, Western North Island) and Lake Pounui (Wairarapa, Southern North Island) have been less studied than the Lower Karori reservoir, but present similar characteristics: cyanobacterial blooms every summer, large perch population, catchments with regenerating forests with limited agricultural land-use. These lake systems, more complex and older than Roto Kawau, require more studies to disentangle the possible drivers behind these cyanobacterial blooms.

1.3.2.3 The need for historical records

While in a few instances there might be historical knowledge from oral histories or written reports, in most cases there are none, and there are very few long-term monitoring records. This limited information makes it challenging for managers

to design effective restoration or mitigation strategies. One opportunity is to use paleolimnology to explore historical changes in cyanobacterial communities, when they have not been documented by regular monitoring (section 1.6.2). It is possible to study general cyanobacterial abundances in sediment cores using their pigments (e.g., zeaxanthin, echinenone; e.g., Buchaca et al., 2011; Deshpande et al., 2014; Hobbs et al., 2021), but to date studies have not been able to link a specific pigment to single species. Furthermore, pigments degrade over time, which could give the impression that total cyanobacteria levels are increasing. Molecular studies may help solve this issue by identifying the DNA of specific species. This topic is discussed in the following sections.

1.4 Traditional methods to assess the state of lakes

Lakes can be monitored regularly to see how their ecological state (their health) evolves. The degree of nutrient enrichment in a lake is used to classify lakes into specific trophic states since the works of Naumann (1919). Trophic states range from low nutrient levels (microtrophic, oligotrophic), medium nutrient levels (mesotrophic), to high nutrient levels (eutrophic, supertrophic). Dissolved oxygen (bottom waters) and macrophyte covers are negatively correlated with nutrient levels, so that the lakes categorised as eutrophic / supertrophic are the most degraded, affected by phytoplankton blooms, and their waters generally cannot be used for drinking. Several indices have been developed to assign a lake to a trophic status, such as Carlson's trophic state index (TSI; Carlson, 1977), the "Naumann index" Chapra et al., 1981, and a New Zealand version taking into account nitrogen levels – the Trophic Level Index (TLI; Burns et al., 1999; Burns et al., 2000). The proxies used vary depending on the index, the TSI using three proxies: chlorophyll-a from pigment levels, total phosphorus, and Secchi depth. The Naumann index uses the same proxies as the TSI and adds primary production, and the TLI uses the same proxies as the TSI and adds total nitrogen (since lakes in the Southern Hemisphere are also limited by nitrogen). Low numbers calculated by these indices indicate low nutrient

levels and a good water quality (microtrophic, oligotrophic) while higher numbers indicate lower water quality (mesotrophic, eutrophic, supertrophic).

Other parameters are important in understanding lake water quality, including different forms of nitrogen, dissolved reactive phosphorus, trace metals, carbon (dissolved and particulate), dissolved oxygen (DO), pH, and temperature. Some of these biochemical measurements are now taken automatically and *in-situ* by monitoring buoys every 10-15 minutes and immediately transmitted on-land. They are composed of a weather station on top, and a multitude of water quality sensors always in the water. This is very useful to study short-lived processes such as algal growth, or unexpected / unknown processes such as water mixing, or more specifically the small hydrothermal eruptions in Lake Taupo (New Zealand).

However, if the lake is very large, a single-spot monitoring may not be enough to assess the state of the lake. Water quality can also be assessed by the general colour of the water, using satellite remote sensing. Decades of aerial imagery can be used since the launch of satellites around the Earth, and changes in water colour can be assessed on cloud-free days. Water colour is mainly affected by the amounts of suspended sediment, chlorophyll-a, and dissolved organic particles: no or few particles will lead to a deeper blue colour, high chlorophyll level will lead to green waters, and high sediment loads will colour the water with yellow / orange shades. This data is already used in Italy (Giardino et al., 2019), the United States of America (Topp et al., 2021), and New Zealand (Lehmann et al., 2018). In New Zealand, satellites can monitor the whole country in eight days (Allan et al., 2011).

Finally, monitoring the physical presence (or absence) of living organisms by observing them or capturing them is a widespread method for assessing the ecological condition of lakes. Diving surveys can help monitor benthic organisms such as macrophytes, sponges, mussels, but they require professionals with the right set of skills. Likewise, taxonomists are needed to identify phytoplankton, zooplankton, fish, and any other species of interest. Another proxy which emerged about a decade ago is the study of DNA molecules found in the environment (Taberlet et al., 2012). Molecular methods have been developed worldwide to target up to a whole clade or

down to a specific species, however the application for routine monitoring varies a lot depending on the country and the target.

1.5 Molecular techniques applied to lake monitoring

1.5.1 The rise of environmental DNA

Environmental DNA (eDNA) refers to the genetic material that can be found in the environment: intracellular (from living or dead organisms, discarded cells), or extracellular - when cells have released their DNA (Cristescu et al., 2018; Taberlet et al., 2012; Taberlet et al., 2018). As such, eDNA can come from tissues or lone cells, blood, saliva, and excretions, and it can be found in bulk environmental samples ranging from sediment, faeces, gut content, water and air (Barnes et al., 2016). In each medium sampled, DNA sequences can be analysed and, if identified, will give information on which species were present in the sample. For these methods to work, DNA needs first to be analysed from a known organism, so that its name can be linked to its DNA sequences. Studies have been working on this sequencing effort for decades, and now databases are available to link environmental DNA sequences to a myriad of known organisms. This means that it is now possible to identify macro-organisms without capturing them as well as micro-organisms without morphological identification (examples in the following section). If the species are hard to identify with other proxies (e.g., bacteria, picocyanobacteria) or if it is preferable to not capture them (e.g., elusive, to reduce unnecessary stress), these methods are very powerful. Furthermore, since there is no need for taxonomical expertise, they are quicker and cheaper than traditional monitoring methods. However, molecular methods and eDNA are no silver bullet, there are a number of pitfalls and precautions to take into account when using them.

1.5.2 Field applications of eDNA and the importance of sample types

Molecular techniques applied to eDNA have been used in numerous studies looking to detect anything from a specific species to a whole community. In aquatic environment, water samples are the most common, to investigate fish, molluscs, amphibians, and phytoplankton (Ardura et al., 2015; Turner et al., 2015). Many invasive species are tracked this way in Northern America, where carp, catfish, zebra mussels, and many others threaten local food webs but can be hard to detect using traditional methods (Sepulveda et al., 2019b; Darling et al., 2011). The lifespan of eDNA in water, especially free-floating extracellular DNA, depends on environmental factors such as temperature, pH, and UV irradiation, keeping eDNA viable for a few hours to a few days (Tsuji et al., 2017; Troth et al., 2021; Strickler et al., 2015; Pilliod et al., 2014; Eichmiller et al., 2016; Barnes et al., 2014). Levels of eDNA can also be related to the biomass of a specific species following semi-linear relationships (Takahara et al., 2012), however it does not provide information about the target's behaviour or life cycle stage. Comparisons from one study to another are also difficult if the environmental parameters (DNA degradation rates, hydrology) are different.

Sedimentary DNA (sedDNA) is a more recent field which has been applied in cave systems, terrestrial soils, and aquatic sediment over the last decade (Turner et al., 2015; Sakata et al., 2020). It has the advantage that DNA is preserved for longer since extracellular DNA binds to sediment particles, thus preventing its degradation to some degree (Turner et al., 2015). Furthermore, in lakes, hard cells such as cysts and eggs generally sink to the lakebed, where they will be detected with sedDNA. Studies have found varying levels of sedDNA compared to water samples, sometimes suggesting containing higher levels than sediment and vice versa higher detection rates (Valdez-Moreno et al., 2019; Buxton et al., 2018) and sometimes lower (Turner et al., 2015). It would be interesting to compare detection rates between water and sediment samples in different lakes, to see whether the discrepancies are due to a

difference in methodology or hydrology. Furthermore, comparing different species would highlight whether there could be discrepancies in detection rates due to their ecology. Surface sediment can record eDNA deposited over the last days, months (fish eDNA remained for up to three additional months in the sediment compared with water in Buxton et al. (2018), even years, until it gets buried under new layers of sediment. These layers can be sampled and recovered as sediment cores, which can be studied to retrace the history of the local ecosystem. The DNA found in these layers is called sedimentary ancient DNA (sedaDNA), and and will be discussed in section 1.6.2.

1.5.3 Which genes to target

DNA can only come from three locations within a cell: nuclear/plasmid DNA for all organisms, and chloroplastic DNA and / or mitochondrial DNA for eukaryotes (Figure 1.4). Molecular methods can target different regions or genes depending on the aim. Studies exploring a wide range of organisms target universal regions such as ribosomal DNA (rDNA), since these code for important molecules well conserved across the tree of life. For example, plasmid 16S rDNA is a universal region to target prokaryotes, and depending on the exact sequence it is possible to target all bacteria, archeae, or a more specific phyla of prokaryotes (such as cyanobacteria). Similarly, the nuclear 18S rRNA gene is a region of choice to target eukaryotes in general. These sequences are so conserved that they can be found across prokaryotes and eukaryotes: since organelles were originally prokaryotic cells which were assimilated by a eukaryote and became permanent subcellular compartments (endosymbiosis theories; McFadden, 2001), some genes can be found across both prokaryotes and eukaryotes (e.g. cyanobacterial 16S rRNA gene can be found in the chloroplasts of flowering plants and microalgae). However, universal regions are often too general to yield taxonomic resolution at species level. Therefore, targeting genes coding for species-specific or clade-specific sequences (barcodes) means targeting more specific species and cellular processes. The specificity can vary, allowing studies to target

a whole family (e.g., all fishes), or only a specific species and no other (e.g., *Perca fluviatilis*). Introns or non-transcribed sequences can be interesting regions for intra-specific variations, they are very variable because they are not coding for proteins, thus not restricted by evolutionary pressure. In eukaryotes, these regions are present both in nuclear DNA and within organelles. For example, the Internal Transcribed Spacer (ITS) located between the small and large subunits of ribosomal DNA is generally used as a barcode for fungi, since it is well conserved yet variable enough to target all fungi specifically and give species-specific identification. Some genes in organelles have also mutated a lot since their endosymbiosis and can be species-specific, such as the p6 loop of the trnL gene in chloroplasts (Taberlet et al., 2012), the CO1 and Cytochrome b (Cytb) gene for eukaryotes (mitochondrial DNA), 12S mitochondrial rRNA gene for animals, or rbcL, MatK, RuBisCo for plants (Figure 1.4).

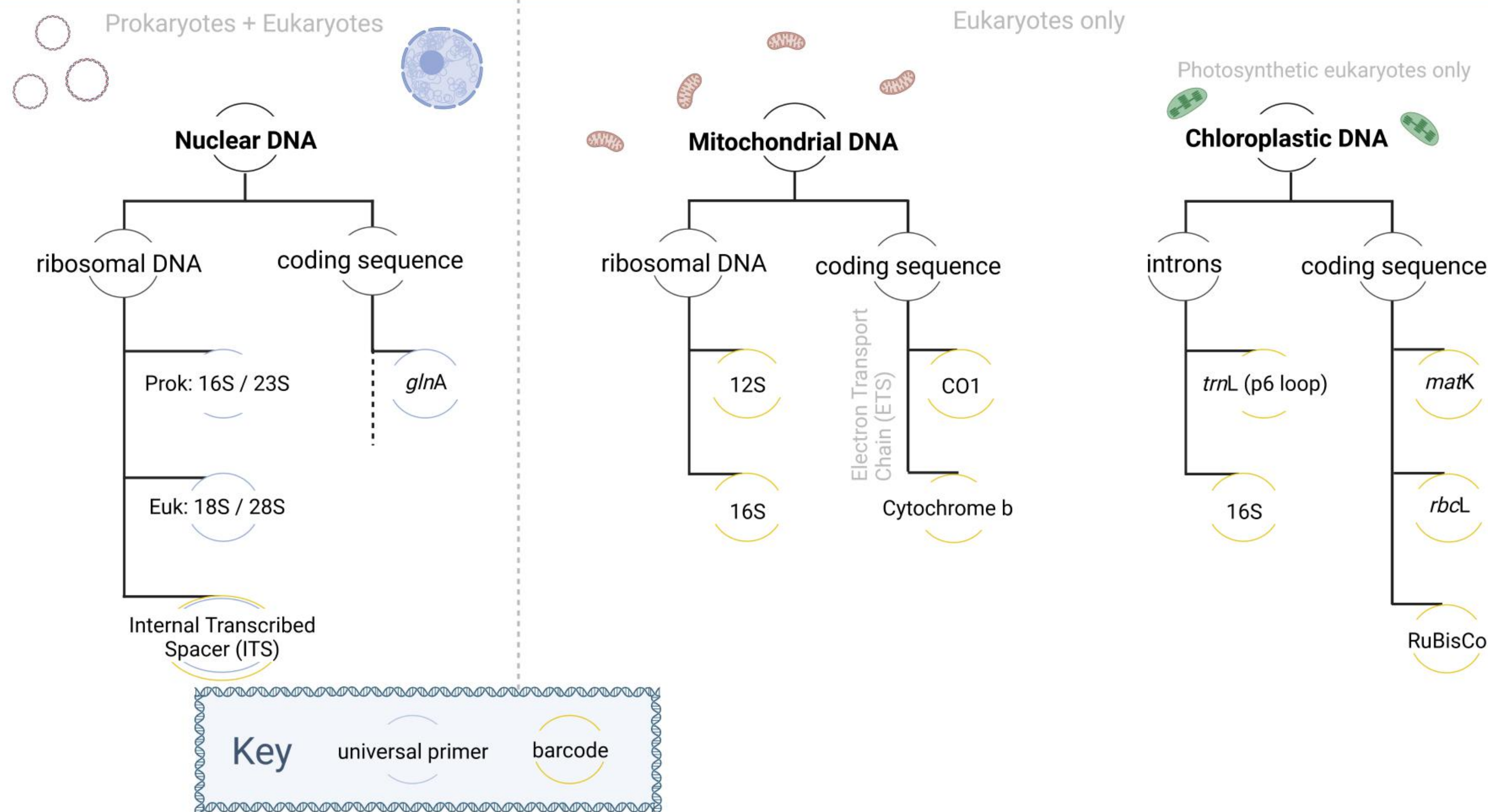

Figure 1.4: Non-exhaustive list of the main regions targeted by environmental DNA studies within an organism's genome. The ITS region in nuclear ribosomal DNA is used for fungi, dinoflagellates / zooxanthellae in corals, hence the double coding (universal primer yet can be species-specific). The dotted line in the coding DNA sequences from nuclear DNA illustrate the myriad of specific genes that can be targeted, whether they be conserved (enzymatic activity) or highly variable.

1.5.4 Sequencing techniques

One of the oldest sequencing techniques still in use today is Sanger sequencing. This is the method used when the DNA of an organism is still unknown, and it needs to be sequenced. A gene or region is targeted using Polymerase Chain Reaction (PCR) and degenerated primers, and amplicons (DNA sequences from PCR) up to 1,000-1,500 base pairs (bp) are generated. The DNA sequenced is matched with the organism it came from (barcoding), and this information is added in databases for future studies.

Progress in sequencing technologies in the last 10 years now enables the use of high-throughput barcoding on multiple organisms within the same sample. This is very useful to study community compositions and their relative abundances within a sample. DNA metabarcoding uses the principles of barcoding, PCR and High Throughput Sequencing (HTS) to identify numerous organisms in bulk / environmental samples. When the reference libraries contain enough references, short sequences (200 to 400 bp) from environmental samples can be amplified, sequenced, and assigned to the organisms previously barcoded. This powerful tool is a useful way to study environmental samples at various taxonomic levels, from whole kingdoms to specific families.

However, PCR can introduce bias in community compositions by preferential replication of some sequences (Fonseca et al., 2022). Shotgun sequencing is a more recent technique which does not use PCR. It sequences all short fragments present in a sample without using primers, and any fragments are then tentatively matched to a reference library, though many are left unassigned since they may come from a region that has not yet been sequenced. To target rare sequences and be able to assign them to an organism, it is now possible to use capture-target shotgun sequencing, which uses 'baits' to target specific fragments, but without PCR (Armbrecht et al., 2021). However, these sequencing options are still quite expensive, therefore metabarcoding is still used when dealing with a lot of samples, and if DNA sequences are not too degraded. Other sequencing methods exist (e.g. pyrosequencing, MinIon), but they

are not used as much in the field of environmental sciences.

1.5.5 Quantification techniques

Molecular techniques have also been developed to reliably determine how many copies of a DNA sequence are present in a sample. These techniques are especially useful in comparison with microscope techniques, as they can count how many cells are in a sample by targeting a gene which only has one copy per cell. When targeting cells with similar biovolumes, DNA quantification can be a proxy for biomass (Takahara et al., 2012).

Two main techniques can be used to count gene copies: real-time quantitative PCR (qPCR), and more recently digital droplet PCR (ddPCR). Both rely on fluorescent dyes being incorporated in or attached to the sequences during DNA replication (PCR) to count DNA sequences. Quantitative PCR needs standards of known concentrations to calibrate its quantification and measures the increase in fluorescence during the amplification process. It can be influenced by other molecules in the samples such as humic acids which act as PCR inhibitors, thus hindering the detection of target sequences (Sidstedt et al., 2020). Droplet digital PCR is more sensitive than quantitative PCR since it measures a true quantification, not based on standards. PCR reactions are separated in c. 20,000 droplets floating in oil before amplification, which theoretically separates the sample so that each droplet contains zero or one copy of the target sequence. Amplification is carried out and the droplets are then read in a third machine, the final level of the fluorescence indicating if and how many gene copies are present in each droplet. The many droplets serve as technical replicates, which are then normalised with a Poisson distribution to obtain a concentration in gene target copies per µL of PCR mix. Due to sample separation by the droplets, ddPCR can accurately quantify DNA even with moderate levels of inhibition (Dingle et al., 2013; Rački et al., 2014).

1.6 Historical insights from paleolimnology

When studying past events, researchers can either turn towards people and their records (oral, written, drawn) or to physical remnants. The latter are particularly useful when human records are incomplete or non-existent, and they are the focus of many fields such as archaeology for human societies, palaeontology for fossils from past biota, and many others. Another field less known by the public is paleolimnology, also a long-established field of research, which studies old sediments layers formed on lakebeds. This field relies on biostratigraphy, the fact that sediment layers are being deposited on a regular basis at the bottom of lakes, the latest layer stacking on top of the previous one, burying it and isolating it in time from the aquatic compartment. A wealth of information about the past states of a lake can be found in these successive layers (Figure 1.5). The material (organic or inorganic) can have originated from the lake itself from organisms and processes present in the lake, or organisms and inorganic particles coming in the lake from its catchment. If left relatively undisturbed, these sediment layers can provide an accurate stratigraphic archive of past biota and other environmental conditions (Figure 1.5).

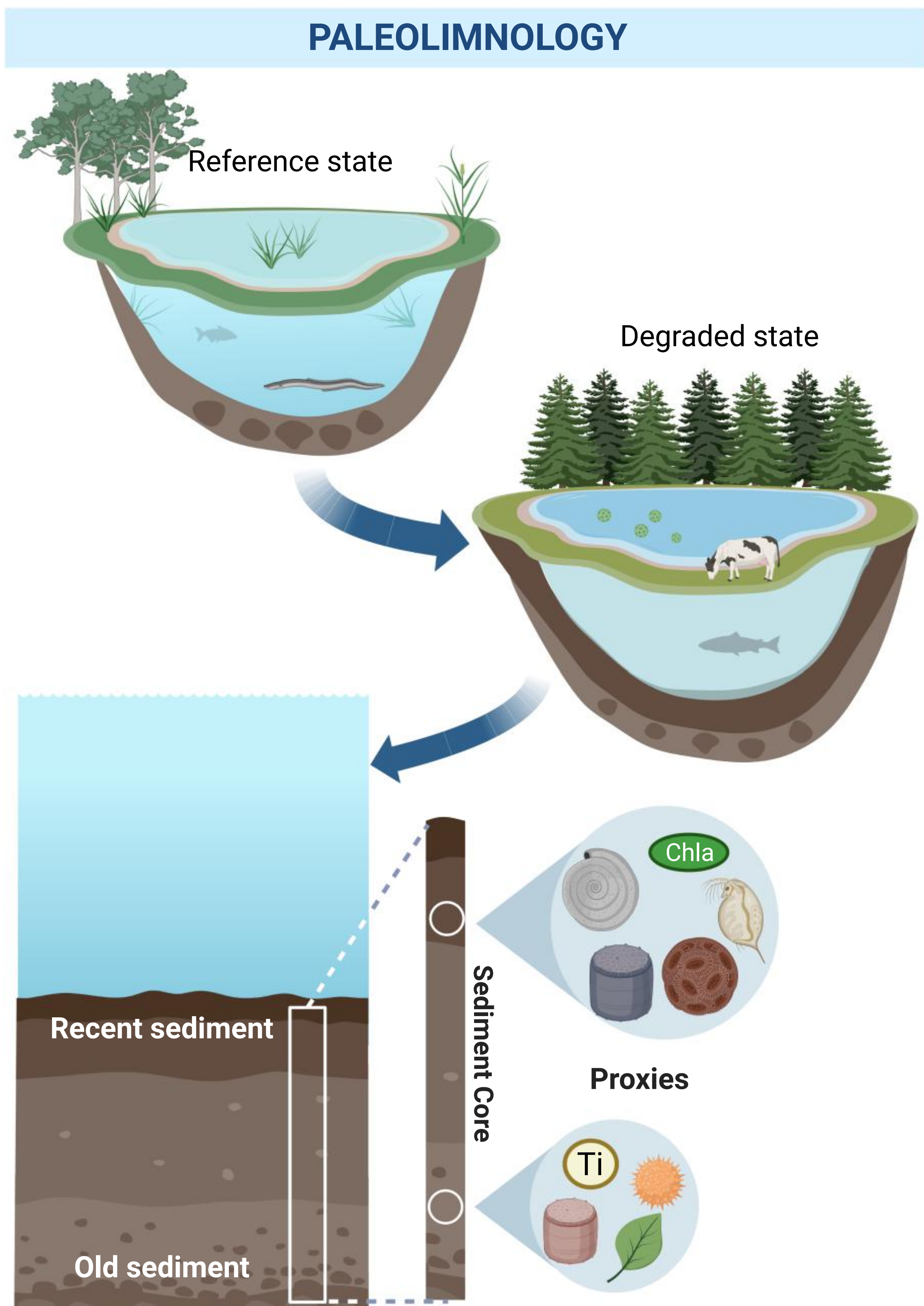

Figure 1.5: Particles from the catchment and from within lakes deposit over time at the bottom and capture lake histories. Sampling these different layers can help reconstruct individual lake histories.

1.6.1 Traditional proxies

Different proxies can be used to retrace the past state(s) of a lake. Fossils and other hard remains such as diatoms, pollens, chironomids (non-biting midges), charcoal and plant material, are traditionally identified to study specific components of lake food-webs (Figure 1.6). Diatoms have been widely used for decades due to their very resistant silica frustules, which allows species-specific identification and inferences about past nutrient levels. Pollens provide insights about past vegetation in the catchment and therefore past climate. On large timescales, it is possible to observe changes in climate based on vegetation changes. Charcoal indicates significant fires in the catchment, where closeness to the lake is related to bigger charcoal particles found in the sediment. Pigments and biomarkers found in bulk sediment samples can also by measured. Chlorophyll-a is often used as a proxy for photosynthetic productivity, and other pigments bring more details on community composition. Cyanobacterial pigments are well-known, and though they do not allow species-specific identification they can at least give an indication of total cyanobacteria abundance (Romero-Viana et al., 2010). Biomarkers are lipid molecules which can be linked back to specific clades, for example to archaea (Wörmer et al., 2014), microalgae (Grossi et al., 2003), and cyanobacteria (Naeher et al., 2012). Recent progress in scanning technology now allows the scanning of pigments (hyperspectral scanning) and many elements (xrf scanning) without damaging the sediment core. X-ray fluorescence (XRF) scanning is being increasingly used to retrace biological and geological processes. The elements are best used as ratios (e.g., silica / titanium rather than silica alone) to correct for degradation processes or organic vs inorganic processes. Studies are still working out which ratios are reliable proxies of which processes, for example silica / titanium is a proxy for biogenic silica from diatoms, manganese / iron is a proxy for anoxia, etc. A full list has bee compiled by Davies et al. (2015).

Several methods can be used to date sediment layers. Plant material such as leaves and twigs can be analysed for their ^{14}C composition, which places a date

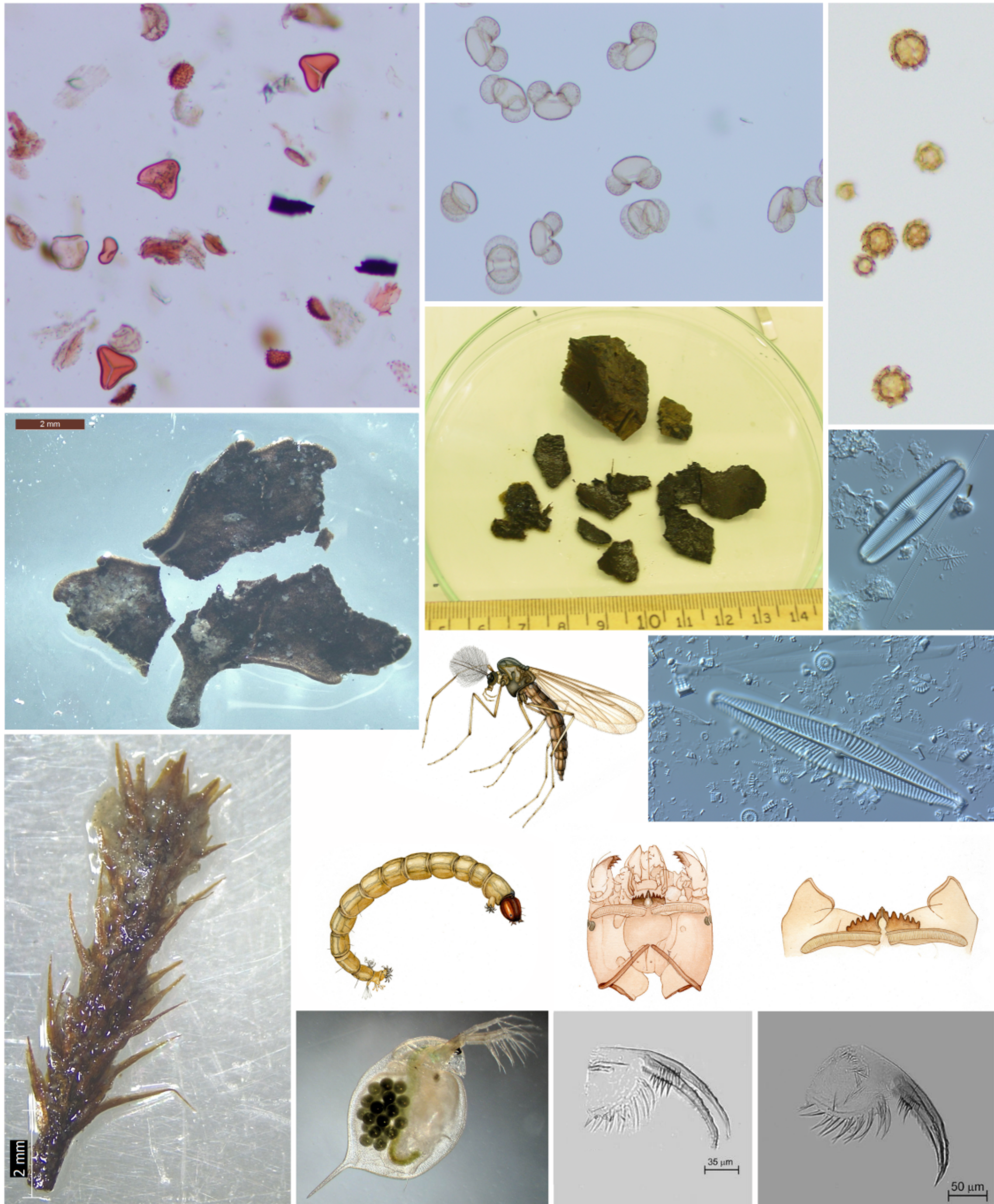

Figure 1.6: Examples of traditional paleolimnological proxies. From left to right and top to bottom: pollens (NZ native tree ferns, pine, dandelion), plant macrofossils, diatoms (*Sellaphora* sp., *Navicula* sp.), chironomid (adult, larvae, larvae head capsule), cladocerans (whole, jaw macrofossils). Pollens are about 50 µm wide, diatoms about 100 µm long, and chironomid head about 400 µm (0.4 mm) wide. Pollen photos from Dr. Xun Li, diatom photos from Dr. Rose Gregersen, plant macrofossils from Jenny Dahl (GNS Science), chironomid representations from Lizzie Harper (lizzieharper.co.uk), *Daphnia magna* photo from cabidigitallibrary.org, *D. longispina* complex and *D. pulex* complex post-abdominal claws from Korosi et al. (2012).

on when they last segregated carbon. In turn, the date can be assigned to the sediment layer in which the plant was found. This method can be done for any organic material, but it is sometimes difficult to find any in some sediment cores. It is also possible to analyse atmospheric lead in the sediment itself, in particular Lead210 (^{210}Pb), since the half-life of its by-product can indicate how long ago it was deposited. In the Northern Hemisphere, Lead210 is analysed by alpha spectrometry due to high abundances of atmospheric lead, but in the Southern Hemisphere the sediment has to be sieved and measured by gamma spectrometry. In more recent sediment from the West Pacific region, it is possible to analyse Cesium (^{137}Cs) since it indicates the start of nuclear bomb testing (1946 (USA) or 1966 (FR)).

Besides the dating of elements, natural disturbance events can help putting a date on specific layers. For example volcanic eruptions, which deposit ash layers across the landscapes. These can be identified as tephra in sediment cores and dated, thus helping to understand the core's sedimentology, and giving a record of volcanic eruptions. The same conclusions can be made with earthquakes, tsunamis, fires, and other major disturbances, though they generally need to be cross-checked with independent records.

1.6.2 Combination of paleolimnology with environmental DNA

Since most traditional paleolimnological proxies are based on hard / fossilised remains present in a sediment core, very limited inferences could previously be made about soft-bodied organisms. However, research over the last decade has highlighted that another molecule can be recovered from sediment: DNA. With tremendous improvements in molecular methods, it has become possible to extract DNA bound to many types of substrates and to potentially target any organism present in environmental samples. Many paleolimnological studies have already been published on that subject, relating their success in reconstructing historical communities in and around lakes using sedimentary ancient DNA (sedaDNA) from sediment cores.

Photosynthetic organisms have been the main focus of sedaDNA studies so far,

especially cyanobacteria and terrestrial plants (Table 1.1). This focus probably stemmed from the need to solve environmental issues (e.g., cyanobacteria blooms), the abundance of genetic material compared to other communities (e.g., fish), and the availability of other datasets as a mean of comparison (e.g., pollen for plants, monitoring data for cyanobacteria). Furthermore, photosynthetic communities in buried sediment are robust targets to avoid studying organisms that could potentially still be living in sediment samples. Issues such as climate change, lake eutrophication, or simply checking what was the reference condition of the lake, could be answered using sedaDNA and different molecular methods (metabarcoding, shotgun sequencing, or quantitative PCR).

Table 1.1: List of studies using sedimentary ancient DNA **in lake sediment cores**, per community and per continent. Africa and Oceania were merged to reduce the width of the table. Studies mentioned on the 'Fungi' row focused exclusively on fungi, as opposed to studies who detected fungi along with other micro-eukaryotes. This list includes papers that were published after the beginning of this thesis (2019).

	Europe	**Americas**	**Asia**	**Africa & Oceania**
Bacteria	Archeae: Ahmed et al. (2018) and Thorpe et al. (2022)	Stoeva et al. (2014) and Vuillemin et al. (2016) Poulain et al. (2015)	Archeae: Yang et al. (2015), Li et al. (2019), and Xu et al. (2022)	Oceania: Matisoo-Smith et al. (2008), Brasell et al. (2021), Pearman et al. (2021b), Pearman et al. (2021a), Cochrane (2017), Parrish (2020), Caird (2021), Gregersen et al. (2022), and Short et al. (2022)
Cyanobacteria	Domaizon et al. (2013), Savichtcheva et al. (2015), Monchamp et al. (2016), Monchamp et al. (2018), and Monchamp et al. (2019)	Escalera et al. (2014), Pal et al. (2015), Zastepa et al. (2017), Pilon et al. (2019), Dodsworth (2020), Erratt et al. (2021), and Hobbs et al. (2021)	Yan et al. (2019), Yan et al. (2020), Zhang et al. (2021), and Zhang et al. (2023)	Africa: Ye et al. (2011) Oceania: Picard et al. (2022b) and Picard et al. (2022a)

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Table 1.1: List of studies using sedimentary ancient DNA **in lake sediment cores**, per community and per continent. Africa and Oceania were merged to reduce the width of the table. Studies mentioned on the 'Fungi' row focused exclusively on fungi, as opposed to studies who detected fungi along with other micro-eukaryotes. This list includes papers that were published after the beginning of this thesis (2019). (Continued)

Cyanobacteria (continuation)	Capo et al. (2019a), Monchamp et al. (2021), and Nwosu et al. (2021)	Mejbel et al. (2021), Hobbs et al. (2022), and Dodsworth (2020)		
Diatoms			Stoof-Leichsenring et al. (2015) and Stoof-Leichsenring et al. (2020)	Africa: Stoof-Leichsenring et al. (2012)
Micro-eukaryotes	Capo et al. (2016), Capo et al. (2017a), Capo et al. (2017b), Kisand et al. (2018), Capo et al. (2019a), Capo et al. (2021b), Keck et al. (2020), and Barouillet et al. (2022)		Li et al. (2019) and Zhang et al. (2021)	Africa: Epp et al. (2010)
Fungi	Talas et al. (2021)		Hippel et al. (2022) and Seeber et al. (2022)	

Continued on next page

Table 1.1: List of studies using sedimentary ancient DNA **in lake sediment cores**, per community and per continent. Africa and Oceania were merged to reduce the width of the table. Studies mentioned on the 'Fungi' row focused exclusively on fungi, as opposed to studies who detected fungi along with other micro-eukaryotes. This list includes papers that were published after the beginning of this thesis (2019). (Continued)

Plants	Alsos et al. (2018), Parducci et al. (2013), Giguet-Covex et al. (2014), Pansu et al. (2015), Parducci et al. (2015), Paus et al. (2015), Parducci et al. (2019), Alsos et al. (2016), Anderson-Carpenter et al. (2011), Voldstad et al. (2020), Nota et al. (2022), and Messager et al. (2022)	Pedersen et al. (2013), Boessenkool et al. (2014), Epp et al. (2015), and Crump et al. (2021)	Heinecke et al. (2017), Clarke et al. (2019), Clarke et al. (2020), Liu et al. (2021b), Schulte et al. (2021), Liu et al. (2021a), and Hippel et al. (2022)	Africa: Bremond et al. (2017)
Metazoans	Fish: Olajos et al. (2018) and Messager et al. (2022)	Fish: Waldman et al. (2004), Stager et al. (2015), and Nelson-Chorney et al. (2019)		Africa: Dommain et al. (2020) Oceania: Matisoo-Smith et al. (2008)

Studies on metazoans using sedaDNA have been very rare. Zooplankton primers are currently not well designed for all ecoregions, and sequences can be difficult to assign to taxonomic entities when regional fauna are poorly represented in databases. Fish communities are harder to target due to the low levels of genetic material in sedaDNA samples. Interestingly, two studies on fish mentioned in Table 1.1 both looked at the history of fish populations to know which species was native between two salmonids (Nelson-Chorney et al., 2019) and when was the introduction date of a whitefish species (Olajos et al., 2018). Despite the methodical challenges, these studies highlight the possibility of sedaDNA to inform on native vs non-native species presence on a historical timeframe.

Sedimentary ancient DNA studies are heavily biased towards northern latitudes, especially Europe and North Americas. Before the start of this thesis (2019), very few studies had been published on the use of sedaDNA from lake sediment cores in the Oceania region (Matisoo-Smith et al., 2008; Cochrane, 2017). Future research on sediment cores will provide information on whether there have been historical changes e.g., due to Māori or European settlement in New Zealand. Establishing a baseline for New Zealand communities would be helpful to see how much degraded lakes have changed from that baseline and which species / clades are usually found in this ecoregion. From an ecological perspective, it may help to understand how the different communities are changing as lakes become more eutrophic, and possibly contributing to a negative feedback loop.

1.7 New Zealand

Though all lakes across the globes are impacted by human activities, the specifics of these impacts vary depending on the ecoregion. New Zealand, an island nation in the South-West Pacific, presents some peculiarities compared to the rest of the world, therefore this section focuses on lakes of New Zealand.

1.7.1 History of New Zealand and summary of ecosystem modifications

New Zealand has been isolated from all other land masses for 80 to 100 million years (Waters et al., 2006). At that time it separated from Gondwana (a grouping of Antarctica and Australia), carrying the local biota, and since then its flora and fauna have evolved in a unique direction. The native fauna is dominated by birds, including non-flying species, with many insects and reptiles species. Iconic species such as the flightless kiwi (*Apteryx* spp, Ratites) are well known, though other species are just as surprising, such as the reptile tuatara (*Sphenodon punctatus*) which is from the ancient lineage of Rhynchocephalia, distinct from lizards. The only three native species of land mammals are bats. The freshwater fauna is just as interesting and mostly endemic, the fish are mostly nocturnal, with many representatives of the *Galaxias* genus, and few from the *Gobiomorphus* genus (bullies), and rare species such as mudfish (*Neochanna* spp.), torrentfish (*Cheimarrichthys fosteri*) and pouched lamprey (*Geotria australis*). Some species are migratory and diadromous, such as the eels (*Anguilla australis* and *Anguilla dieffenbachii*) and a few galaxiids: banded kōkopu (*Galaxias fasciatus*), giant kōkopu (*Galaxias argenteus*), shortjaw kōkopu (*Galaxias postvectis*), īnanga (*Galaxias maculatus*), and kōaro (*Galaxias brevipinnis*).

New Zealand sits on the Zealandia continent, jointly on the Pacific tectonic plate (c. two thirds of the South Island and Steward Island) and on the Australian tectonic plate (c. one third of South Island and the North Island). The subduction of the Pacific plate under the Australian one leads to the formation of volcanoes in the North Island, and of high alpine mountains (the Southern Alps) in the South Island. Being surrounded by the sea, three main types of natural disturbances can therefore occur regularly: volcanic eruptions, earthquakes, and tsunamis. These events have been key in shaping what is nowadays New Zealand. Nowadays, the climate for New Zealand's mainland (North Island, South Island, Stewart Island) ranges from sub-tropical in the North to cold alpine in the South. Mean temperatures range

from c. 11 to c. 20 °Celsius, and mean precipitations range from 66 to 145 mm per month (NIWA).

Human arrival was very late in New Zealand, compared to other continents. Māori were the first to discover the country, coming from Polynesia using wakas (wooden canoes). Archaeological research and Māori knowledge retrace their arrival in two waves, c. 1250 and c. 1350 AD. Some parts of the country were settled later than others. Māori brought a few non-native species with them, such as rats, dogs, and few vegetables. They burned about 30% of the native forests to travel more easily and to aid in defending their homes. These fires can be seen in sedimentary records as small to big charcoal pieces. Māori took care of their environment for future generations, in a role of guardianship / protection (kaitiakitanga). Ecosystems were managed for future harvests, for the respect of their life force (mauri), and for the danger they may represent. Knowledge is still passed down orally, and today it is called Mātauranga Māori. This knowledge applies to many disciplines in a holistic view, different but complimentary to Western Science. It is being increasingly valued today in ecology, as a source of information on the historical state of New Zealand ecosystems, how Māori used to live and use their resources sustainably.

Europeans first learned of New Zealand through the travels of Abel Tasman in 1642, but the ships did not stay around. At that time, the Māori population was estimated to be about 100,000. A second explorer, James Cook, sailed to New Zealand in 1769 and made successful contact with Māori, leading to the British settlement of New Zealand which was formalised in 1840 with the signing of the Treaty of Waitangi. Since then, many ecosystems have been modified. Many non-native species were introduced, some with negative impacts on native ecosystems. Forests were further burned to clear the land, native trees were felled for timber and the non-native Monterey pine (*Pinus radiata*) was selected by the forestry industry as a rustic and fast-growing species. Rivers were dammed to create hydroelectrical power and water reservoirs, fields were enriched with superphosphate fertiliser to make up for the general poor quality of New Zealand soils. More recently, animal husbandry shifted from sheep to dairy cows, and nitrogen fertiliser was added to

pastures for increased productivity. Cities and factories were built over time, with poor to non-existent sewage treatment in the beginning. Since 1769, the population of New Zealand has grown from around 100,000 to 5 million today, for an area of 268,021 km^2 (StatsNZ, 2022).

1.7.2 New Zealand lakes

Since human arrival is so recent in New Zealand, human impact on freshwater systems is relatively recent, but it has been continuous and has intensified over the years. In comparison, most natural disturbances such as storms, earthquakes, tsunamis, are short in duration (pulse disturbances) and there is often enough time between events for systems to recover (e.g. Brasell et al., 2021). In the 1950s, people noticed the water quality of some lakes starting to change, and limnology (the study of lakes) started to develop in New Zealand to investigate the factors behind these shifts. Today, despite the c. 3,800 lakes found in New Zealand, only about 2.5% are monitored (*New Zealand's Environmental Reporting Series: Environment Aotearoa 2022* 2022). Monitoring is biased towards lowland lakes since they are more accessible, and also towards lakes known to have water quality issues, therefore it does not paint a representative picture of the global New Zealand lake health.

Of the 101 lake sites monitored regularly between 2016 and 2020, more than half (62.4%) are eutrophic or supertrophic, 25.7% are mesotrophic, and 11.9% are oligotrophic/microtrophic (*New Zealand's Environmental Reporting Series: Environment Aotearoa 2022* 2022). Modelling predicts that these numbers are quite different when looking at all lakes: most lakes are either eutrophic/supertrophic (45.8%) or mesotrophic (51.8%), with only very few lakes microtrophic to oligotrophic (2.4%) (*New Zealand's Environmental Reporting Series: Environment Aotearoa 2022* 2022). These statistics are alarming, considering that lake water quality is significantly lower in lakes with human-modified landcover in their catchment (*New Zealand's Environmental Reporting Series: Environment Aotearoa 2022* 2022). Modelling data indicated that levels of total phosphorus are high in 13.6% of lakes, total nitrogen

in 35.6% of lakes, and levels of chlorophyll-a high in 42.7% of lakes.

A total of 210 lakes had enough data to study their macrophytes between 2007 and 2016. Their conditions were evenly distributed: excellent/high ecological conditions (33%), moderate condition (31%), and poor unvegetated conditions (36%). However, a total of 189 out of these 210 lakes (90%) have non-native macrophytes (Ministry for the Environment & Stats NZ, 2019). Native macrophytes are being outcompeted by invasive ones, with 39 aquatic weeds declared pests (unwanted organisms and invasives). In 2012, only 10.1% of the 168 surveyed lakes having macrophytes had only native macrophytes in them and 2.4% of the vegetated 168 lakes have lost all natives, with only exotic / invasive macrophytes remaining (*New Zealand's Environmental Reporting Series: Environment Aotearoa 2022* 2022).

A lesser known but interesting biotic driver is the change in animal behaviour after landscape modification, and its impact on lake health. For example, a study has documented that an increase in bird populations in Southland could lead to an increase in nutrient and subsequent eutrophication (Woodward, 2013). Vegetation clearance by humans led to the opening of the vegetation cover, which meant waterfowl could have easier access to the lake's margin, so their numbers increased, and their faeces could have led to the eutrophication of Alexander Lake (Southland, New Zealand).

1.7.3 A growing invasive condition

Changes in food webs can deeply affect aquatic ecosystems. This can happen during natural catastrophes (e.g., volcanic eruptions), or through human activities. In New Zealand, many aquatic species have been introduced since European arrival. New Zealand is now considered one of six global hotspots for non-indigenous fish introductions (>25% of all freshwater fish species present; Leprieur et al., 2008). Fish have been introduced for aquaculture purposes in the marine environment, and for angling / recreative / biological control purposes, as well as accidental releases from aquaria practices, in freshwater. The impact of aquaculture will not be discussed

here. There are 20 species of freshwater fish 'introduced and naturalised' in the country (Table 1.2), with population numbers increasing since their introduction (c. 1870 for most species; Dunn et al., 2018; Collier et al., 2015). In contrast, there are only 40 native fish with a determinate taxonomic status (Dunn et al., 2018) including numerous endemic and cryptic fish (quite small, mostly solitary, nocturnal and secretive). The North Island has a higher number of introduced species than the South Island, and studies have found many lakes across the country containing at least one to two introduced species (Drake et al., 2011), some lakes with up to six introduced fish species (Rowe, 2007). The continued spread of invasive fish in lakes is known to promote ecosystem shifts and biodiversity loss, through effects such as competition, predation, food-web modifications, and habitat alterations (Collier et al., 2015).

A particular non-native fish of interest is the European or redfin perch (*Perca fluviatilis*). It was introduced in New Zealand in the 1870s from Australia. Perch are voracious fish, reproduce fast in the right conditions, and change their diet when they reach a certain size. The larvae and juveniles eat small zooplankton, and when they grow over c. 11-15 cm they prey on other fish (including juvenile perch). Perch do not have an equivalent in the New Zealand native food chain, and their only predators are brown trout (*Salmo trutta*) and native eels. However, brown trout are not present in all lakes, and eel populations are declining. Therefore, in some lakes, perch populations can be very large, and they may significantly affect zooplankton communities and their ability to graze on phytoplankton (see section 1.3.2.2). Perch are considered noxious fish throughout most of the country, except in the Auckland and Waikato regions where they are still considered a sports fish.

Finally, invasive aquatic plants are also destabilising native ecosystems. While *Hydrilla verticillate* is almost under control, species such as *Lagarosiphon major*, *Egeria densa*, and hornwort (*Ceratophyllum demersum*) are still spreading, with stems up to several meters in length. Trout ova were wrapped in *Elodea canadensis* leaves to protect them from desiccation during travels, and released in lakes with the leaves of *E. canadensis*, leading to a nation-wide spread of this aquatic weed.

Table 1.2: Exotic fish species introduced in New Zealand and currently present. All species belong to the Actinopterygii class. Family, genus and species name are specified. Other species have been introduced but have not established and therefore not presented here. Silver carp and grass carp are introduced but not reproducing. Sources: Collier et al. (2015) and McDowall (2008)

Cyprinidae - carps

Cyprininae

- Goldfish (*Carassius auratus*) (1870s)
- Koi carp (*Cyprinus carpio*) (1800s, wild in 1960s)

Leuciscinae

- Orfe (*Leuciscus idus*)
- Rudd (*Scardinius erythrophthalmus*) (1960s)
- Tench (*Tinca tinca*) (1870s)

Squaliobarbinae

- Grass carp (*Ctenopharygnodon idella*)

Xenocyprinae

- Silver carp (*Hypophthalmichthys molitrix*)

Ictaluridae - Catfish

- Brown bullhead catfish (*Ameiurus nebulosus*) (1870s)

Gobiidae – Gobies

- bridled goby (*Arenigobius bifrenatus*)

Percidae – common perch (*Perca fluviatilis*) (1860s)

Poeciliidae / Livebearers

Poeciliinae

- Gambusia (*Gambusia affinis*) (1930s)
- Sailfin molly (*Poecilia latipinna*)
- Guppy (*Poecilia reticulata*)
- Caudo (*Phalloceros caudimaculatus*)
- Swordtail (*Xiphophorus helleri*) (may be extinct since 1999)

Salmonidae - Salmonids

- Rainbow trout (*Oncorhynchus mykiss*) (early 1880s)
- Brown trout (*Salmo trutta*) (1880s)
- Brook char (*Salvelinus fontinalis*) (late 1870s)
- Lake char / mackinaw (*Salvelinus namaycush*) (1906)
- Atlantic salmon (*Salmo salar*) (1864, close to extinction in NZ)
- Chinook salmon / King Salmon (*Oncorhynchus tshawytscha*) (early 1900s)
- Sockeye salmon (*Oncorhynchus nerka*) (1902)

1.8 Thesis objectives and outline

The sedaDNA studies mentioned earlier (Table 1.1) are heavily biased towards northern latitudes, especially Europe and North Americas. The New Zealand ecoregion has evolved differently from other landmasses due to its long isolation, therefore comparisons with overseas studies are not always applicable. Human settlement is relatively recent and general European impacts are well-known, therefore sedimentary records only need to target the last 1,000 years to get a record of pre-human times, Māori settlement, and European settlement. Multiple drivers can affect lake health, and lake eutrophication has been a hot topic in New Zealand for the past decade, especially with the increase of potentially toxic algal blooms which have led to the temporary closure of drinking and swimming spots (e.g. Lake Hayes, Lake Taupo). However, the impact of non-native species is more controversial since some species have become local economical assets, and the onset of their impact is not well documented. Sediment cores and sedaDNA from New Zealand lakes will provide information on whether there have been historical changes due to Māori or European settlement in New Zealand. Establishing a baseline for New Zealand communities would be helpful to see how much degraded lakes have changed from that baseline and which species / clades are usually found in this ecoregion. From an ecological perspective, it will helpful to understand how the different communities are changing as lakes become more eutrophic, and possibly contributing to a negative feedback loop.

The purpose of this thesis is to investigate how well eDNA and sedaDNA can retrace historical changes in New Zealand lakes, with a particular focus on cyanobacterial blooms and their links with exotic fish introduction such as the European Perch (*Perca fluviatilis*). The development of specific methods will be necessary, such as investigating how well molecular methods can distinguish lakes undergoing cyanobacterial blooms versus those who do not (Chapter 2), how well molecular methods related to traditional proxies such as pigments for historical cyanobacterial abundances (Chapter 3), and how well fish DNA can be detected in lake surface sed-

iment compared to surface water (Chapter 4). Chapter 4 is a precursor to detecting fish sedimentary ancient DNA in sediment cores, which is investigated by another PhD student (Thomson-Laing et al, unpublished). These investigations will support the final goal of this thesis, determining what led a lake with low intensity land-use but with introduced perch to be dominated by cyanobacterial blooms in recent decades (Chapter 5). All findings will be summarised and discussed in Chapter 6.

This PhD thesis is composed of six chapters:

- Chapter 1 (the current chapter) provides background on the various aspects covered in this thesis and highlighting the gaps in scientific knowledge.
- Chapter 2 presents the first historical reconstruction of cyanobacteria communities from sedimentary DNA in New Zealand. The 16S rRNA gene was amplified from sedimentary ancient DNA found in sediment cores from six lakes and analysed using metabarcoding and quantitative PCR (digital droplet PCR - ddPCR).
- Chapter 3 compares two paleolimnological proxies for cyanobacteria in sediment cores from four lakes: sedimentary ancient DNA (16S rRNA gene with ddPCR) and cyanobacteria-specific pigments (Canthaxanthin, Echinenone, Myxoxanthophyll, Zeaxanthin with High Performance Liquid Chromatography - HPLC).
- Chapter 4 compares environmental DNA (eDNA) detection rates of invasive fish in contemporary samples: surface water and surface sediment. Fourteen sites were surveyed in three lakes to map the occupancy of two invasive fish (European perch and rudd). Fish DNA was amplified with species-specific primers (ddPCR) and analysed with occupancy modelling.
- Chapter 5 explores whether the introduction of the European Perch (*Perca fluviatilis*) led to the decline in health of a lowland lake with minimal land-use (Lake Pounui) using historical reconstructions of bacteria (16S rRNA gene), microeukaryotes (18S rRNA gene), metazoans (CO1 gene), and macrophytes (*rbc*L and *trn*L genes), and other paleolimnological proxies.

- Chapter 6 summarises this thesis' findings.

Chapter 2

Drivers of historical shifts in cyanobacterial communities: the impact of human activities in six contrasting lakes

2.1 Preliminary note

This paper was published in Scientific Reports in July 2022 under the following reference:

Maïlys Picard, Xavier Pochon, Javier Atalah, John K. Pearman, Andrew Rees, Jamie D. Howarth, Christopher M. Moy, Marcus J. Vandergoes, Ian Hawes, Samiullah Khan, and Susanna A. Wood (July 27, 2022a). "Using metabarcoding and droplet digital PCR to investigate drivers of historical shifts in cyanobacteria from six contrasting lakes". In: *Scientific Reports* 12.1. Number: 1 Publisher: Nature Publishing Group, p. 12810. ISSN: 2045-2322. DOI: 10.1038/s41598-022-14216-8

It was published in Open Access under a Creative Commons Attribution 4.0 International License. The content of this section is therefore an exact copy of the published article, except that (1) the ORCID numbers of the authors and the lists of keywords and of references were omitted, (2) due to the difference in bibliography

styles, the references in Table 2.1 were omitted for clarity's sake, and (3) for consistency with the rest of the thesis, the citations were reformatted and the numbering of figures and tables adjusted.

2.2 Author list and author contributions

Maïlys Picard (M.P.), Xavier Pochon (X.P.), Javier Atalah (J.A.), John K. Pearman (J.K.P.), Andrew Rees (A.R.), Jamie D. Howarth (J.D.H.), Christopher M. Moy (C.M.M.), Marcus J. Vandergoes (M.J.V.), Ian Hawes (I.H.), Samiullah Khan (S.K.), and Susanna A. Wood (S.W.)

S.W. and M.P. conceived and designed the study. A.R., J.D.H., C.M.M., M.J.V., S.K. provided the lake sediment cores. M.P. processed the samples and M.P., J.K.P., and J.A. analysed the data. X.P., I.H., A.R., J.D.H., M.J.V., J.A., J.K.P. provided guidance during the study and assisted with draft manuscripts. All authors reviewed the manuscript.

2.3 Abstract

The frequency and intensity of cyanobacterial blooms is increasing worldwide. Multiple factors are implicated, most of which are anthropogenic. New Zealand provides a useful location to study the impacts of human settlement on lake ecosystems. The first humans (Polynesians) arrived about 750 years ago. Following their settlement, there were marked landscape modifications which intensified after European settlement about 150 years ago. The aims of this study were to reconstruct cyanobacterial communities in six lakes over the last 1000 years and explore key drivers of change. Cyanobacterial environmental DNA was extracted from sediment cores and analysed using metabarcoding and droplet digital PCR. Cyanobacteria, including potentially toxic or bloom forming species, were already present in these lakes prior to human arrival, however their overall abundance was low. Total cyanobacteria abundance and richness increased in all lakes after European settlement but was very pronounced

in four lakes, where bloom-forming taxa became dominant. These shifts occurred concomitant with land-use change. The catchment of one deteriorated lake is only moderately modified, thus the introduction of non-native fish is posited as the key factor driving this change. The paleolimnological approach used in this study has enabled new insights into timing and potential causes of changes in cyanobacterial communities.

2.4 Introduction

Freshwater ecosystems throughout the world are currently experiencing an increase in frequency and intensity of cyanobacterial blooms(Paerl et al., 2009; Paerl et al., 2012; Huisman et al., 2018). Various causes have been identified, such as increased human disturbances including enhanced erosion caused by deforestation and land-use intensification, and major shifts in food-webs due to the introduction of non-native species (such as fish and macrophytes, Reissig et al., 2006; Britton et al., 2010). A further compounding factor is climate change, which is resulting in warmer water temperatures, longer periods of stratification, greater frequency of severe weather events such as storms or droughts, and reduced length of ice cover, all of which have also been implicated in the rise of cyanobacterial proliferations (Paerl et al., 2012; Beaulieu et al., 2013; O'Neil et al., 2012; Paerl et al., 2008). Many bloom-forming species produce toxins collectively known as cyanotoxins, which can affect the reproduction and behaviour of aquatic organisms and have led to poisoning, or in extreme cases, death of humans and animals (Chorus et al., 1999; Sukenik et al., 2015). Considerable effort has therefore been placed on developing lake management and restoration plans for water bodies experiencing cyanobacterial blooms (Ibelings et al., 2016; Paerl, 2014; Rastogi et al., 2015). Unfortunately, historical records are very limited and monitoring programmes often start once a lake is already in an advanced state of degradation. There is uncertainty as to whether the recent increase in cyanobacterial blooms is entirely due to eutrophication and environmental change, or to a greater awareness and more monitoring programs con-

tributing to these new bloom reports (Ewing et al., 2020). Increasing knowledge on how lake ecosystems, their catchments and regional climates have changed historically, and identifying links with cyanobacterial blooms, may help in the development of informed and successful restoration actions.

New Zealand is an island nation in the South Pacific that has been isolated from all other landmasses for over 80 million years. The first humans to set foot on the mainland were from Polynesia (Māori), and only arrived around 1250–1350 AD (McGlone et al., 1999; Brooking et al., 2004; Wilmshurst et al., 2008). Following their arrival, there were marked landscape modifications in some regions of the country. For example, burning of native forests (McGlone, 1983; McGlone, 1989; McWethy et al., 2010; McWethy et al., 2014) reduced overall coverage by about 50% (McWethy et al., 2010; McWethy et al., 2014). Land clearance intensified with the first major wave of European settlement beginning in the 1840s (Brooking et al., 2004), coupled with farming practices which used fertilisers leading to nutrient runoffs, urbanisation releasing contaminants in waterways, and the introduction of many non-native freshwater species affecting native food-webs (Star, 2003; Clark, 1949; Wilmshurst, 2007).

Paleolimnology provides a suite of methods that allow inferences to be made about a lake's historical condition—its past biological communities, water quality, and vegetation changes in and around the lake. For example, shifts in biological communities, such as midges, diatoms, or vegetation, can be indicative of past disturbance events (Smol, 1985; Rees et al., 2008; Epp et al., 2010; Buchaca et al., 2011). Paleolimnological reconstructions have traditionally relied on microfossil indicators, however molecular methods targeting environmental DNA (eDNA) now allow a wider range of micro- or macro-organisms to be investigated in sediment cores, particularly soft-bodied organisms. While DNA can be extracted from the organism, it can also be recovered when it is bound to extracellular matrices in the sediment (exDNA, Cristescu et al., 2018). Recent work has shown that it is possible to use DNA from sediment (sedDNA), and ancient DNA from sediment (sedaDNA) to reconstruct the historical dynamics of various lake organisms including mammals,

fish, and plants (Giguet-Covex et al., 2014; Alsos et al., 2016; Nelson-Chorney et al., 2019; Capo et al., 2021a). High-Throughput Sequencing (HTS) coupled with metabarcoding analyses allows for a large number of DNA sequences to be amplified directly from environmental samples and these can be used to characterise entire communities (Shokralla et al., 2012; Taberlet et al., 2012; Thomsen et al., 2015; Keeley et al., 2018). A recent study used metabarcoding to reconstruct cyanobacterial diversity over the last 200 years in Lake Greifensee and Lake Zürich, Switzerland (Monchamp et al., 2016). Their results were congruent with an independent dataset obtained by microscopic identification of water samples for the last 40 years. Furthermore, quantitative PCR (qPCR) or droplet digital PCR (ddPCR) have also been successfully applied to paleolimnological studies for various targets including cyanobacteria and genes involved in cyanotoxin production in several countries (Pal et al., 2015; Dodsworth, 2020; Rinta-Kanto et al., 2009; Zastepa et al., 2017).

New Zealand has 3820 natural lakes greater than a hectare (Schallenberg et al., 2013), and due to its recent human settlement it provides a particularly useful location to study the ecological impacts of human settlement on lake ecosystem. It is possible to obtain sediment from pre-human times and reconstruct the past c. 1000 years with a relatively short sediment core (1–2 m) for most small lakes. Understanding the past of lakes is important because less than 200 of New Zealand's lakes have regular water quality monitoring programmes in place, most of which have been underway for less than two decades. It is estimated that 46% of all lakes are eutrophic (*Our freshwater 2020 | Ministry for the Environment* n.d.) with many experiencing cyanobacterial blooms. The direct impact of anthropogenic activities is thought to be the main driver, but climate change may also be contributing. Over the last century, land temperatures have increased by 1 °C on average, droughts and floods have become more frequent and severe (*Our freshwater 2020 | Ministry for the Environment* n.d.).

The aims of this study were to reconstruct cyanobacterial communities in six New Zealand lakes over a period spanning approximately 1000 years using sediment cores, ddPCR and metabarcoding, and to identify key drivers that may have led

to increases in cyanobacteria abundance or shifts in community structure. We hypothesised that: (1) cyanobacterial abundance and composition would start to shift during the period following Māori settlement in lakes where the vegetation cover in the catchment had changed. However, the most pronounced change would occur post European settlement with bloom-forming species becoming more prevalent in lakes which had experienced land use change; and (2) in lakes with little modification in vegetation cover in their catchment, the increases in abundance and changes in composition would be related to the presence of non-native carnivorous fish having a top-down effect on the food chain. While we acknowledge that climate change is likely to have some impact on the study lakes, we do not directly explore this in this study.

2.5 Methods

2.5.1 Sampling

Sediment cores were collected from six lakes in New Zealand: Rotoehu, Pounui, Wairarapa, Paringa, Hayes, and Johnson (Figure 2.1, Table 2.1). These lakes were chosen because they have a wide variety of catchment modifications, span a range of trophic levels, have varying histories of cyanobacterial blooms, and contain different species of non-native fish (Table 2.1). The lakes span a range of climatic regions (Table 2.1).

One sediment core was retrieved close to the deepest point of each lake (Table 2.1) using an Uwitech gravity corer with 2 m-long, 90-mm diameter polyvinyl chloride barrels. The exception was Lake Paringa, where the sediment core was collected using a Mackereth corer (Mackereth, 1958) and sealed within plastic barrels (50 mm internal diameter). All barrels were cleaned with 2% sodium hypochlorite (bleach) prior to coring. Core lengths ranged from 0.80 m to 5.52 m (Table 2.1). After retrieval, the cores were sealed, stored at 4°C and in darkness for up to 4 weeks until sub-sampling.

2.5.2 Sample processing

In a room separate from where all DNA work was undertaken, the cores were split in half using a manual saw and a guillotine. They were photographed and described in detail to relate sediment type and colour and the presence of organic material. To prevent cross-contamination caused by the splitting of the cores, the top 2–3 mm of one half-core per lake was carefully removed with a sterile spatula. Sub-samples (c. 0.5 g) were taken from the centre of the half-core using a sterile spatula at various depths down the core. In general, sub-samples were taken every 1–2 cm in recent sediments and every 4–5 cm in older sediments (Supplementary Table D.1), resulting in 206 sediment subsamples. Sub-samples were kept frozen (-20°C) and in the dark until DNA extraction. Sub-samples were also collected from a range of depths for pollen and charcoal analysis in all lakes as well as carbon-14 dating for Lakes Paringa and Pounui. These analyses are described elsewhere (Howarth et al., 2012; Trodahl et al., 2016; Cochrane, 2017; Khan et al., n.d.) and a brief summary of methods is provided in Supplementary Table D.2.

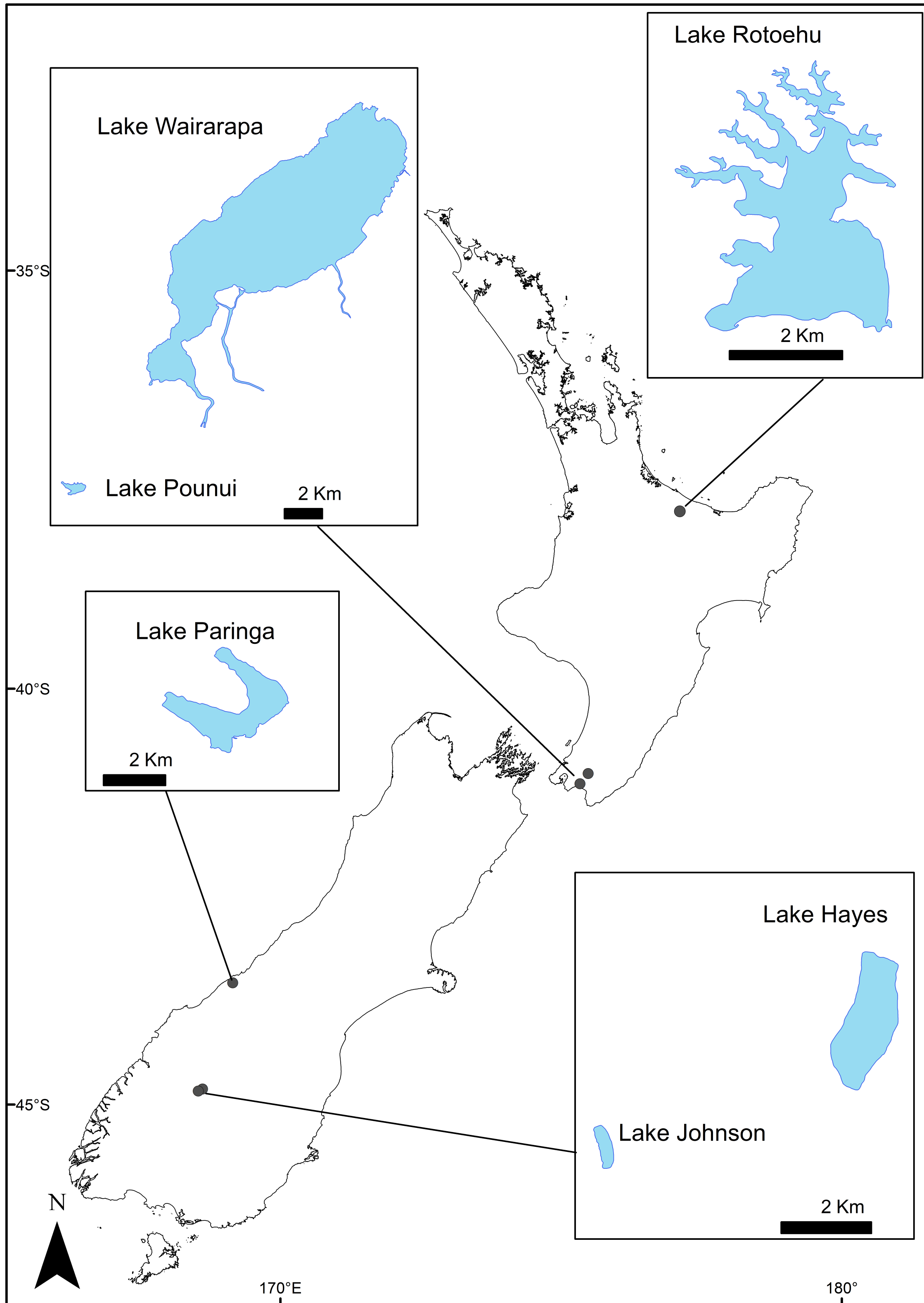

Figure 2.1: Location of the six study lakes, situated within the North and South islands of New Zealand and spanning 7° of latitudinal gradient. Black dots in each lake indicate coring sites. Map created using ArcGIS version 2.8 (https://www.esri.com) by Kati Doehring, Cawthron Institute.

Table 2.1: Lake characteristics. Note that the FENZ ID is related to the Freshwater Ecosystems of NZ (FENZ) geo-database, and the Trophic Level Index (TLI) is based on total nitrogen, total phosphorous, water clarity, and chlorophyll-a. Rainfall and temperature values are the highest and lowest mean monthly values over one year, data from NIWA (NIWA, 2018). Lakes are ordered from left to right by increasing lake trophic status.

	Paringa	**Pounui**	**Rotoehu**	**Hayes**	**Johnson**	**Wairarapa**
FENZ ID	46,725	229	40,188	54,190	53,707	1708
Latitude (S)	43°43'07.0"	41°20'40.0"	38°00'54.4"	44°58'28.6"	45°00'06.5"	41°13'31.6"
Longitude (E)	169°23'17.5"	175°06'51.8"	176°31'53.4"	168°48'40.7"	168°43'55.8"	175°12'58.0"
Rainfall (mm/month)	200–300	75–137	92–136	50–75	50–75	75–137
Temperature (°C/month)	7.4–15.6	8.9–17.7	7.8–17.9	3–15.8	3–15.8	8.9–17.7
Coring date	December 2017	August 2016	April 2019	November 2017	November 2017	June 2017
Core total length (m)	5.52	1.30	1.52	0.82	0.80	2.00
Lake elevation (masl)	16	14	296.9	324.8	392.1	1.3
Lake max depth (m)	52	9.6	13.5	33	27	2.5
Lake surface area (km^2)	4.75	0.46	7.90	2.74	0.25	77.37
Lake total volume (km^3)	0.29	0.002	0.035	0.31	0.002	0.64
Lake type	Glacial	Tectonic	Volcanic	Glacial	Glacial	Riverine

Continued on next page

Table 2.1: Lake characteristics. Note that the FENZ ID is related to the Freshwater Ecosystems of NZ (FENZ) geo-database, and the Trophic Level Index (TLI) is based on total nitrogen, total phosphorous, water clarity, and chlorophyll-a. Rainfall and temperature values are the highest and lowest mean monthly values over one year, data from NIWA (NIWA, 2018). Lakes are ordered from left to right by increasing lake trophic status. (Continued)

Mixing type	No data	Polymictic	Polymictic	Monomictic	Monomictic	Isothermal
Current catchment area (km^2)	79.09	6.27	73.85	44	1.90	654.25
Current catchment composition	Native forest and shrubs	Native forest and a bit of pasture	Pasture, urban, native forest	Pasture and grasslands, urban, bit of native forest	Mainly pastoral land, bit of native forest and shrubs	Pasture, urban, native forest
Trophic status—TLI	Oligotrophic (2020)	Eutrophic (2014)	Eutrophic (2018)	Eutrophic (2018)	Eutrophic (2018)	Supertrophic (2018)
Reported cyanobacterial blooms	Not monitored	Yes	Yes	Yes	Yes	Yes
Non-native carnivorous fish species	Brown trout (*Salmo trutta*) in 1950s, Chinook/Quinnat salmon (*Oncorhynchus tshawytscha*) from 2011	Rainbow trout (*Oncorhynchus mykiss*) from 1938 to 1958, European perch (*Perca fluviatilis*) in the 1960s	Rainbow trout (*O. mykiss*) around 1900	Brown trout (*S. trutta*) and European perch (*P. fluviatilis*) from 1870s	European perch (*P. fluviatilis*) from 1880s, rainbow trout (*O. mykiss*) from 1962	European perch (*P. fluviatilis*) unknown date

2.5.3 DNA extraction and amplification

Each step of molecular analyses (DNA extraction, PCR or ddPCR set-up, template addition, PCR/ddPCR analysis) was conducted in separate sterile laboratories dedicated to these steps, with sequential workflow to ensure no cross-contamination. Rooms dedicated to DNA extraction, PCR set-up, or template addition were equipped with ultra-violet sterilisation which was switched on for at least 15 min before and after each use. The PCR/ddPCR set-up and template addition was always undertaken in laminar flow cabinets with HEPA filtration. Aerosol barrier tips (Axygen, USA for PCR or epT.I.P.S., Eppendorf, Hamburg, Germany for ddPCR) were used throughout.

Approximately 0.25 g of sediment from each sub-sample were weighed in the first tube provided in the DNeasy Power-Soil™ DNA Isolation Kit (QIAGEN, Germany) and exact weights recorded. Environmental DNA was extracted following the manufacturer's protocol. DNA extraction was performed in batches of eight to ten samples, including a negative control every two batches which contained all the reagents but no sediment. DNA concentrations and quality were measured using a spectrophotometer (Eppendorf AG, Hamburg, Germany).

2.5.4 Quantifications of total cyanobacteria

Droplet digital Polymerase Chain Reaction (ddPCR) was used to quantify total cyanobacteria from each sample using the CYAN 108F and CYAN 377R primers targeting an approximate 270 base-pairs (bp) region of the 16S rRNA gene (Rinta-Kanto et al., 2005). All samples were diluted for quantification by ddPCR; dilutions ranged from 1/10 to 1/1000. The ddPCR was undertaken using a BioRad QX200 system. Each ddPCR reaction included 10 µL of 2 x BioRad QX200 ddPCR EvaGreen Supermix, 0.2 µL of each primer at 10 µM, 4 µL of diluted template DNA, and 7.6 µL of DNA/RNA-free water (Life Technologies) for a total reaction volume of 22 µL per well, loaded on a semi-skirted ddPCR 96-well plate. The BioRad QX200 droplet generator partitioned each reaction mixture into tens of thousands of

nanodroplets by combining 20 µL of the mixture with 70 µL of Evagreen droplet oil. After processing, this resulted in a total nanodroplet volume of 40 µL, which was transferred to another semi-skirted ddPCR 96-well plate for amplification using specific thermo-cycling conditions (Supplementary Table D.3). The plate was analysed on the QX200 droplet reader instrument. For each ddPCR plate run, at least one negative control (containing all reagents and DNA/RNA-free water instead of template DNA) and one positive control (genomic DNA extracted from a cyanobacteria culture) were included. Droplet digital PCR droplet counts were fitted to a Poisson distribution by the QuantaSoft Analysis software (BioRad), resulting in target DNA concentration (gene copies/µL) which were then standardised to DNA gene copy numbers per gram of sediment using the following formula:

$$\begin{aligned} Target\ concentration\ (gene\ copies/g)\ = \\ \frac{\frac{nbcopies}{\mu L} \times \frac{Total\ Mastermix\ volume(22\,\mu L)}{Template\ DNA\ volume(4\,\mu L)}}{sediment\ sample\ weight \times (1 - water\ content)} \\ \times \frac{dilution\ factor\ (10 - 1,000) \times DNA\ extraction\ volume(100\,\mu L)}{sediment\ sample\ weight \times (1 - water\ content)} \end{aligned} \tag{2.1}$$

2.5.5 Reconstructing cyanobacterial communities with metabarcoding

Polymerase Chain Reactions (PCRs) were performed using the cyanobacteria-specific primers CYB359-F and CYB784-R (Nübel et al., 1997). These primers amplify an approximately 400 bp fragment of the V3-V4 regions of the cyanobacterial 16S rRNA gene. Sedimentary DNA samples were amplified in PCRs of 48 µL volume each, containing AmpliTaq Gold® 360 Master Mix (Life Technologies), 360 GC enhancer (Life Technologies), Bovine Serum Albumin (Sigma), primers at 10 µM, DNA/RNA free water (Life Technologies), and template DNA (Supplementary Table D.3). Batches contained 20 samples, including a negative and positive control, PCR cycling conditions are detailed in Supplementary Table D.3.

Amplicon products, including all negative extraction and PCR controls, were

visualised on a 1.5% agarose gel electrophoresis stained with Red Safe DNA Loading Dye (iNtRON Biotechnology Inc, Kyungki-Do, Korea), and UV illumination to ensure amplification of a single ˜400 bp product. PCR products were purified (Agencourt AMPure XP Kit; Beckman Coulter, CA, USA), quantified (Qubit® 2.0 Fluorometer; Invitrogen, CA, USA), diluted to 5 ng.μL^{-1} and submitted for sequencing to Auckland Genomics (University of Auckland). Sequencing adapters and sample-specific indices were added to each amplicon via a second short round of PCR using the Nextera™ Index kit (Illumina, CA, USA). Amplicons were pooled into a single library and paired-end sequences (2 × 250 bp) were generated on a MiSeq™ instrument using the TruSeq™ SBS kit (Illumina, CA, USA). Sequence data were automatically demultiplexed using a MiSeq Reporter (v2), and forward and reverse reads were assigned to samples.

2.5.6 Bioinformatic analyses

The R software(R Core Team, 2020) and RStudio software (RStudio Team, 2020) were used for all bioinformatic and statistical analyses. The Tidyverse v1.3.0 and its associated packages (Wickham et al., 2019) were used for data manipulation and exploration. All plots were drawn using the ggplot2 package (Hadley Wickham, 2016) unless stated otherwise. Primer sequences were removed with Cutadapt (Martin, 2011) (Anaconda environment adapted in R) allowing 1 bp of mismatch. The DADA2 package (Callahan et al., 2016) was used for general sequence quality assessment, quality profiles plots, and for the full amplicon workflow (quality filtering, merging of paired-end reads, dereplication, chimera identification, sample inference, and taxonomy assignment). Reads were truncated at 225 and 215 bp for forward and reverse reads respectively. The maximum number of "expected errors" (maxEE) per read was set at two for forward reads and four for reverse reads. Other parameters were set to default. The first 108 bp of the sequences were then used to calculate a parametric error matrix for forward and reverse reads each, which were checked for convergence. Sequences were dereplicated and sequence variants were inferred

by pseudo-pooling based on their respective error matrix. Singletons were discarded and remaining paired-end reads were merged with a maximum mismatch of 1 bp and a required minimum overlap of 10 bp, producing a sequence table. Samples from all sequencing runs were then merged into one sequence table. Amplicon lengths were filtered so that only those within the range of 379 and 403 bp were retained and checked for chimeras using the consensus method, resulting in Amplicon Sequence Variants (ASVs). Taxonomy was assigned from Kingdom to Genus for each ASV using a training set from the SILVA database r138 (Quast et al., 2013; Yilmaz et al., 2013; Glöckner et al., 2017). If the prediction of the SILVA classifier was estimated to be correct at 85% or more (min bootstrap), then the taxonomy for that rank was assigned to the ASV.

The phyloseq package (McMurdie et al., 2013) was used to prepare the sequence table and associated information and undertake some analyses (merging, rarefaction with rarefy_even_depth, richness measures with estimate_richness). All non-bacterial reads (eukaryotes, mitochondria, chloroplasts) were removed from the dataset. Rarefaction curves were plotted using the vegan package (Oksanen et al., 2019) to visualize sampling depth for each sample. Rarefaction of the entire dataset was undertaken at 10,400 reads per sample using phyloseq on all remaining bacterial reads through random sub-sampling with no replacement. Cyanobacterial-only ASVs were selected after rarefaction for further univariate analysis (richness). After rarefaction, some samples appeared to have no cyanobacteria ASVs because their very low numbers of cyanobacterial reads were removed during rarefaction. Multivariate analyses were therefore undertaken on unrarefied samples, where photosynthetic cyanobacterial reads counts were transformed to relative reads counts (%) per sample.

2.5.7 Defining phases and age models

Pollen and charcoal data were used to assign specific occupation periods which are referred to throughout this study as: Pre-Human (PH), Evidence of Māori Settle-

ment (EMS, from approximately 1300 AD), and Post-European Settlement (PES, from approximately 1840 AD) phases (Supplementary Figs. D.1 to D.5). The main markers for the start of the EMS were peaks in charcoal and bracken fern spores, both of which are indicators of landscape disturbance (McGlone et al., 1999; McWethy et al., 2010). The primary indicator for the start of the PES phase was the appearance of exotic plant pollens such as the Monterey pine (*Pinus radiata*), sheep's sorrel (*Rumex acetosella*), and willow (*Salix* spp.) (Williams et al., 2006). The catchment of Lake Paringa is relatively undisturbed and for this lake the palynology did not provide any useful information on the start or finish of the eras therefore the age model was used to assign these (see below).

Age models from 14C were developed in other studies for the sediment cores of Lake Pounui and Lake Paringa (Cochrane, 2017; Howarth et al., 2012, presented in Supplementary Tables D.4 and D.5) and correlated and applied to the cores collected in this study. The cores from Lakes Rotoehu, Hayes, Johnson, and Wairarapa were not dated. In Lake Rotoehu, an approximately 5-cm tephra layer corresponded to the 1886 eruption of Mount Tarawera provides a defined point in time.

2.5.8 Statistical analyses

Cyanobacteria abundances (number of 16S rRNA gene copies per gram of sediment) from ddPCR data were reconstructed across lakes. The number of photosynthetic cyanobacteria (also now commonly called oxyphotobacteria) ASVs (species richness) from metabarcoding data were calculated and plotted as downcore profiles; the number of species within each Order and Class (photosynthetic or not) were plotted as barplots, and the relationship between cyanobacteria abundance and photosynthetic richness for individual lakes was assessed using scatterplots and Spearman's correlation.

Three binary categories of perturbations were assigned to each sample. The categories were: (1) land clearance as the start of large-scale vegetation disturbance—indicated by peaks of charcoal and/or bracken fern spores, which were in-

dicative of burning, (2) land-use change – characterised by increases in pollen from non-native grasses and/or pine during the PES phase, and (3) presence of carnivorous non-native fish (trout or perch). Catchment-related perturbations were observed in all lakes apart from Lake Paringa, indicated by increased in charcoal, bracken spores, and/or terrestrial non-native pollen (Supplementary Figs. D.1 to D.5). When the sediment cores were dated, the dates of non-native fish introduction in each lake were determined directly or indirectly from historical documentation or oral historical reports (Howarth et al. (2012) for Lake Paringa; Rawhiri Smith pers. comm. for Lake Pounui). Where no dating was available (Rotoehu, Hayes, Johnson, Wairarapa), non-native fish introduction was assumed to be associated with the start of the PES phase.

Generalised least squares (GLS) models were then used to investigate the drivers associated with changes in historical cyanobacteria total abundance and photosynthetic richness. GLS were chosen over other statistical methods to account for the temporal autocorrelation between proximate down-core samples (Simpson, 2018). Five candidate predictor variables were initially considered (Supplementary Table D.6): Phase (three levels: PH, EMS, and PES), Non-native Fish (binary), Native Vegetation Change (binary), Land-Use Intensification (binary) and Lake (six levels: Hayes, Johnson, Paringa, Pounui, Rotoehu, and Wairarapa). Because all predictors were highly correlated and collinear, in particular non-native fish presence and land-use intensification, their effects were summarised by the three phases: PH, EMS—encompassing the beginning of native vegetation clearance, and PES—encompassing non-native fish introduction and land-use intensification. Though not all lakes were affected by these pressures (Lakes Paringa and Pounui have not undergone significant changes in their catchment), this proved to be the best option for modelling purposes. Models were fitted using Phase and Lake as fixed effects and with a continuous first-order autoregressive process (corCAR1), with sub-sample core depth nested within Lake to correct for autocorrelation. Because cyanobacteria abundances consisted of strictly positive and right-skewed continuous data while richness was mildly right-skewed, they were log and

square-root-transformed, respectively. Model assumptions of normality, homogeneity of variances and independence were checked by inspecting normalised residuals, including using autocorrelation function plots. Only photosynthetic cyanobacteria were selected for the multivariate analysis.

Shared ASVs across the dataset, across lakes, across phases, and across phases within lakes were investigated, and visualised using Venn diagrams (Chen et al., 2011). Changes in relative read abundance in the cyanobacterial community structure were plotted for each phase within each lake at genus level using stacked barplots. To further understand specific changes in cyanobacteria versus picocyanobacteria across time and lakes, all ASVs identified at Genus level were assigned to a cell diameter factor: either >3 µm, or <3 µm (picocyanobacteria), as a well as a toxin category (none or potentially toxic, Supplementary Table D.7). Community changes at genus level were visualised with barplots and aligned with dendrograms using the packages rioja (Juggins, 2020) and ggdendro (Vries et al., 2022). Variations in ASV community structure were then visualised using principal coordinates analyses ordination plots (PCoA) based on Bray–Curtis dissimilarities using the vegan package (Oksanen et al., 2019). Difference in community structure were explored using a distance-based permutational analysis PERMANOVA (Anderson, 2001) based on Bray–Curtis dissimilarities of the relative abundance community data and 999 permutations. The experimental design consisted of Lake and Phase as fixed factors (Anderson, 2006). Significant terms were then investigated using pair-wise comparisons with the PERMANOVA t-statistic and 999 permutations. Tests for homogeneity of multivariate dispersions based on Bray–Curtis distances (Anderson, 2006) were used to compare distances to the multivariate centroids between Lake, and between Phase within individual lakes.

2.6 Results

2.6.1 General richness patterns

A total of 21,545 bacterial ASVs were recovered from the 208 samples sequenced. Of these, 1361 corresponded to all cyanobacteria and 1199 to photosynthetic cyanobacteria. After rarefaction (10,400 reads), a total of 1144 cyanobacterial ASVs remained, of which 928 were photosynthetic cyanobacteria. Several samples were discarded because they either did not have enough sequence reads to meet the rarefaction threshold (Supplementary Fig. D.6, two samples), or they did not have any cyanobacterial reads following rarefaction (thirteen samples), or any photosynthetic cyanobacterial reads (further five samples).

Cyanobacterial ASVs were assigned to two different classes: Vampirovibrionia (previously Melainabacteria candidatus (Soo et al., 2014), non-photosynthetic cyanobacteria) and Cyanobacteria (photosynthetic cyanobacteria). Cyanobacteria ASVs were found in every lake and were amplified in 193 instances in the rarefied dataset (i.e., 93.7% of investigated [n = 206] samples). There were no common photosynthetic cyanobacteria ASVs across all samples, with only three ASVs belonging to the genus *Cyanobium* found in all lakes. When aggregating all samples by phase, there were 34 shared ASVs across the three phases (PH, EMS, PES), belonging to the genera *Cyanobium* (25 ASVs), *Dolichospermum* (2 ASVs), *Microcystis* (4 ASV), *Synechocystis* (1 ASV), and 3 ASVs unassigned at Genus level (1 unknown Nostocales, 1 unknown Cyanobacteriales and 1 unknown Nostocaceae). An assessment of individual lakes showed that in Lakes Hayes and Johnson a single ASV was found in all samples (a different one in each lake), taxonomically assigned to the genus *Cyanobium*. In all lakes the highest number of shared ASVs was between the PES and EMS phases (Supplementary Fig. D.7). Lake Wairarapa was the only lake that did not have a core community across all phases (Supplementary Fig. D.7). Shared taxa differed from one lake to the other, though they belonged mainly to the picocyanobacteria genus *Cyanobium*. Total Cyanobacteria, Vampirovibrionia and

(photosynthetic) Cyanobacteria ASVs varied among lakes, with the highest numbers recorded in Lake Johnson (Table 2.2). A total of 47.8% cyanobacterial ASVs were unclassified at Genus level (493 out of 1031), including 297 ASVs that corresponded to photosynthetic cyanobacteria.

Table 2.2: Cyanobacterial Amplicon Sequence Variants (ASVs) per lake. Results are given for total cyanobacteria and per Class.

	Paringa	Pounui	Rotoehu	Hayes	Johnson	Wairarapa	Total
Number of samples	29	36	29	31	37	30	187
Total Cyanobacteria	116	134	254	272	359	142	1,034
Cyanobacteria	80	115	225	223	280	129	839
Vampirovibrionia	36	19	29	49	79	13	195

The majority of Vampirovibrionia ASVs could only be assigned at Order level (Caenarcaniphilales, Gastranaerophilales, Obscuribacterales, and Vampirovibrionales). Vampirovibrionia ASVs were found in every lake but only occurred in 104 out of the 193 samples and their richness differed greatly between lakes (>40 ASVs in Lake Hayes and Johnson and <40 ASVs in the other lakes, Supplementary Figs. D.8 and D.9). The Order Gastranaerophilales was the most common (7–72 ASVs per lake), followed by Obscuribacterales (4–11 ASVs per lake). The other two Orders (Caenarcaniphilales and Vampirovibrionales) were poorly represented and were not found in all lakes (Supplementary Fig. D.9). Due to the lack of ecological knowledge on Vampirovibrionia, no further analysis was conducted.

The rarefied cyanobacterial photosynthetic dataset was composed of 8 orders, 21 families, and 51 genera across all lakes and all samples. The most common Cyanobacteria Order was Synechococcales, followed by Cyanobacteriales (formerly Nostocales; Supplementary Fig. D.8). The number of Synechococcales ASVs was variable across lakes by an order of ten (55–153 ASVs per lake), and it was similar for Cyanobacteriales (14–120 ASVs per lake). All other photosynthetic cyanobacteria Orders were poorly represented (≤4 ASVs per lake) and were not detected in all lakes.

2.6.2 Cyanobacterial richness and abundance profiles

The richness (number of ASVs) of photosynthetic cyanobacteria increased significantly in all lakes during recent times compared to their historical levels (Figure 2.2). Overall, Lakes Hayes and Johnson had the highest richness and Lake Wairarapa the lowest (Figure 2.2 and 2.4). Shifts in richness after European arrival were particularly notable in lakes known to currently experience cyanobacterial blooms (Hayes, Johnson, Pounui, Rotoehu). This was particularly obvious in Lake Rotoehu (PH = 0–6 ASVs, PES = 9–65 ASVs), while Lakes Hayes and Johnson displayed a very high richness in recent times (80–90 ASVs at the top of the core). Despite having moderate 'European land-use' in its catchment, Lake Pounui also showed an increase in richness. Timing of shifts were different for Lakes Pounui (1̃900s) and Paringa (1̃700s). No specific dates could be approximated for Lakes Rotoehu, Hayes, Johnson, and Wairarapa due to the absence of age models, though the increase in richness coincided with land-use intensification in Lake Johnson. The richness profile of Lake Wairarapa was more consistent with the timing of phases, with a slight increase to 8 ASVs on average after EMS until near the top of the core, with a marked increase in the top sample (43 ASVs).

Cyanobacterial abundance (16S rRNA gene copy numbers per gram of dry sediment) also increased in all lakes during recent times compared to historical levels (Figure 2.3). These increases were particularly prominent in Lakes Johnson and Pounui (up to 6̃,000 times their historical levels), medium in Lakes Hayes, Rotoehu, and Paringa (around 500 times their historical levels), and a slight increase in Lake Wairarapa, which was mostly in the surface sample. Timing of shifts in cyanobacterial abundance were different for all lakes: Lake Paringa suddenly shifted in the mid 1900's, Lake Pounui underwent a steady though steep increase post European arrival, while Lake Hayes and Johnson steadily increased with land-use intensification. Increase in abundance were more recent for Lakes Rotoehu and Wairarapa (top of the cores). Lastly, though the sediment core of Lake Hayes did not include the PH phase, its levels of cyanobacterial abundances during EMS were up to 1000

times higher than in the other lakes at the same time (Figure 2.3 and 2.4).

When all lakes were combined, richness was positively correlated with 16S rRNA cyanobacterial gene copy numbers ($r = 0.74$, $p < 0.001$). Relationships were also significant when each lake was analysed individually (from $R = 0.49$ for Lake Wairarapa to $R = 0.82$ for Lake Pounui, all $p<0.01$; Supplementary Fig. D.10).

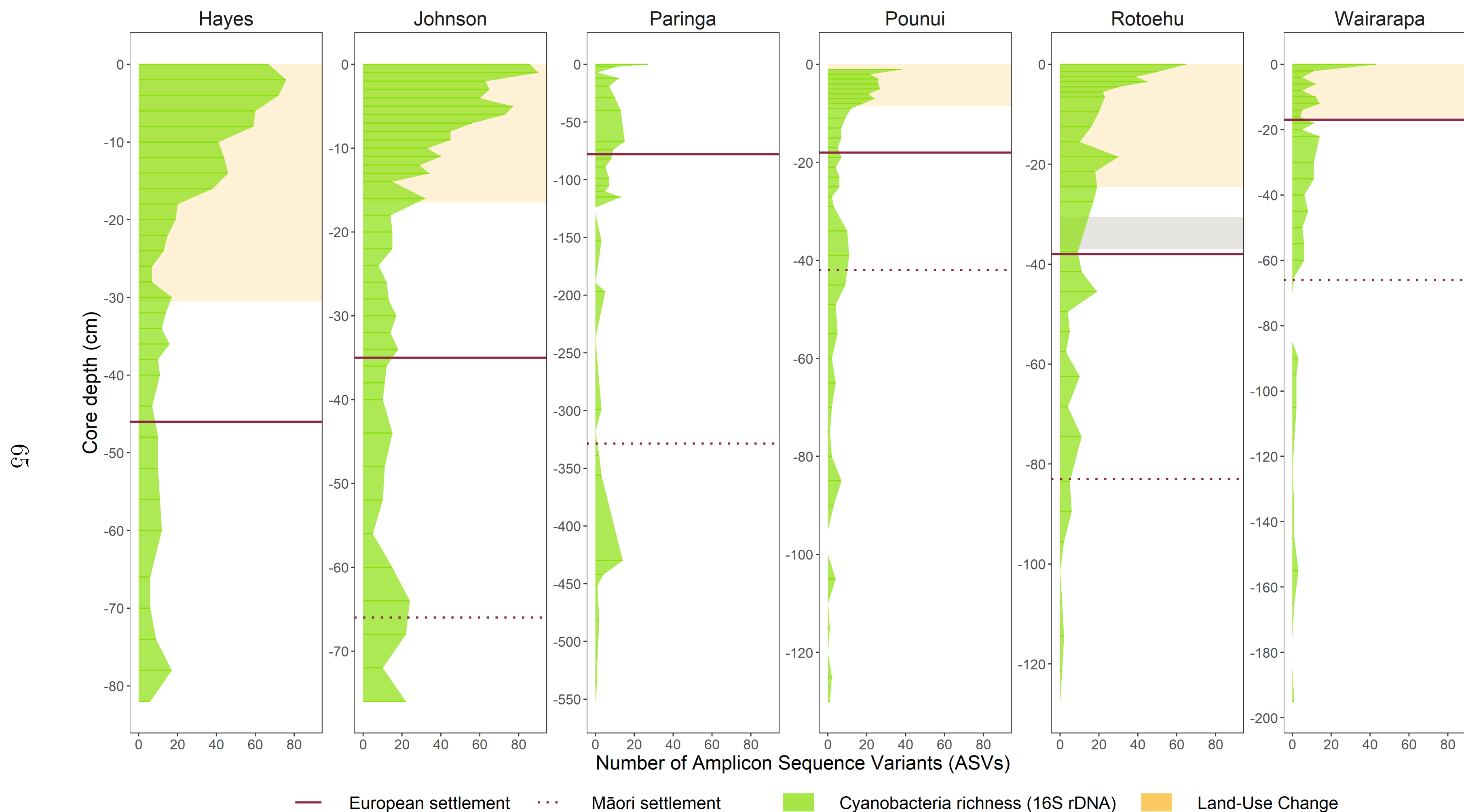

Figure 2.2: Richness of photosynthetic cyanobacterial Amplicon Sequence Variants (ASVs) from metabarcoding data, cyanobacterial 16S rRNA gene. The horizontal lines separate the three phases, pre-human (PH; bottom), evidence of Māori settlement (EMS; middle), and post European settlement (PES; top); note: the sediment core from Lake Hayes does not include any PH phase. Yellow shades indicate the depths associated with land-use intensification in relevant lakes and the grey shade indicates the 1886 Tarawera tephra layer in Lake Rotoehu.

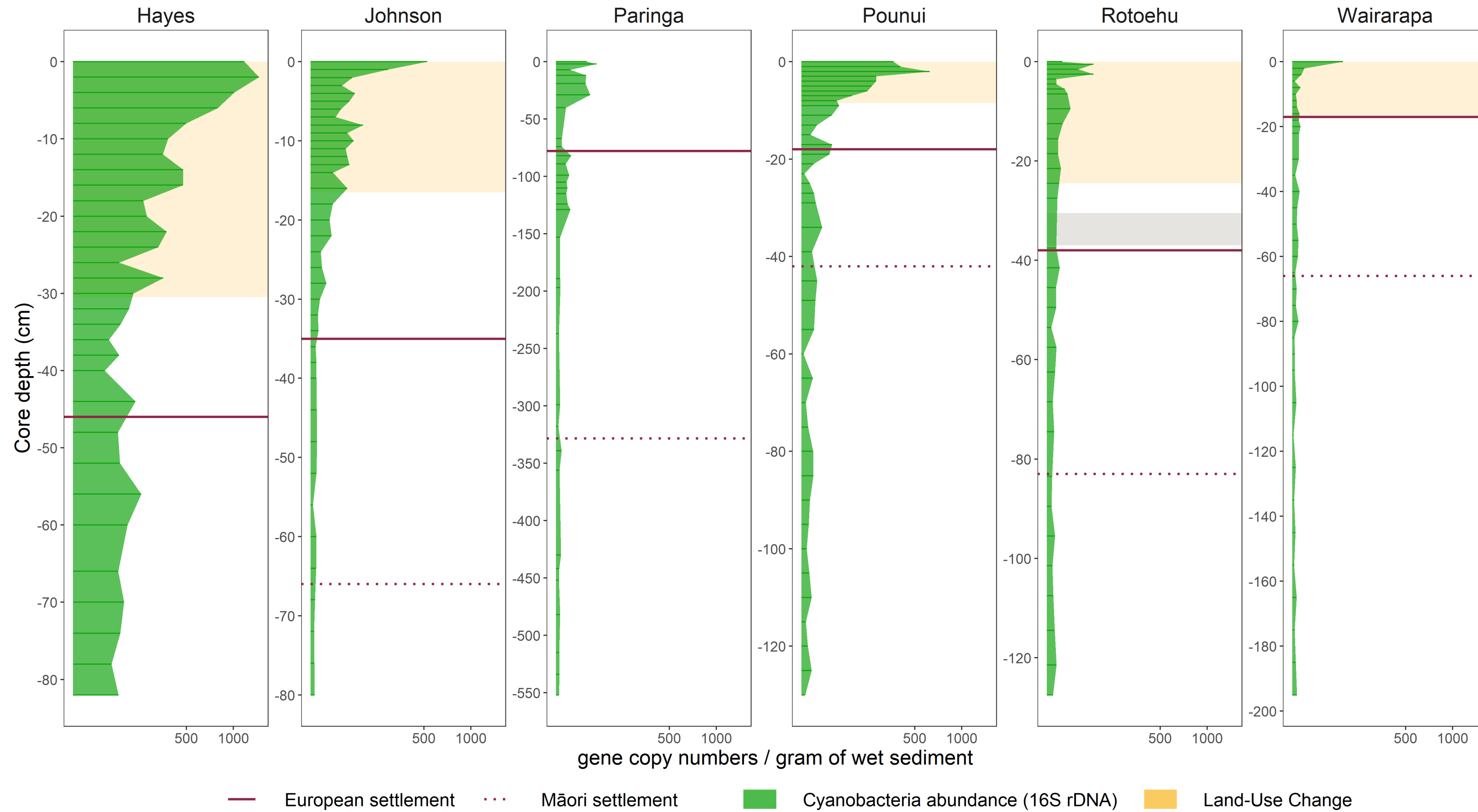

Figure 2.3: Cyanobacterial concentrations from droplet digital PCR data. Sediment depth profiles of cyanobacterial 16S rRNA gene copy numbers per gram of dry sediment, y-axis shown on square-root scale. The horizontal lines separate the three phases, pre-human (PH; bottom), evidence of Māori arrival (EMS; middle), and post European arrival (PES; top); note: the sediment core from Lake Hayes does not include any PH phase. Yellow shades indicate the depths associated with land-use intensification in relevant lakes, the grey shade indicates the 1886 Tarawera tephra layer in Lake Rotoehu.

2.6.3 Drivers of shifts in cyanobacterial community

Both cyanobacteria abundances and photosynthetic richness tended to increase with land clearance, land-use change, and the introduction of non-native (carnivorous) fish (Figure 2.4). Generalised least squares (GLS) models only incorporated Phase and Lake since the other predictors were highly correlated. These models showed a significant effect of Phase on cyanobacteria abundance and richness ($p<0.01$); compared to EMS, richness and abundance were significatively higher during PES and lower during PH (Table 2.3, (Figure 2.4). When comparing all lakes to Lake Paringa (the most pristine lake), GLS models revealed that all lakes had a significantly higher abundance than Lake Paringa apart from Lake Wairarapa which had a significantly lower abundance (Table 2.3, (Figure 2.4). Only Lakes Johnson and Hayes had a significantly higher richness (Table 2.3). When comparing lakes to one another with pairwise comparisons, the models revealed that overall, Lakes Hayes, Johnson and Pounui had a significantly higher 16S rRNA gene copy abundance than Lakes Paringa and Wairarapa (Supplementary Table D.8), while Lake Johnson had a significantly higher richness than Lakes Paringa and Wairarapa (Supplementary Table D.9).

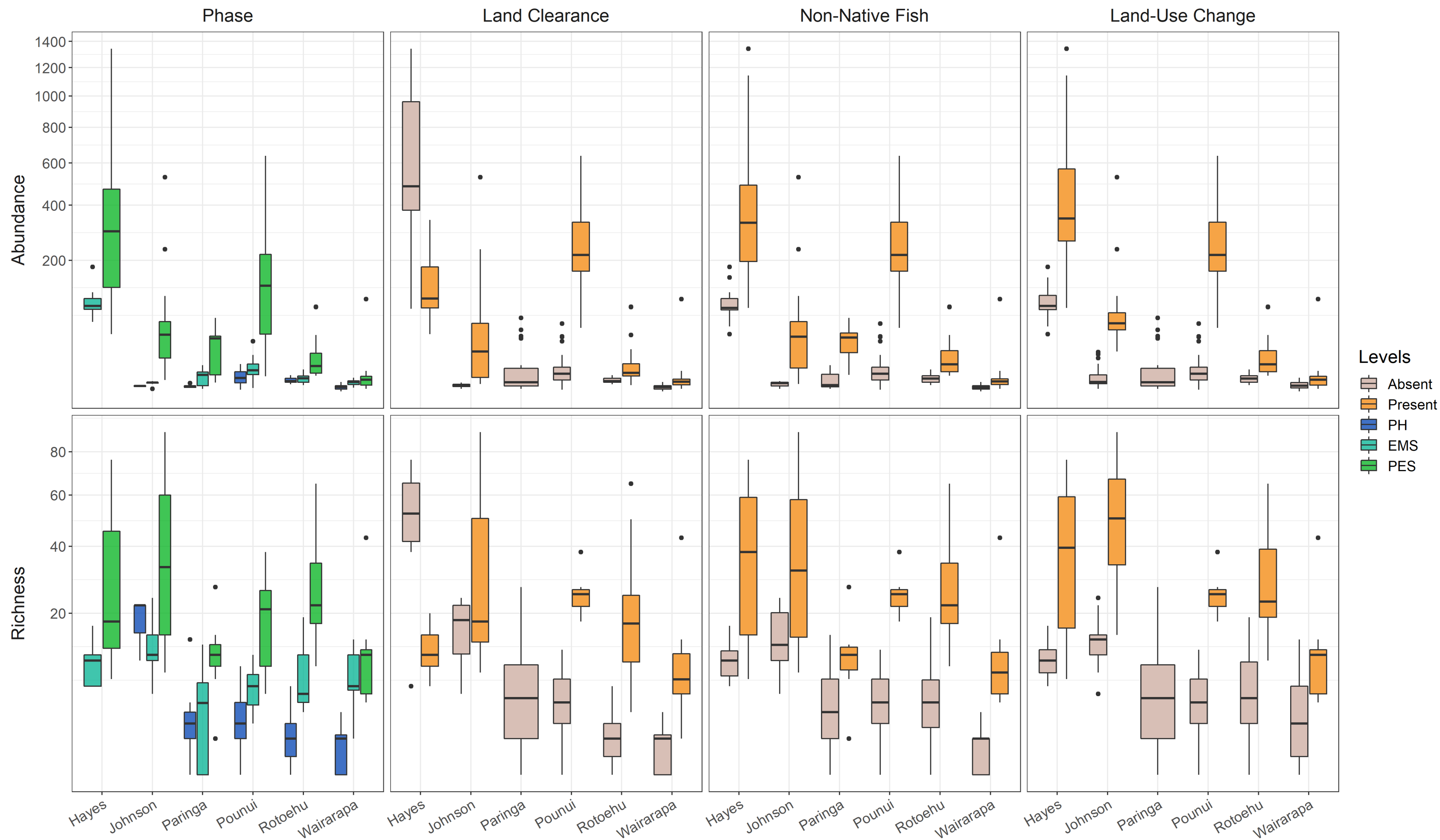

Figure 2.4: Differences in each response (cyanobacteria abundance or richness) depending on the lake and predictor variable. Cyanobacteria abundance was measured by droplet digital PCR (ddPCR–16S rRNA gene copy numbers/gram of dry sediment, log10 y-axis scale) while cyanobacteria richness was calculated from metabarcoding data (number of photosynthetic cyanobacteria Amplicon Sequence Variants per sample, square-root y-axis scale). PH = Pre-Human, EMS = Evidence of Māori Settlement, PES = Post European Settlement.

Table 2.3: Results of the generalised least squares (GLS) models for cyanobacteria abundance (from droplet digital PCR data) and richness (from metabarcoding data). PH = pre-human, EMS = evidence of Māori settlement, PES = post European settlement. PES and PH were compared to EMS since the Lake Hayes core does not have a PH phase, while all lakes were compared to Lake Paringa (most pristine lake). Significant p values ($\alpha < 0.05$) are shown in bold.

	log(Abundance)			sqrt(Richness)		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	12.91	12.32–13.49	<0.001	1.98	1.29–2.67	<0.001
Phase PES	1.64	1.04–2.24	<0.001	1.04	0.39–1.69	0.002
Phase PH	-1.13	-1.75– -0.51	<0.001	-1.05	-1.77– -0.32	0.005
Lake Pounui	4.28	3.32–5.25	<0.001	0.62	-0.49–1.73	0.27
Lake Rotoehu	1.62	0.68–2.57	0.001	0.90	-0.21–2.01	0.11
Lake Johnson	1.44	0.60–2.28	0.001	2.57	1.27–3.87	<0.001
Lake Hayes	1.13	0.29–1.97	0.009	1.76	0.47–3.05	0.008
Lake Wairarapa	-1.08	-1.86 – -0.30	0.007	0.21	-0.79–1.20	0.68
Observations		209			206	

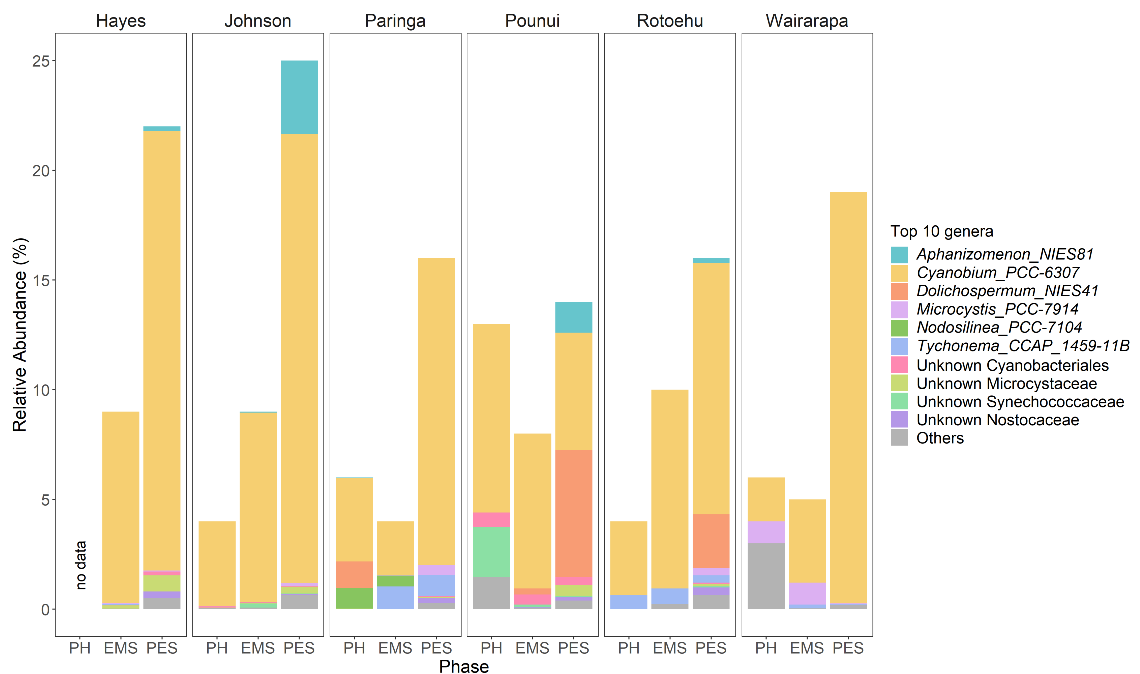

Figure 2.5: Relative abundance of the top 10 most abundant photosynthetic cyanobacterial genera across all lakes, less abundant genera are grouped as "Others". All samples were grouped by Phase within each lake. PH = Pre-Human; EMS = Evidence of Māori Settlement; PES = Post-European Settlement. The sediment core from Lake Hayes does not include any PH phase.

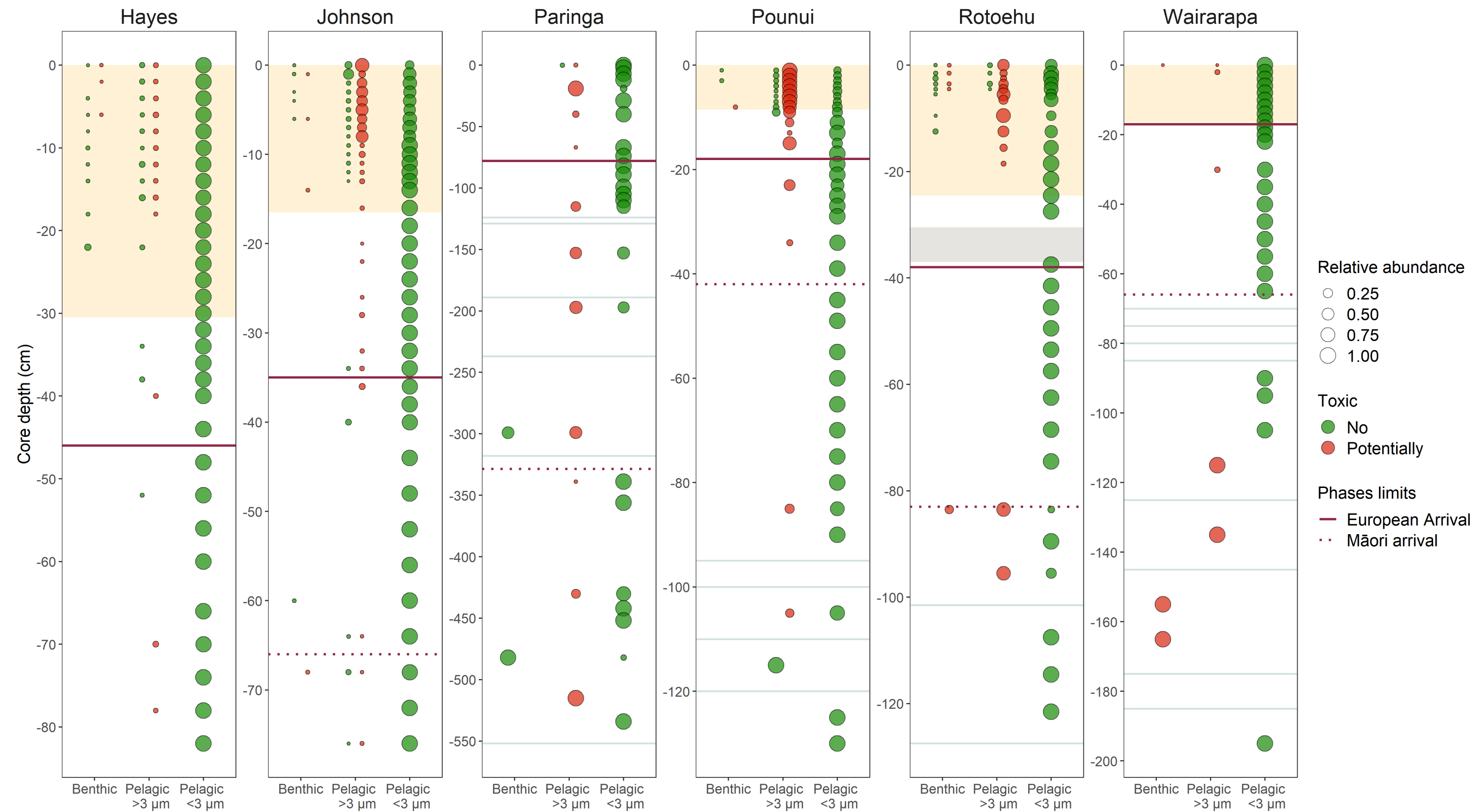

Figure 2.6: Relative abundance profiles of photosynthetic cyanobacterial genera classified by size (>3 µm or <3 µm—picocyanobacteria) and potential toxin production. Relative abundance is plotted as a continuous variable and the legend gives an indication of size for a set of abundances. Potential for toxin production of each genus was inferred from the literature. Phase delimitation is indicated by horizontal purple straight and purple dotted lines (see legend). Horizontal grey lines indicate samples with no cyanobacteria sequences after rarefaction. Yellow shades indicate land-use intensification in relevant lakes. Grey shade in the Lake Rotoehu core indicates the Tarawera tephra (1886).

2.6.4 Historical and spatial variability in cyanobacteria community structure

Photosynthetic cyanobacterial reads were selected from the unrarefied dataset for the remainder of the analyses (n = 190 samples). *Cyanobium*, a picocyanobacterial genus, dominated the cyanobacterial community in almost all lakes and phases (Figure 2.5 and 2.6). Other picocyanobacteria genera, such as *Synechocystis*, were present intermittently in low abundance in Lakes Pounui, Hayes, and Johnson (mainly PES). The main non-picoplanktonic genera were the potential toxigenic *Aphanizomenon*, *Microcystis*, and *Dolichospermum* and these became dominant in Lakes Johnson and Pounui in in the PES phase (Figure 2.5 and 2.6). These were found in at least one sample in most lakes. *Aphanizomenon* and *Microcystis* were found in all lakes and mostly in the PES phase; *Dolichospermum* was restricted to Lakes Pounui (PES), Rotoehu (PES), Johnson (PES) and Paringa (PH), although relative abundances differed greatly among lakes. Other relatively common pelagic genera were only found in specific lakes: *Geminocystis* in Lake Hayes (PES), Johnson (PES and PH), and Rotoehu (PES), *Tychonema* in all lakes except Lake Pounui. Some benthic taxa were also identified, such as *Phormidium* and *Geitlerinema* in Lake Wairarapa, *Calothrix* in one sample from Lake Rotoehu (EMS), *Nodosilinea* in Lakes Paringa (EMS + PH, one sample each), Johnson (PES), and Wairarapa (PES, one sample), and *Oscillatoria* in Lake Paringa (EMS). Some potentially interesting taxa (high enough relative abundance) could unfortunately not be identified down to genus level, especially in Lakes Pounui and Hayes (Unknown Cyanobacteriales, Microcystaceae, and Synechoccaceae, Fig. 5). Overall, toxic taxa were present in all lakes prior to human arrival, however their relative abundance increased in Lakes Hayes, Johnson, Pounui, and Rotoehu in the PES phase. They became continuously occurrent from European arrival in Lakes Pounui and Johnson, and shortly after land-use intensification in Lakes Rotoehu and Hayes. In general, picocyanobacteria relative abundance remained constant, although slight decreases were observed in Lakes Johnson and Pounui as potentially toxic taxa became dominant (Fig. 6,

Supplementary Fig. D.11). The CONISS analysis showed that timings of shifts in the community composition varied depending on the lakes, though the main shift seemed to coincide with land-use intensification for Lakes Rotoehu, Hayes, and Johnson (Supplementary Fig. D.11).

The PERMANOVA test showed that there were significant differences in community structure across lakes ($F_{189,5}$ = 11.726, p<0.01), and pairwise comparisons revealed that all lakes were significantly different from one another (p<0.01). The test for homogeneity of multivariate dispersions showed that these differences could be due to differences in the mean distance from lake centroids among lakes ($F_{F184,5}$ = 7.5613, p<0.001). The PCoA ordination revealed three distinct clusters: Hayes and Johnson; Pounui, Paringa and Rotoehu; and Wairarapa by itself (Figure 2.7A). Only Lake Hayes's dispersion was significantly different from Lakes Rotoehu, Paringa, and Pounui (Tukey's post-hoc test on homogeneity of multivariate dispersion, p<0.05).

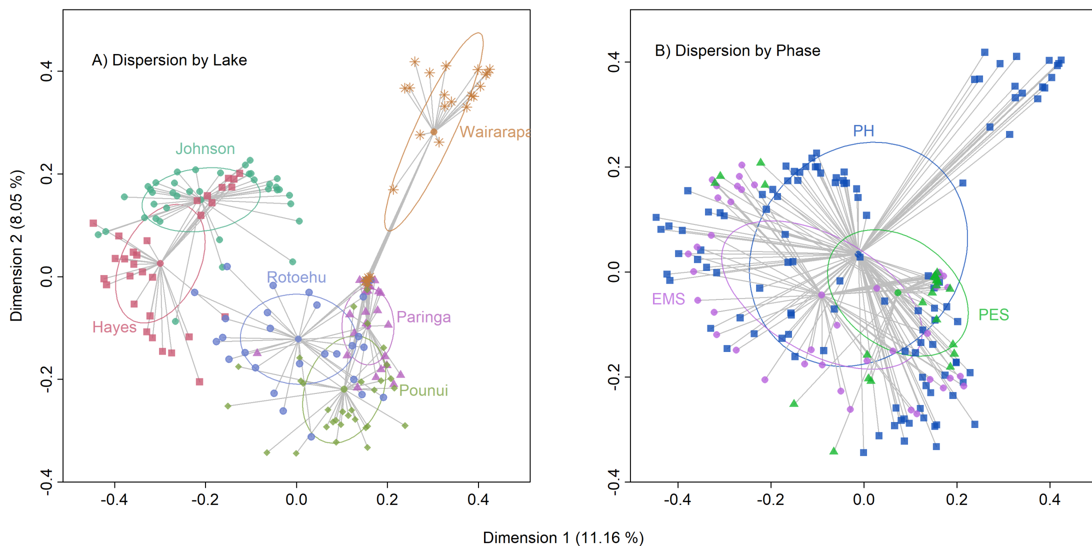

Figure 2.7: Principal coordinates analysis (PCoA) ordination plots of cyanobacterial communities based on Bray–Curtis dissimilarities for all six study lakes (**A**) and all phases (**B**). The ellipses indicate the mean distance from centroids, whereas the vectors show the distance to the centroid of each group.

When data from all lakes was combined, there was no clear evidence of a differentiation of cyanobacterial assemblages by phase (Figure 2.7B). When each lake was analysed individually, phases tended to be quite distinct and significatively dif-

ferent from one another (Supplementary Fig. D.12, Supplementary Tables D.10 and D.11). All phases were different from one another in Lakes Pounui, Rotoehu, Hayes and Johnson; the PH phase was not different from EMS only in Lakes Paringa and Wairarapa (Supplementary Table S10). While some of the results may be affected by heterogeneous dispersion (Supplementary Table D.11), the data indicate that the cyanobacterial community changed Post-European settlement (PES vs. EMS) in all lakes except Lake Pounui and Lake Rotoehu (Supplementary Fig. D.11, Supplementary Tables D.10 and D.11).

2.7 Discussion

2.7.1 General patterns

We inferred a large diversity of cyanobacteria present within our study lakes. Interestingly, many ASVs were identified as picocyanobacteria (small—usually less than 3 µm, unicellular cyanobacteria), and in particular *Cyanobium*, regardless of lake or phase. It is unclear whether their high numbers are due to; (1) primer biases (i.e. the primers preferentially bind to these taxa and/or these taxa have higher 16S rRNA copies in the genome compared to other cyanobacteria), (2) more variability in their 16S rRNA sequences (which resulted in our bioinformatics pipeline separating them as different ASVs), (3) better cell or DNA preservation, or (4) if they are actually present in high concentrations in the environment. It is also possible that some cyanobacteria sequences are wrongly assigned as *Cyanobium* in the SILVA database, leading to an over-representation of this genus in our results. Given their small size and difficulties with identifying them, it is also highly likely they are commonly overlooked in traditional light microscopy analysis of lake samples (MacKeigan et al., 2022). A study of cyanobacterial diversity in 143 New Zealand lakes identified an equally high number of operational taxonomic units and associated sequence reads of picocyanobacteria and suggested that this group has been overlooked in most limnological studies in this country (Wood et al., 2017). Other recent ecology

studies (Becker et al., 2007; Sánchez-Baracaldo et al., 2008) have highlighted similarly diverse freshwater autotrophic picoplankton communities and suggested a need for further characterisation with particular regard to their response to environmental change. Although relative abundances of picocyanobacteria remained relatively constant across phases, there were differences in picocyanobacteria structure among lakes and between phases at the ASV level. Because so little is known about the ecology of these taxa, it is not feasible to make ecological inferences based on these shifts. Enhancing knowledge on their ecology would greatly assist in understanding the observed changes and may allow these taxa to be used as early indicators of change in lake ecosystems.

Potentially toxic genera and/or well-known bloom-forming species (e.g., *Microcystis, Dolichospermum, Aphanizomenon*, or *Phormidium*) were present in all lakes including before human arrival (except Lake Hayes, no pre-human period reconstructed). This corroborated previous findings that potentially toxic cyanobacteria have been present historically in lakes, albeit in low abundance or sporadically (Zastepa et al., 2017; Pilon et al., 2019). However, while bloom-forming species were occasionally common in some samples in pre-human times according to the metabarcoding data, this does not necessarily mean they were actually abundant in the lake. Rather, this could be an artifact due to few cyanobacteria sequences in the samples, since metabarcoding relies on relative abundance. This is reinforced by the ddPCR data which does not indicate high abundance of cyanobacteria in the pre-human period. Furthermore, shifts to dominance by potential bloom-forming species were only observed in recent times in lakes that are presently eutrophic (Pounui, Rotoehu, Hayes, Johnson), a trend which was reflected by an increase in total cyanobacterial abundance in the same lakes. Lake Wairarapa was the only exception, having general low levels of cyanobacteria despite its current supertrophic status.

Some non-photosynthetic cyanobacteria (Vampirovibrionia Class) were identified by the metabarcoding data. They mostly occurred in the top half of the cores, and could generally only be identified at Order level. It is likely that they were also detected by the ddPCR assay, and they may account for some of the observed in-

creases in abundance and richness. Further research is needed to enhance knowledge on their ecological role.

2.7.2 Lake-specific cyanobacteria histories

Total cyanobacterial abundance and richness significantly increased in all six study lakes over the 500–1500 years reconstructed (depending on the sediment core). However, it was most evident after the arrival of Europeans in Lakes Hayes, Johnson, Pounui, and Rotoehu. Although the GLS models could not specifically test the relationship between land-use and cyanobacteria response, the exploration of the data suggests that both abundance and richness increased significantly post European settlement, particularly for Lakes Hayes and Johnson. Palynology data from the cores used in this study shows that there was a marked decrease in native forests in the catchments of Lakes Hayes, Johnson, and Rotoehu, coinciding with the periods of increasing cyanobacterial abundance.

All lakes presented in this study have some historical environmental/social records, though the degree of knowledge varies across lakes (summarised in Supplementary Table D.12). In Lake Hayes, cyanobacteria abundance has increased steadily since European settlement (1862, *Queenstown's Pioneering Beginnings* 2017) and especially since the early 1900s with the conversion of much of the lower catchment into sheep pasture. Furthermore, superphosphate fertiliser application began in the 1950s; by 1961 there had been significant drainage of the wetlands in the catchment, and by the 1970s Lake Hayes was considered eutrophic. This increase in trophic state aligned with the first reports of cyanobacteria blooms (1970–1972, *Dolichospermum flos-aquae*, Burns et al., 1974), and our own observations of potentially toxic cyanobacteria in the second half of the Post-European Settlement phase (PES, top 20 cm of the core). Similarly, the catchment of Lake Johnson, near Lake Hayes, was spared from urban development, but forest clearing from c. 1895 led to pasture development and application of superphosphate fertiliser from c. 1955 (Burns et al., 1974). Subsequently, potentially toxic taxa appear continuously in the sedi-

ment core with European settlement ($\tilde{4}5$ cm), and in higher proportion in the top 8 cm. Cyanobacterial richness and abundance both increased with land-use intensification, which may be related to superphosphate application (1955). Lake Rotoehu, the northernmost lake of this study, receives phosphorus-rich waters from geothermal input(s), has numerous shallow arms and generally quite warm waters (min 8 °C, max 22 °C, Fish, 1970). Its name in Te Reo Māori translates as "murky/cloudy (ehu) lake (roto)", which could suggest that phytoplankton biomass might already have been high when Māori were living around the lake. However, the data in the present study indicate that if this was the case, it was likely not due to cyanobacteria, as historic levels were relatively low. The first cyanobacterial blooms were observed in the 1960s, coinciding with the first changes in land-use (forest and scrubs converted to pasture, Fish, 1970; *Lake Rotoehu – Lakes Water Quality Society* 2021). In the 1960–70s, Lake Rotoehu was considered mesotrophic but a marked decrease (4.2 m) in lake level in 1993 is thought to have increased nutrient levels and possibly enhanced cyanobacterial blooms, which have occurred on an annual basis since then (Bay of Plenty Regional Council et al., 2007). While we do not have any dates for the core of Lake Rotoehu apart from the tephra (1886, 30.5 to 37 cm), potential bloom-forming species also appear continuously from 18.5 cm to the top.

The results from these three lakes are in agreement with previous studies that have indicated a link between land use change, increased nutrient runoff, and cyanobacterial blooms and associated toxins. For example, in Baptiste Lake (Alberta, Canada) an increase in microcystin gene copies was correlated with nutrient enrichment (Zastepa et al., 2017). Similarly, increases in agriculture in the surrounding landscape of Anderson Lake (Washington, USA) were linked with the first appearance of toxic *Dolichospermum* blooms (Hobbs et al., 2021), and saxitoxin gene copies increased in Laguna Blanca (Maldonado, Uruguay) after forestry developed in its catchment (Escalera et al., 2014). Further work is required to fully identify the drivers of change as the definition of land-use intensification was categorical (presence/absence) rather than a quantitative measure of change in the catchment and was highly colinear with non-native fish introduction. The analysis would be enhanced by including

more lakes and more variables that were not available for all the lakes studied.

In contrast to the previous three lakes, Lake Pounui provides an interesting anomaly to the proposed link between land-use change and the development of cyanobacterial blooms. Palynological data (Cochrane, 2017) and historic knowledge (*Retrolens - Historical Imagery Resource* 2021) suggest that land clearance only occurred within a part of the catchment and mostly stopped after a few decades. Aerial photographs show that land clearing in the southern side of the catchment had already occurred in 1941 (*Retrolens - Historical Imagery Resource* 2021), but the native forest had mostly regenerated by 1961. A possible link to the severity of cyanobacteria blooms in this lake is the introduction of rainbow trout (*Oncorhynchus mykiss*) and the European perch (*Perca fluviatilis*) into Lake Pounui and associated streams and lakes in the wider catchment, from 1938 and the 1960s, respectively (Fish and Game NZ, historic records; Rawhiri Smith, pers. comm.).

Non-native fish can disrupt native food webs (Strayer, 2010; Hall et al., 2000) and have a top-down impact on cyanobacteria (Gehrke et al., 1994). Pelagic food webs are quite simple in New Zealand, as they are lacking strongly piscivorous fish (Burns et al., 1998; Rowe, 2004). Voracious zooplanktivorous fish such as juvenile perch are absent from the indigenous fauna, and when introduced, they can effectively shift zooplankton communities so that smaller species (e.g. rotifers) become dominant, thus reducing grazing pressure on phytoplankton (Gliwicz et al., 1989). *Perca fluviatilis* have few effective predators in New Zealand, albeit they are cannibalistic, and, as such, their populations are often stunted and mostly composed of juveniles, therefore enhancing zooplanktivory and reducing grazing on phytoplankton (Vanni et al., 1990). Brown trout (*Salmo trutta*) and perch were introduced to Lake Hayes in the 1870s, while for Lake Johnson, perch was introduced in the 1880s and rainbow trout have been stocked annually since 1962. Lake Rotoehu also contains rainbow trout, which were introduced between 1898 and 1903 (McKinnon, 2015). Only a handful of studies have explored the trophic effects of perch (*P. fluviatilis*) introductions in New Zealand (Smith et al., 2006; Smith et al., 2007), and more detailed food-web related research is required to fully understand the wider

impacts of the introduction of these species on cyanobacterial blooms in these lakes.

Analysis of the metabarcoding data was required to further understand the recent increases in cyanobacterial abundance in Lakes Paringa and Wairarapa. The vegetation in the catchment of Lake Paringa is relatively unmodified (Howarth et al., 2012), therefore we anticipated there would be no change in cyanobacterial abundance at this site. Conversely, the catchment of Lake Wairarapa has been highly modified (Bunny et al., 2014; Trodahl et al., 2016) but only very sporadic and localised cyanobacterial blooms have been reported (e.g. *Dolichospermum lemmermannii* in 2008, Bunny et al., 2014). Lake Wairarapa is a very shallow lake with constant sediment resuspension; therefore any labile material could be thoroughly degraded before it settled down on the lakebed. This might have an impact on sedaDNA recovery and analysis. The metabarcoding data indicated that total increase in cyanobacteria copy numbers in these two lakes was due to picocyanobacteria. Picocyanobacteria are abundant in the oceans and often in oligotrophic lakes, but more recently they have also been reported in high numbers in eutrophic lakes, particularly coastal lakes and lagoons (Caroppo, 2015; Pulina et al., 2017). Picocyanobacteria are sensitive to high light intensities103, and the low-light conditions in both Lakes Paringa and Wairarapa (respectively brown waters due to tannins, and turbid waters from sediment resuspension) could potentially explain their abundance and dominance in these lakes. The increase in abundance in picocyanobacteria in Lake Paringa occurred in the 1960s. This coincides with the introduction of *S. trutta* (brown trout) in the 1950s, and the construction of a road in 1958 along the south-eastern side of the lake, thus enhancing human access to the lake. The dramatic increase in picocyanobacteria communities in the top 3 cm of the core in Wairarapa could suggest this is a recent change, possibly representing a further decline in the health of this lake.

2.7.3 Other potential drivers of change

In addition to the impact of land-use change, and non-native fish introduction, many other factors impacted these lakes. For example, Lake Wairarapa have been significantly affected by hydrological changes, such as changing the main inflow away from the lake in the 1960s. Earthquakes can also have significant impacts on lakes, potentially triggering landslides, especially in mountainous terrain (Keefer, 2002; Fan et al., 2019). Landslides increase sediment yields directly in the lake, and tributaries flowing in to the lakes. The Alpine fault line near Lake Paringa has a magnitude 8 earthquake approximately every 300 years (Howarth et al., 2012), with at least four earthquakes occurring during the time period covered by the sediment core. Based on the results in this study, these earthquakes do not always appear to align with major shifts in cyanobacterial abundance or composition in Lake Paringa. One exception seems to be the 1717 Alpine fault earthquake, which is followed by a slight increase in cyanobacteria abundance. In 1855, a major earthquake (est. M 8.2, Manighetti et al., 2020) struck the Wairarapa region where Lake Wairarapa and Lake Pounui are situated, as well as smaller one in 1942 (max M 7.2 and 6.8, Mcsaveney et al., 1989). In Lake Pounui, these events were concomitant with the discrete appearance of *Synechococcus* after these two events, and post 1855 there was a shift to *Dolichospermum* which is now dominating the cyanobacteria community. However, Europeans also started more intensive use of the land in the catchment around the same time and it is therefore not possible to correlate these changes in community structure with earthquakes specifically.

The impact of climate change was not assessed in this study because its timing overlaps with the main anthropogenic stressors studied here, making it extremely challenging to tease apart the impacts of climate change from other stressors. Further proxies which provide more detailed indications of climate change are required. Furthermore, focusing on lakes not affected by land-use, such as high-altitude alpine lakes, would yield more significant results. It is likely that climate change is enhancing the blooms. In New Zealand, average air temperatures have risen by 1.13 °C (±

0.27) from 1909 to 2019 nationally and there are more extreme, storm/rainfall events and longer periods of droughts since the beginning of the twentieth century (*Our atmosphere and climate* 2020). It is extremely unlikely that this change in temperature alone is responsible for driving the changes in cyanobacterial abundance and composition observed in this study. If it was the case, blooms would be observed in Lake Paringa despite no major catchment changes, which is not the case.

2.7.4 Caveats when interpreting sedimentary DNA data

Although sedaDNA approach are now increasingly used in paleolimnology, there are a number of caveats that need to be considered when interpreting the data. Many of these are well acknowledged challenges with the use of molecular approaches for analysing eDNA from a variety of sample matrices including primer biases, appropriate selection of universal (or targeted) primers and incomplete reference databases (Capo et al., 2021a; Beng et al., 2020; Freeland, 2016).

One issue that requires further investigation in paleolimnological studies is DNA degradation. Degradation leads to DNA fragmentation; however it is currently unknown how quickly this occurs. Studies on contemporary samples indicate that amongst others, hight amounts of light (UV irradiance), high temperatures, acidic and oxic conditions contribute to DNA decay (Barnes et al., 2014; Barnes et al., 2020; Corinaldesi et al., 2008; Eichmiller et al., 2016; Strickler et al., 2015; Seymour et al., 2018). A recent study suggests that sedaDNA degradation mainly occurs over the first 250 years after sediment deposition (Dommain et al., 2020), however this research was undertaken in a tropical swamp (Uganda) and thus the environment conditions (especially temperature and pH) are very different to the lakes studied here. It is likely that degradation rates would be much slower rates in deeper, temperate lake sediments. DNA degradation in lake sediment cores needs further exploration, using methodologies such as shotgun sequencing which shows the degree of fragmentation in DNA sequences.

A puzzling observation in the present study was the higher ASV richness in all

lakes in the PES phase, albeit this was most pronounced in the lakes that experienced the greatest increase in cyanobacterial abundance. This is somewhat contrary to expectations, given that cyanobacterial blooms are generally dominated by one or few species. There are several plausible explanations for this observation. Sediments core samples integrate time, they capture activity across an entire year and over multiple years of within lake biological activity. Cyanobacterial blooms primarily occur in summer months (Jöhnk et al., 2008), yet the sediment samples capture an annual signal, thus it is feasible that richness escalates during non-bloom periods. A study which focused on collecting regular water samples for at least a year is required to determine whether seasonal differences in cyanobacterial ASV abundance drive the observed increase in ASV richness. Additionally, in this study we took 1-cm wide depth slices for most lakes, which we estimate captures a period of 2–5 years in Lakes Hayes and Johnson, approximately 10 years in Lake Pounui, and 0.5 cm slices in Lake Paringa which captures about one year of time. During these most recent periods there have been rapid shifts in many environmental variables not measured in this study such as nutrients, chemicals, and toxins. It is feasible that the high species richness may truly represent a community that is adapting and diversifying to respond to an increasing number of new pressures. A further possibility is that DNA degradation is accounting for the reduction of richness in deeper samples. In metabarcoding studies a large proportion of ASVs are rare and have a low number of reads (Sogin et al., 2006). In this study 18 photosynthetic cyanobacterial ASVs (out of 839) accounted for 50% of the reads after rarefaction. As DNA deeper in the core is subjected to degradation pressures for a longer period, there is a likelihood that the rare ASVs are more likely to be lost compared to more recent samples, thus decreasing richness.

2.8 Conclusion

Through the study of six different lakes over $\tilde{1}000$ years, this research has provided valuable new insights into changes in cyanobacterial communities in a range of lake

types in New Zealand. Although cyanobacteria were present in these lakes prior to human arrival, including potentially toxic or bloom forming species, their overall abundance was low. Some changes in cyanobacterial abundance and structure occurred following Māori settlement in New Zealand, but the most pronounced shifts happened post European settlement, concomitant with land use change. The inclusion of Lakes Pounui and Paringa, where there has been only moderate (Pounui) or minimal (Paringa) land use change in the catchment, highlights that there are multiple drivers of shifts in cyanobacterial communities. While we speculate that the introduction of non-native carnivorous fish played a role in changing cyanobacterial communities, further work exploring multiple trophic levels is required. Our results demonstrate the high utility of combining ddPCR and metabarcoding for investigating shifts in cyanobacterial abundance and structure in lakes over many centuries. Paleolimnological studies utilising sedaDNA offer the potential to track the history of cyanobacteria, and when used in combination with other paleolimnological proxies the data can provide insights into the drivers of change.

2.9 Data availability

The metabarcoding dataset generated and analysed during the current study is available in the Sequence Read Archive (SRA) repository (BioProject PRJNA855252).

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Chapter 3

Comparison between cyanobacterial eDNA and pigments in lake sediment cores

3.1 Preliminary note

This paper was published in Microorganisms in January 2022 under the following reference:

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It was published in Open Access under a Creative Commons Attribution 4.0 International License. The content of this section is therefore an exact copy of the published article, except that (1) the ORCID numbers of the authors and the lists of keywords and of references were omitted,(2) for consistency with the rest of the thesis, the citations were reformatted, the numbering of figures and tables adjusted,

the language was adapted to British English, and (3) following comments from the examiners, section 3.6.4 and Figure 3.3 were modified to reflect that Spearman's correlation (ρ) is indeed the correlation measure used in this this study (written as R^2 in the published article).

3.2 Authors and author contributions

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Conceptualisation, M.P., S.A.W. and J.P.; formal analysis, M.P.; funding acquisition, S.A.W. and M.J.V.; investigation, M.P., S.A.W. and J.P.; methodology, M.P.; resources, M.P., S.A.W., M.J.V., L.R., J.D.H. and J.P.; supervision, S.A.W., I.H., X.P. and J.P.; validation, M.P.; visualisation, M.P.; writing—original draft, M.P., S.A.W. and J.P.; writing—review and editing, M.P., S.A.W., X.P., M.J.V., L.R., J.D.H., I.H. and J.P. All authors have read and agreed to the published version of the manuscript.

3.3 Abstract

Understanding the historical onset of cyanobacterial blooms in freshwater bodies can help identify their potential drivers. Lake sediments are historical archives, containing information on what has occurred in and around lakes over time. Paleolimnology explores these records using a variety of techniques, but choosing the most appropriate method can be challenging. We compared results obtained from a droplet digital PCR assay targeting a cyanobacterial-specific region of the 16S rRNA gene in sedimentary DNA and cyanobacterial pigments (canthaxanthin, echinenone, myxoxanthophyll and zeaxanthin) analysed using high-performance liquid chromatography in four sediment cores. There were strong positive relationships between the 16S rRNA gene copy concentrations and individual pigment concentra-

tions, but relationships differed among lakes and sediment core depths within lakes. The relationships were more consistent when all pigments were summed, which we attribute to different cyanobacteria species, in different lakes, at different times producing different suites of pigments. Each method had benefits and limitations, which should be taken into consideration during method selection and when interpreting paleolimnological data. We recommend this biphasic approach when making inferences about changes in the entire cyanobacterial community because they yielded complementary information. Our results support the view that molecular methods can yield results similar to traditional paleolimnological proxies when caveats are adequately addressed.

3.4 Introduction

Information about past events, such as historical weather trends and the effects of anthropogenic actions, can provide valuable insights to understand the present and to predict the future (Balmford et al., 2005; Cox et al., 2002; Ellis et al., 2013; Ellison et al., 1996; Hazeleger et al., 2015; Lobell et al., 2010) . To learn about past events that happened in and around lakes, paleolimnology has traditionally examined physical remains preserved in lake sediments (Douglas, 2013). These proxies are based on the resistant physical parts of some organisms such as insect mouthparts, pollen and spores from plants, and diatom frustules. Pigments have also been used for decades to retrace historical patterns in photosynthetic communities and can target a wide range of organisms (Charette et al., 2003; Levine et al., 2012; Riedinger-Whitmore et al., 2005; Sanger, 1988; Waters et al., 2005), but they do not allow species-specific identification in the way that diatom frustules do. This used to be troublesome when studying photosynthetic communities which do not leave morphological remains behind when they die, such as cyanobacteria.

Cyanobacteria are photosynthetic prokaryotes that have inhabited Earth for an estimated three billion years (Whitton, 2012). They have recently received increasing scientific and public attention due to the impact of their excessive proliferations

(blooms), which degrade aquatic ecosystem health and often produce life-threatening toxins (Bláha et al., 2009; Carmichael, 2001; Harke et al., 2016; Huisman et al., 2018; Paerl, 2014; Wood et al., 2020). Reports indicate that cyanobacterial blooms are increasing in frequency and magnitude in many waterbodies around the globe. Due to a lack of long-term monitoring records, there is uncertainty as to whether there is an actual rise in blooms or if an increase in awareness is leading to more reports (Ewing et al., 2020). In the case of the former, it is important to be able to pinpoint what triggered this increase, which can be evaluated using paleolimnology. However, unlike diatoms, cyanobacteria have soft cell walls which do not leave microfossils behind; therefore the only proxies available to study them in sediment cores are the molecules they produce. Studies to date have targeted their pigments (Riedinger-Whitmore et al., 2005; Ewing et al., 2020; Vermaire et al., 2017; Pal et al., 2015; Monchamp et al., 2021; Hobbs et al., 2021; Deshpande et al., 2014), toxins (anatoxin, microcystin; Hobbs et al., 2021; Zastepa et al., 2017), lipid biomarkers (e.g., 2-methylbacteriohopanetetrol as a possible biomarker for a freshwater strain of *Synechococcus*; Coolen et al., 2008), and now, their DNA (Pal et al., 2015; Hobbs et al., 2021; Coolen et al., 2008; Monchamp et al., 2018; Monchamp et al., 2016; Mejbel et al., 2021).

Recently, molecular analysis of sedimentary DNA (sedDNA, intra- and extracellular DNA from bulk sediment samples) has been applied to paleolimnological studies (Capo et al., 2021a). These methods can target a range of organisms across a broad spectrum of taxa, with high levels of specificity and sensitivity (Corinaldesi et al., 2008; Domaizon et al., 2017; Wood et al., 2019). However, limited comparisons of sedDNA with traditional paleolimnological proxies (such as pigments for cyanobacteria) have been made so far. One study did reconstruct total cyanobacterial abundances using quantitative PCR (qPCR, targeting a cyanobacterial specific 16S ribosomal RNA (16S rRNA) gene) from the sediment cores of five lakes in Western Quebec (Canada), and compared them to two pigments found in cyanobacteria (echinenone and zeaxanthin; Pal et al., 2015). However, when present, the pigments were not always correlated significantly and/or positively with total cyanobacte-

ria abundance. In another study focusing on the history of cyanobacterial blooms in Anderson Lake (Washington State, USA), cyanobacterial pigments were quantified (specifically echinenone, zeaxanthin, canthaxanthin and myxoxanthophyll) and cyanobacterial sedDNA abundances measured using qPCR (cyanobacterial-specific 16S rRNA gene; Hobbs et al., 2021). No direct comparisons were made, however, their results showed an exponential increase in cyanobacterial sedDNA abundances which was not matched by any of the pigments. An explanation for these discrepancies could be due to differences in the relative abundance of individual cyanopigments observed in different cyanobacteria (Hertzberg et al., 1971). A recent study suggested that using the sum of the cyanopigments could provide a better representation of general cyanobacterial abundance in sediment core samples (Puddick et al., 2021), but this has not yet been tested in comparison to sedDNA molecular analyses. Discrepancies could also reflect differences in degradation rates since DNA and pigments are very different molecules. Further detailed studies with the two methodologies applied in parallel are required to understand these observed discrepancies, and to gain new insights into the complementarity of these methods.

The present study compared a droplet digital PCR (ddPCR) assay, targeting a cyanobacteria-specific region of the 16S rRNA gene, with high-performance liquid chromatography (HPLC) focusing on the concentrations of four pigments largely confined to cyanobacteria (cyanopigments—canthaxanthin, echinenone, myxoxanthophyll and zeaxanthin). Droplet digital PCR is a relatively recent technique that generates an emulsion of approx. 20,000 separate PCR reactions as droplets in an oil solution. The target DNA sequence is amplified as in standard PCRs and then the number of DNA copies in each droplet is counted using fluorescence. It has numerous advantages over traditional qPCR, for example no inhibition assays or standard curves are required, and samples do not need to be run in triplicate since every result is an average of all the individual PCR reactions corrected by a Poisson distribution. Pigment analysis by HPLC separates a complex mixture of compounds in samples (which have been extracted and concentrated) based on the interaction of individual compound species with an adsorbent material (the column packing)

and a gradient of solvent flowing through the column. A photo-diode array (PDA) detector then gives a quantitative measure of each component as they elute from the column, based on their light-absorbance pattern.

In this study, historical cyanobacteria abundances were determined using these two techniques and compared in 132 samples taken from four lake sediment cores, spanning periods of ca. 700 to 900 years. We hypothesised that: (1) there would be strong correlations between cyanobacteria-specific 16S rRNA gene copies (determined by ddPCR) and cyanopigments (determined by HPLC) across all lakes and core depths, and (2) the sum of all cyanopigments would have stronger relationships to cyanobacterial 16S rRNA gene copy numbers compared to individual pigments.

3.5 Materials and Methods

3.5.1 Study sites and sediment core sampling

Four lakes were sampled between September 2018 and July 2019 in the North Island of New Zealand: Lakes Nganoke, Okataina, Pounui, and Rototoa. These lakes were selected because they have good sedimentary records and different contemporary cyanobacterial abundances: Lakes Nganoke and Pounui experience cyanobacterial blooms every summer, while Lakes Rototoa and Okataina are deeper lakes with no cyanobacterial blooms (Table D.14). Sediment cores were retrieved from a site closest to the deepest point of each lake using an Uwitec gravity corer (UWITEC, Mondsee, Austria) and 2 m-long 90-mm diameter polyvinyl chloride barrels (Leda Extrusions NZ ltd, Upper Hutt, New Zealand). All core barrels were cleaned with 2% sodium hypochlorite (bleach) prior to coring. After retrieval, the cores were sealed and stored at 4 °C in darkness for up to 4 weeks until sub-sampling. The Nganoke core was 91 cm long which corresponds to ca. 1130 AD (Parrish, 2020), the Rototoa core was 75 cm long (ca. 1690 AD, unpublished data Table D.13), the Okataina core was 124 cm long (ca. 1720 AD; Caird, 2021) and the Pounui core was 73 cm, dating to ca. 1280 AD (Pearman et al., 2021a). The sediment cores

were split in half using a using a bench mounted Geotek core splitter (Geotek Ltd., Deventry, Northamptonshire, UK) in a dedicated room (no molecular analysis). Due to the guillotine smearing the sediment upon core splitting, 2–3 mm from the surface of one half-core were carefully removed with a large, sterilised spatula (dipped in ethanol and blow-torched). Sediment was subsampled (2–3 g) from the center of the half-core using a sterile plastic scoop or a sterilised spatula at various depths down the cores and frozen (-20 °C) until further use.

3.5.2 Cyanobacteria primers modification

Partial 16S rRNA cyanobacteria sequences of all main types of cyanobacteria (picocyanobacteria, benthic, pelagic bloom-forming) and some non-target bacteria were selected from GeneBank and aligned (140 sequences in total) with MEGAX (Pennsylvania State University, State College, PA, USA; Kumar et al., 2018). This alignment was assembled to assess whether primers CYAN108F and CYAN377R (Rinta-Kanto et al., 2005) adequately amplified all cyanobacteria (a selection of the alignment is shown in Figure D.13). This in-silico analysis revealed that picocyanobacteria such as *Cyanobium gracile* and some *Synechoccocus* had a one-nucleotide difference with CYAN108F and all cyanobacteria species in the alignment had a one-nucleotide difference with CYAN377R (Figure D.13). Both primers were therefore slightly modified to enhance the amplification of as many cyanobacteria as possible while not amplifying other non-target bacteria (Table 3.1). The specificity towards cyanobacteria was further checked in-silico by running both primer sets (old and new) with TestPrime (v 1.0, Ribocon GmbH, Bremen, Germany; Klindworth et al., 2013), which runs an in-silico analysis using the SILVA 16S database.

Table 3.1: Details of the modifications to the CYAN cyanobacteria primer set targeting a region of the 16S ribosomal RNA gene. Nucleotides added or modified are in bold. Total amplicon length is ~283 bp.

Primer	Sequence	Source
CYAN108F	5’-ACGGGTGAGTAACRCGTRA-3’	Rinta-Kanto et al., 2005
CYAN107F	5’-**G**ACGGGTGAGTAACRCGTR**RG**-3’	This paper
CYAN377R	5’-CCATGGCGGAAAATTCCCC-3’	Rinta-Kanto et al., 2005
CYAN377R_mod	5’-CCAT**T**GCGGAAAATTCCCC-3’	This paper

3.5.3 Water content determination

Sediment core samples were thawed in the dark at 4 °C, subsamples (0.5–1 g) were weighed into pre-weighed glass vials, lyophilised (Gamma 1–16 LSC freeze-dryer; Martin Christ Gefriertrocknungsanlagen, Osterode am Harz, Germany), and reweighed. The water content was determined using the following formula:

$$\text{Water content of sediment} = \frac{\text{Wet weight (g)} - \text{Dry weight (g)}}{\text{Wet weight (g)}} \tag{3.1}$$

3.5.4 DNA extraction and droplet digital PCR

The molecular analysis was conducted sequentially in separate sterile laboratories dedicated to each step (DNA extraction, ddPCR set-up, template addition, and PCR amplification) to ensure no cross-contamination. Each room (except for PCR amplification) was equipped with ultra-violet lights on the ceiling for sterilisation, switched on for 40 min before and after each use. Furthermore, ddPCR setup and template addition were undertaken in laminar flow cabinets, with HEPA filtration and 15 min UV sterilisation before and after use. Aerosol barrier tips (epT.I.P.S.,

Eppendorf, Hamburg, Germany) were used throughout.

The DNeasy PowerSoilTM DNA Isolation Kit (QIAGEN, Hilden, Germany) was used for sedDNA extraction. Approximately 0.25 g of wet sediment was weighed in the first tube of the kit (bead tube) and exact weights for each subsample recorded. DNA extraction was performed in batches of 12 samples including a negative control every two batches (all the reagents but no sediment) using a QIAcube (QIAGEN, Hilden, Germany) for automated extraction following the manufacturer's protocol.

Following extraction, cyanobacterial 16S rRNA gene copy numbers were quantified using ddPCR. All samples had to be diluted for adequate quantification; dilution ranged from 1/10 to 1/10,000. The ddPCR workflow was undertaken using a BioRad QX200 system (Bio-Rad laboratories, Hercules, California, United States) following the manufacturer's protocol and the methods described in a previous paper (Picard et al., 2022a). Due to the change in primers, the annealing temperature was lowered to 55 °C for 1 min (full Mastermix composition and cycling conditions in Table D.15). The Lake Nganoke sedDNA samples were analysed using the CYAN108F and CYAN377R (Table 3.1) and the primers designed in this study to compare levels of amplification. Cyanobacteria concentration obtained with the QuantaSoft software were then standardised to DNA gene copy numbers per gram of dry sediment using the following formula:

$$Gene\ copies = \frac{ddPCR \times \frac{22\,\mu\text{L}}{4\,\mu\text{L}} \times DF \times 100\,\mu\text{L}}{sed.\ weight \times (1 - water\ content)} \tag{3.2}$$

where gene copies = cyanobacteria (16S rRNA) gene concentrations (gene copies/gram of dry sediment), ddPCR = concentration of 16S rRNA gene copies per μL, 22 μL was the volume of the MasterMix used, 4 μL was the volume of DNA template added to the PCR reaction, DF = dilution factor (10 to 10,000), 100 μL was the volume the DNA was eluted in during extractions, sed. weight = exact weight of each subsample extracted for DNA (0.25 g), and water content = water content of the core subsample (from Equation 3.1).

The limit of quantification (LoQ) of the new cyanobacterial 16S rRNA primer set was determined using a dilution series of a positive control (DNA extracted from a cyanobacterial-dominated mat) and a negative control (extraction using the PowerSoil kit with no sample added), in duplicate. The LoQ was determined as the cyanobacterial 16S rRNA gene concentration of the lowest dilution where a linear relationship was still measured. At this point there was a clear distinction between positive droplets in the positive control and the cloud of negative droplets observed in the negative control.

3.5.5 Pigment Extraction and High-Performance Liquid Chromatography

Sediment core samples were thawed in the dark at 4 °C, and subsamples (0.5–1.2 g) were extracted three times using acetone and ultrasonication (30 min) in a bath sonicator (Kudos Ultrasonic Cleaner; Shanghai, China) with ice. The extract was dried under a stream of nitrogen gas at 40 °C and stored at -20 °C until analysis. On the day of analysis, the dried extract was resuspended in acetone (0.5 mL) and transferred to a septum-capped amber vial for analysis by HPLC with DAD using an Agilent 1260 system (Santa Clara, CA, USA). Pigments were separated using a C30 column (Develosil RP-Aqueous C30, 5-µm, 250 × 4.6 mm; Phenomenex, Torrance, CA, USA) maintained at 30 °C and a gradient of methanol + 0.1% triethylamine (Solvent A) to 40:60 methanol/isopropyl alcohol + 0.1% triethylamine (Solvent B). Samples were injected (10 µL injection) in 100% Solvent A, which was maintained for 5 min before proceeding in a linear gradient to 65% Solvent B over 35 min. The column was washed with 90% Solvent B for 5 min and re-equilibrated in 100% Solvent A for 5 min between each injection. The flow rate was 1 mL/min throughout the chromatographic gradient. Light absorption data were collected over a 320–800 nm wavelength range, but only specific wavelength ranges were used for compound quantitation during postprocessing (Table 3.2).

Table 3.2: Parameters of the high-performance liquid chromatography analysis of carotenoid pigments in sediment core samples. RT stands for retention time (minutes) and the wavelength is the range (nanometres) used for pigment quantification.

Pigment	RT	Wavelength	Equivalence Factor
Lutein	9.6	435–455	-
Zeaxanthin	10.3	435–455	1.56
Myxoxanthophyll	12.7	460–480	1.26
Canthaxanthin	16.1	460–480	1.06
Echinenone	32.9	435–455	0.63

A five-point mixed standard curve (0.5–20 µg/mL) of lutein (Carotenature, Münsingen, Switzerland) was analysed with each HPLC run along with qualitative standards for each pigment analysed. The lutein standard was calibrated by spectrophotometry at 445 nm using the extinction coefficients described in Roy et al., 2011. Equivalence factors for canthaxanthin, echinenone, myxoxanthophyll and zeaxanthin (Table 3.2) were determined in relation to lutein by analysing standards at known concentrations. These equivalence factors were used for the routine quantification of the other pigments, rather than preparing a standard curve for each HPLC run.

3.5.6 Data Analysis

Analysis was undertaken using R software (v4.0.2, Vienna, Austria; R Core Team, 2020) and RStudio software (v1.3.959, Boston, MA, USA; RStudio Team, 2020). Data manipulation, exploration, and plots were made with Tidyverse (v1.3.1) and associated packages (Wickham et al., 2019).

Differences in 16S rRNA gene copy quantification between the previous PCR primer and the newly developed primer set were investigated using the Lake Nganoke sediment core. Cyanobacterial concentrations obtained from the ddPCR using both primers were first plotted as a down-core profile, and then summarised using paired boxplots. Log10 transformation was undertaken, and a paired t-test was performed on the transformed data to evaluate whether the new primer set amplified a high

number of cyanobacterial 16S rRNA gene copies.

Total cyanopigments were calculated from the sum of the concentrations of each cyanobacterial pigment per lake. All proxies were first visualised as down-core profiles to look at general trends. Last, cyanobacterial 16S rRNA gene copy concentrations from ddPCR data were then compared to cyanopigment concentrations from HPLC using scatterplots and Spearman's correlation. Four samples from the tephra in-wash in Lake Okataina had very low 16S rRNA gene copies levels (lower than the LoQ) and were not compared with cyanopigments. The subsample depths for each core were visualised using colour codes. Differences in correlations to pigments depending on the primer set were represented using scatterplots (Lake Nganoke only).

3.6 Results

3.6.1 Cyanobacterial 16S rRNA gene primer comparison

Modifying the cyanobacteria-specific 16S rRNA ddPCR primers increased the groups of cyanobacteria detected, as revealed by both the in-silico analysis and sediment core test. The in-silico analysis using TestPrime only found two matches for the CYAN108F and CYAN377R primer set when no nucleotide mismatch was allowed: a species of *Calothrix* (cf. *Calothrix* sp. 'muscicolous cyanobiont 5') as well as one sequence of chloroplast (unclassified). The new primer set CYAN107F and CYAN377R_mod amplified (in-silico, no mismatch) 3842 cyanobacteria sequences of the 4700 (82%) in the SILVA database, including 223 genera of photosynthetic cyanobacteria (oxyphotobacteria). Most importantly for our study, the new primer set amplified specific genera found in New Zealand lakes, including the most abundant taxa identified a recent study [42]: *Aphanizomenon, Cyanobium, Dolichospermum, Microcystis, Nodosilinea,* and *Tychonema* (potentially not amplified by the previous primer set). The in-silico analysis indicated that the new primer set CYAN107F and CYAN377R_mod also amplified some nontarget bacteria and

chloroplasts sequences (respectively 60 and 454 sequences), since the amplicon targets a conserved region of the 16S rRNA gene. Plastids from some diatoms could be potentially co-amplified, other plastids were from plants and algae that are non-native to New Zealand and very unlikely to be found in and around the study lakes. Overall, twenty-six plastids were amplified in-silico by the new primer set compared to the original primer set (Table D.16). The sedDNA from Lake Nganoke was used to compare the two primer sets. The concentrations of 16S rRNA gene copies were on average 7.8-times higher with the new primer set (CYAN107F and CYAN377R_mod; Figure 3.1A), and a paired t-test showed this was a significant difference (p-value < 0.01, Figure 3.1B). The downcore profile showed that both primer sets reproduced similar trends overall. For example, there were some common peaks at depths of 0, 4, 13, 19, 33, and 40 cm, but the number of gene copies detected with the new primer set was markedly higher from 55 cm onwards (ca. 1561 AD). Furthermore, when the concentrations of the cyanobacterial 16S rRNA gene (from both primers) were compared to the concentrations of individual cyanopigments also found in the Lake Nganoke sediment core (canthaxanthin, echinenone, myxoxanthophyll and zeaxanthin), stronger relationships were observed using the new primer set ($0.65 \leq R^2 \leq 0.81$ for the new primer set, $0.59 \leq R^2 \leq 0.72$ for the original primer set; Figure D.14). The LoQ for the new primer set (CYAN107F and CYAN377R_mod) was 0.4 16S rRNA gene copies per µL (raw ddPCR value) using DNA extracted from the positive control. When normalised to the standard sediment weight (0.25 g) and the mean water content (70%) using the formula described in the Methods section (Section 3.5.4), the LoQ was 2933 16S rRNA gene copies per gram of dry sediment.

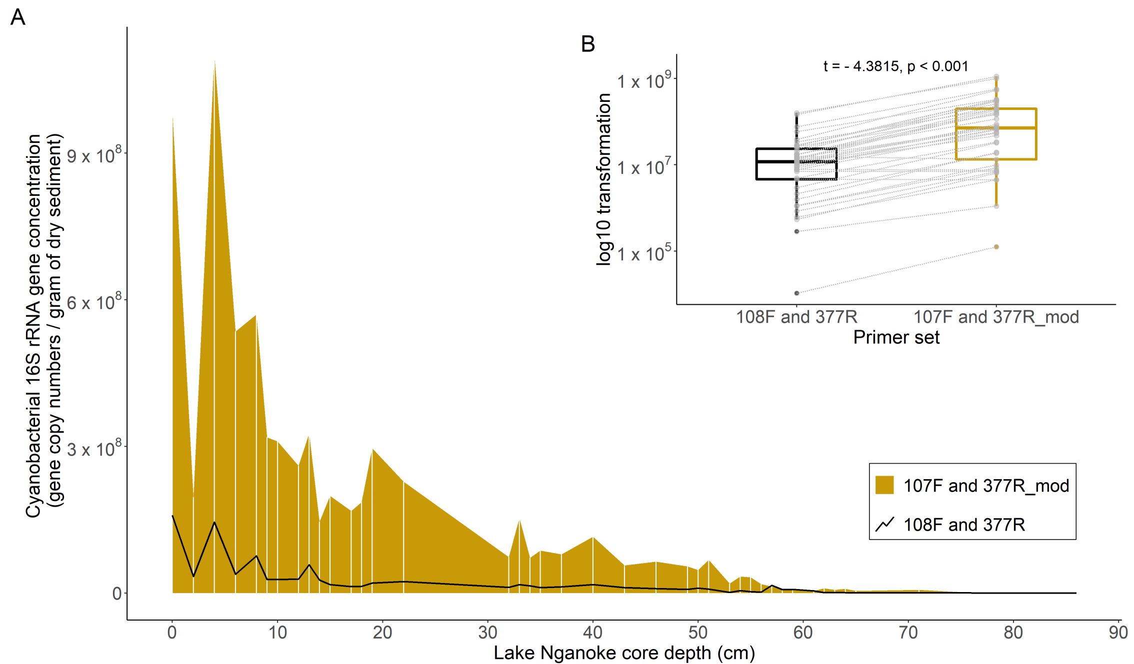

Figure 3.1: (A) Differences in cyanobacterial 16S ribosomal RNA gene copy numbers between the primer set CYAN108F and CYAN377R (Rinta-Kanto et al., 2005) and the primer set developed in this study (CYAN107F and CYAN377R_mod) when applied to sedimentary DNA extracted from a sediment core from Lake Nganoke. White vertical lines show sample depths. (B) A paired t-test was run on the log-transformed data and the results are displayed in the inset. The y-axis is the same as for Figure 1A, cyanobacterial 16S rRNA gene concentration, but with a log10 transformation.

3.6.2 Cyanobacterial 16S rRNA genes in sediment cores

Cyanobacterial 16S rRNA genes were successfully detected in every sample analysed (some at very low levels–minimum 255 copies/g dw). For all lakes, higher cyanobacterial 16S rRNA concentrations were observed at the top of the cores and decreased in the older sediment samples (Figure 3.2). The exception to this was Lake Okataina, where the concentrations observed at the top of the core were lower than other lakes, and levels were relatively stable over the length of the core. Furthermore, the cyanobacterial 16S rRNA gene copy levels measured above and below the tephra from the 1886 Mount Tarawera eruption were similar (Figure 3.2). The highest concentrations of 16S rRNA gene copies were detected in Lakes Rototoa and Nganoke (c. 8.5×10^8), while Lakes Pounui and Okataina showed lower levels (Figure 3.2). There was a substantial nine-fold difference in mean cyanobacterial

16S rRNA gene copy levels between Lake Rototoa and Lake Okataina.

3.6.3 Cyanobacterial pigments

Cyanopigment concentrations were variable across lakes and within the same core; some high pigment concentrations were observed at the bottom of the cores, especially canthaxanthin in all lakes, and all cyanopigments in Lake Pounui (Figure 3.2 and Figure D.15). Zeaxanthin was generally the most abundant cyanopigment across all lakes (max. 32 µg/g dw), followed by canthaxanthin (max. 13 µg/g dw), while myxoxanthophyll and echinenone displayed the lowest levels (max. 6 µg/g dw, respectively). The sum of all four cyanopigments (total cyanopigments) reflected individual pigment trends and was overall highest in Lake Nganoke (mean = 17.5 µg/g dw) and lower in Lakes Okataina, Pounui, and Rototoa (mean = 3.4, 3.6, and 7.1 µg/g dw, respectively). Zeaxanthin and canthaxanthin made the greatest contribution to total cyanopigments (max. 60% and 80%, respectively), while myxoxanthophyll and echinenone had lower contributions (max. 34% and 11%, respectively) (Figure 3.2, Figures D.15 and D.16). Individual cyanopigments were not detected in every sample; for example, in Lake Okataina some layers of tephra inwash (volcanic ash washed in from the catchment) did not yield any cyanopigments, and there was no echinenone nor zeaxanthin detected in the oldest sample (93 cm core depth) of Lake Nganoke (Figure 3.2).

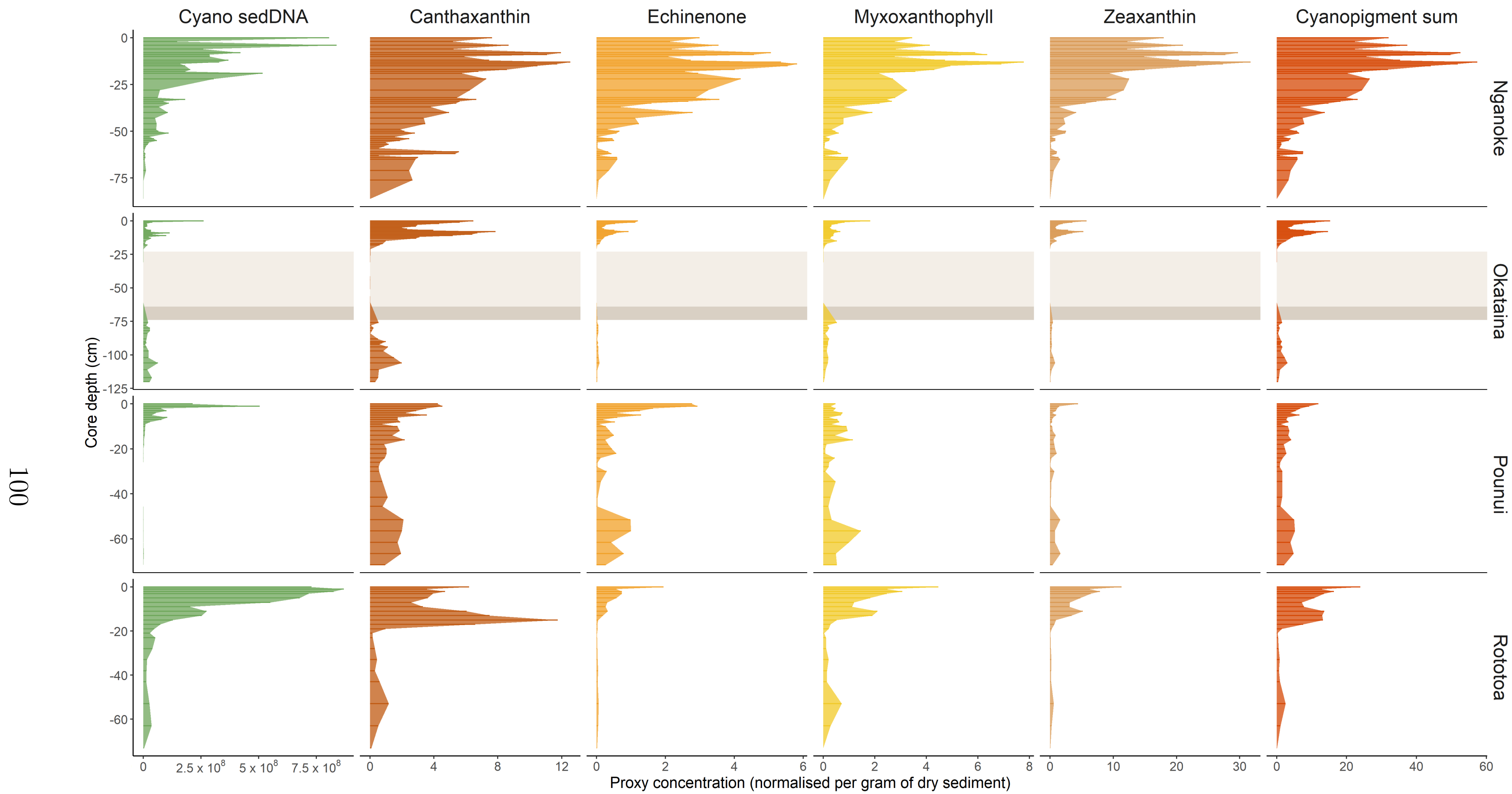

Figure 3.2: Cyanobacterial 16S rRNA gene, individual, and total cyanopigments downcore profiles for each lake. Cyano sedDNA = Cyanobacterial 16S rRNA gene copy numbers per gram of dry sediment, cyanopigment concentration (µg per gram) of dry sediment. Cyanopigment sum refers to the sum of the four individual pigments. The grey shades in Lake Okataina indicate the tephra (dark) from the 1886 Mount Tarawera eruption and tephra in-wash (light) from the catchment, which affected the detection of cyanobacteria.

3.6.4 Proxy relationships within each lake

The relationships between the cyanobacterial 16S rRNA gene concentration and the individual cyanopigments (canthaxanthin, echinenone, myxoxanthophyll and zeaxanthin) were variable. In Lake Nganoke, the Spearman's correlation was strong for each individual cyanopigment ($\rho \geq 0.64$, $p < 0.001$; Figure 3.3). In Lake Pounui, the best correlation was for canthaxanthin (positive, strong, and significant; $\rho = 0.54$, $p < 0.001$) and the worst correlation was for myxoxanthophyll (not significant, $\rho = 0.05$, $p = 0.24$). In Lakes Okataina and Rototoa, significant correlations were observed for each individual cyanopigment ($p < 0.001$), although the strength of the correlation varied ($0.34 \leq \rho \leq 0.8$). To overcome the differences observed between individual cyanopigments, the sum of all cyanopigments was compared with cyanobacterial 16S rRNA gene copy concentrations. Total cyanopigments were always positively and significantly correlated to 16S rRNA gene copies for all lakes ($0.43 \leq \rho \leq 0.73$, $p < 0.001$, Figure 3.3) and were more consistent than any individual cyanopigment. The correlation of total cyanopigments to 16S rRNA gene copies was the best-equal for Lake Okataina (alongside canthaxanthin and echinenone). In Lakes Nganoke and Pounui, total cyanopigments was second best (behind zeaxanthin and canthaxanthin, respectively). In Lake Rototoa, total cyanopigments had the third highest Spearman's correlation coefficient, but this was close to that of the individual pigments demonstrating stronger relationships ($\rho = 0.71$ for total pigments, 0.76 for myxoxanthophyll, 0.8 for zeaxanthin).

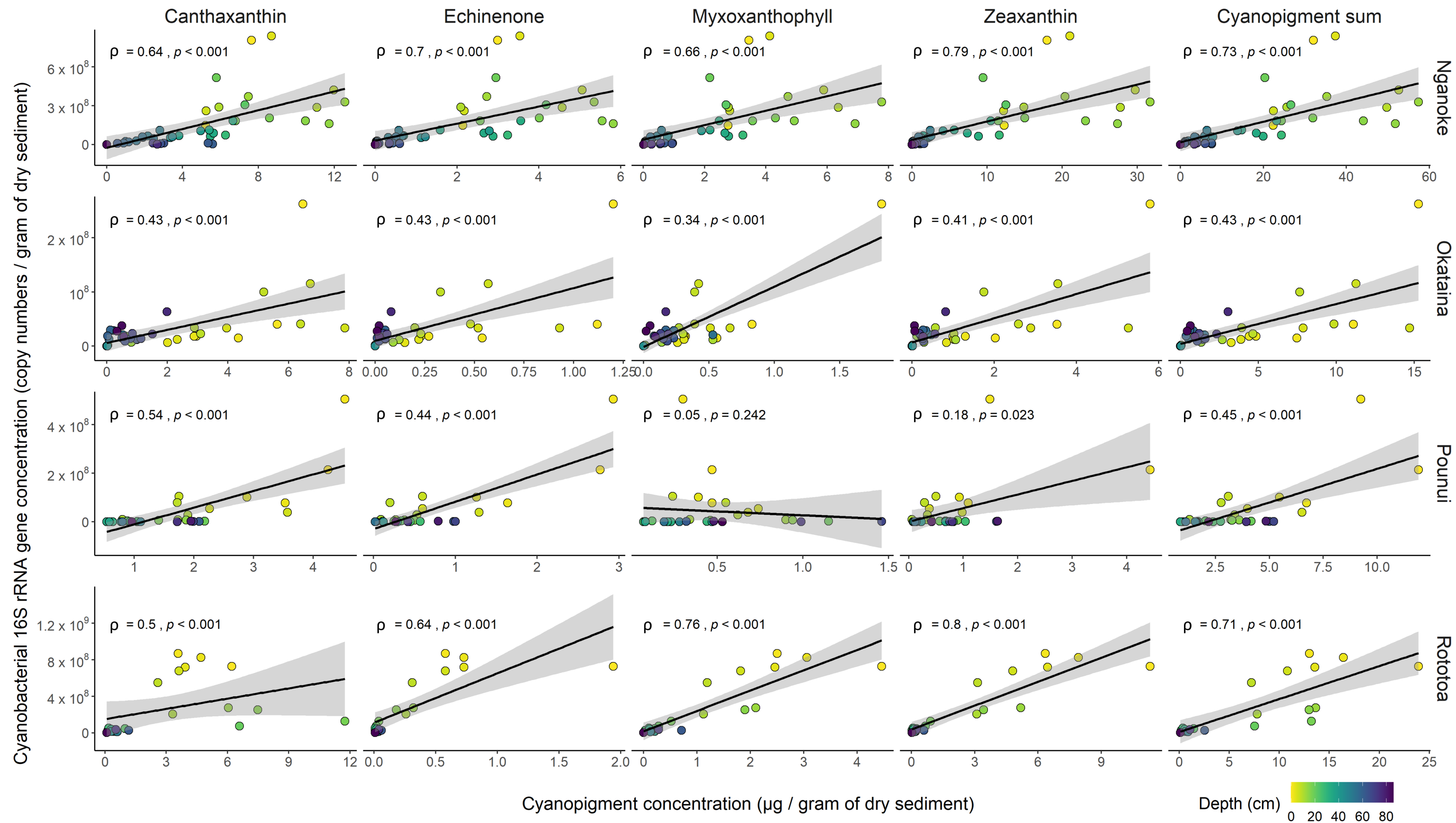

Figure 3.3: Relationships across lakes between cyanobacterial 16S rRNA gene concentrations (gene copy numbers per gram of dry weight of sediment) from droplet digital PCR data and concentrations of individual cyanopigments and total cyanopigments from high-performance liquid chromatography (µg per gram of dry weight of sediment). Relationships (ρ) and p-values calculated using Spearman's correlation test are displayed, colour gradient shows the depth of each core sub-sample.

3.7 Discussion

3.7.1 Cyanobacterial-specific primer design and testing

Initial explorations suggested that the original cyanobacterial 16S rRNA primer set (CYAN108F and CYAN377R; Rinta-Kanto et al., 2005) was unlikely to amplify a wide range of cyanobacteria in the sediment samples when no mismatches were allowed. These primers were first designed more than 15 years ago. Since then, there has been a rapid increase in the number of 16S rRNA sequences available in databases and this allowed us to enhance the specificity of PCR primers for this target. The in-silico analysis indicated that with no mismatches allowed, the primer set CYAN108F and CYAN377R would be unlikely to amplify the picocyanobacteria known to occur in New Zealand lakes Picard et al., 2022a. While other cyanobacterial 16S rRNA gene qPCR primers have been developed (Al-Tebrineh et al., 2010), we were unable to adapt these to the ddPCR workflow used in this study, because positive droplets did not segregate away from negative droplets. Therefore, slight modifications were made to the CYAN108F/CYAN377R primer set to enable the amplification of a wider range of cyanobacteria. The amplicon from the new primers was slightly longer than recommended for ddPCR (283 bp vs. <200 bp; Bio-Rad Laboratories, 2018), which resulted in some noise (intermediate droplets between positive and negative droplets) when the samples were too concentrated. However, upon adequate dilution the positive droplets segregated correctly.

The new primers (CYAN107F and CYAN377R_mod) resulted in cyanobacterial 16S rRNA gene concentrations that were on average 7.8-fold higher compared to the original primer set. This was most likely because the new primer set detected a wider range of cyanobacteria taxa, which is of particular value for paleolimnological studies. This allowed an increase in cyanobacteria to be detected from ca. 1560 AD up to present time in the Lake Nganoke sediment core, whereas the previous primer set would have detected an increase much later (ca. 1850s). The downcore 16S rRNA gene profiles produced with the new primer set also yielded a stronger fit

to historical cyanopigment concentrations compared to the original primer set.

3.7.2 Correlation between cyanobacterial 16S ribosomal RNA gene copies and cyanopigments

In general, there were moderately strong positive relationships between the cyanobacterial 16S rRNA gene and cyanopigment concentrations but differences among lakes. A recent study evaluated a range of cyanobacteria species and reported differences in the relative concentrations of individual cyanopigments [36]. In particular, picocyanobacteria (Synechococcaceae) contained lower relative concentrations of echinenone than other cyanobacteria, and bloom-forming genera such as Dolichospermum and Microcystis contained lower relative concentrations of zeaxanthin. Differences in the cyanobacterial community over time were evident in Lake Pounui, where echinenone was more abundant than myxoxanthophyll in the top of the core, and in Lake Rototoa where myxoxanthophyll was more abundant from the middle of the core. These types of shifts in specific cyanopigments have been well documented in other lakes in the literature (Vermaire et al., 2017; Hobbs et al., 2021; Deshpande et al., 2014) and have been theorised to be associated with changes in the cyanobacterial community.

Pal et al., 2015 also applied cyanobacteria-specific qPCR primers and pigment analysis to five sediment cores from Western Quebec (Canada). The relationships between DNA and pigment analyses (echinenone and zeaxanthin) showed high between-lake variability, with some positive and some negative correlations. Although no explanation was provided for this, it could be because only two 'cyanopigments' were targeted, because of the primers used (CYAN108F and CYAN377R; which may not detect all taxa), or due to the use of qPCR (which is more susceptible to inhibition). In contrast, Hobbs et al. (2021) found no pigment patterns to mirror cyanobacterial 16S rRNA gene copy numbers in a core from a eutrophic lake in Washington (USA), particularly at the top of the core where copy numbers increased in the absence of pigment increase. Reasons for the discrepancy are un-

known, although we note that a different primer set (CyanoReal16S) was used for these ddPCR analyses. Because of the limited number of studies currently conducted in this area, using a variety of primers and pigment choices, it is currently difficult to comprehensively explain the different results observed between these studies and the current study.

3.7.3 The sum of all cyanopigments - a better proxy for total cyanobacterial biomass?

As mentioned above, different cyanobacterial taxa contain varying relative abundances of canthaxanthin, echinenone, myxoxanthophyll, and zeaxanthin. A recent study analysed pigments in 34 cyanobacterial cultures and found, for example, that strains of *Dolichospermum* sp., *Nodularia spumigena*, and *Cuspidothrix issatchenkoi* produced high levels of canthaxanthin, while *Planktothrix* sp. produced more myxoxanthophyll than other cyanobacteria (Puddick et al., 2021). Furthermore, myxoxanthophyll was the only pigment not detected in all (10 out of 34) studied cultures; it was only detected in all of the picocyanobacteria (Synechococcaceae), as well as *Planktothrix* sp. and one *Microcoleus autumnalis* culture. For this reason, if only one or a few cyanopigments are analysed, a marked portion of cyanobacterial biomass may go unaccounted for. In the present dataset, this may partially explain why the strongest relationship between individual cyanopigment concentrations and 16S rRNA gene concentrations varied across lakes. To overcome these biases and to draw inferences on the whole cyanobacteria community, we propose that future studies should analyse the suite of cyanobacterial pigments presented here (or a broader suite) and evaluate the sum of these pigment concentrations.

When analysed individually or in combination with one another, the pigments may also provide some information on the composition of the cyanobacterial community. For example, a recent study showed that ratios of selected pigments could be used to assess the relative abundance of picocyanobacteria and bloom-forming cyanobacteria in a sample (Puddick et al., 2021). Given that these data are required

when calculating the total cyanopigments values, it allows multiple levels of information on the cyanobacterial community to be obtained from the HPLC pigment analysis.

3.7.4 Reasons for discrepancies between the two methods

As noted above, the relationship between cyanobacterial 16S rRNA gene copy concentrations and individual cyanopigment concentrations varied across lakes and were not always strong. There are many plausible reasons for these discrepancies, primarily related to the fundamental difference in what each technique measures. The ddPCR assay measures the number of copies of the cyanobacteria-specific 16S rRNA gene in a sample, akin to a traditional microscopic cell count. However, some cyanobacteria can have multiple copies of the 16S rRNA gene within their genomes (e.g., two copies in *Microcystis* and picocyanobacteria such as *Synechococcus, Synechocystis*, and four copies in *Nostoc*; Schirrmeister et al., 2012; Větrovský et al., 2013); therefore, in these instances cyanobacterial abundance will be overestimated. Using an approach where ddPCR is coupled with a community characterisation technique such as metabarcoding may help in establishing periods where taxa with multiple 16S rRNA gene operons exist, allowing data to be normalised. However, this would be time-consuming and challenging because the exact number of 16S rRNA gene operons is not known for all species. Challenges linked to the 16S rRNA gene such as non-specificity and multiple copy numbers could be solved by targeting other genes such as those involved in pigment production, which are generally single copy. However, this would not solve other issues previously mentioned, such as the lack of databases, which would be required for designing universal primers. Besides, in contrast to gene copy numbers, pigment quotas are affected by cell size, irradiance, and nutritional status, in addition to species-specific characteristics (Serive et al., 2017). In the same way that ddPCR is close to an estimate of cell numbers, pigments are more likely to approximate biomass, albeit modulated by growth conditions and taxonomy. For example, two recent studies indicated that

the cyanobacterial communities in at least some of the study lakes included large numbers of picocyanobacteria (Picard et al., 2022a; Puddick et al., 2021). These are very small (<1 µm) and can be highly abundant even in oligotrophic lakes (Callieri, 2008; Caroppo, 2015). The ddPCR assay will give an even weight (provided they have the same number of 16S rRNA gene copies) to a picocyanobacteria cell as to a larger non-picocyanobacteria cell, whereas pigments will not.

A further reason for discrepancies between the two methods is that neither is truly cyanobacteria-specific. It is likely that the ddPCR primers are amplifying nontarget sequences, such as DNA from chloroplasts, while potentially missing some cyanobacteria. Likewise, some ‘cyanopigments’ are produced by organisms that are not cyanobacteria (e.g., zeaxanthin is known to be produced by some eukaryotic microalgae; Serive et al., 2017). Furthermore, the relationships between the two proxies were most variable near the top of the sediment cores. Both pigments and DNA are susceptible to degradation, and this is most evident at the top of sediment cores (Rydberg et al., 2020) where sharp declines in both proxies were observed in our data. However, the bottom of the Lake Okataina core had the highest 16S rRNA gene copies levels compared to the other cores, but the pigments show a different trend across lakes. This could be due to age differences across sediment cores, since the bottom of the Okataina core was younger than other cores (ca. 1700s in Lake Okataina vs. ca. 1300s or older in Lakes Nganoke and Pounui; Parrish, 2020; Caird, 2021; Pearman et al., 2021a; Table D.13). It is also likely that the rates of degradation vary between DNA and pigments, which would explain the discrepancies observed in downcore profiles. A strategy that could be used to minimise the impact of degradation on the results and is commonly applied in paleolimnological analysis is the normalisation of the data across samples, for example to total DNA, chlorophyll-a, or total pigments. Given that the aim of this study was to directly compare the methods in terms of concentration of cyanobacteria per sample, this was not undertaken here. A final consideration is that lake sediments vary in the degree and types of inorganic and organic material they contain, and this may change down a sediment core as it reflects changes in the landscape around a lake. The presence

of some compounds, for example, humic acid, can make extracting high-quality DNA and pigments challenging. For example, Pal et al., 2015 were unable to detect zeaxanthin and echinenone in a lake sediment core and proposed it could be due to the high humic acid content of the sediment core.

3.7.5 Comparing the pros and cons of ddPCR and HPLC to track historic cyanobacteria abundances

Pigment analysis using HPLC has been used for many decades in paleolimnological studies (Charette et al., 2003; Levine et al., 2012; Riedinger-Whitmore et al., 2005; Sanger, 1988; Waters et al., 2005), providing a wealth of comparative data. In contrast, ddPCR is a relatively new method (Hindson et al., 2011; Pinheiro et al., 2012), and a limited number of studies have used cyanobacteria-specific ddPCR assays. As discussed above, methodological choices, such as primer sets and data normalisation, need optimising. Evaluation of the relative value of these two techniques is thus based on different amounts of information. Capital expenditure for both techniques is relatively high (c. USD 57,000 /EUR 48,000 for HPLC systems and c. USD 121,000 USD/ EUR 102,000 for the QX200 automated ddPCR workflow in 2021). Robotics can be used during the DNA extraction step, making this process markedly quicker and more cost/time efficient than pigment extraction. Analysis of ddPCR samples is also quicker, with a batch of up to 96 samples being run in about four hours compared to HPLC which may only be able to analyse one sample per hour (depending on the chromatography adopted). Whilst ultra-performance liquid chromatography (UPLC) systems can be used to reduce the analysis times for cyanopigments, we have found that UPLC systems cannot achieve the required sensitivity and separation-efficiency for complex sediment extracts. Based on our analysis, including technician time, the cost per sample is approximately seven times cheaper on the ddPCR compared to HPLC when a batch of more than 20 samples is processed. As noted above the two methods provide different information on cyanobacteria, 16S rRNA gene copies is a proxy for cell density and pigments for

biomass, and each comes with caveats that need to be considered during interpretation. An advantage of ddPCR is that primers can target any gene of interest provided there is prior knowledge on its sequence, and multiple assays can be undertaken in one run (multiplexed) with limited additional costs. For example, to provide insights into the composition of the cyanobacterial community or target problematic species, multiplexed ddPCR assays could have up to four targets (QX200 system) or more targets (modern ddPCR systems becoming available now). Potential targets could be total cyanobacteria (using the cyanobacterial 16S rRNA gene as described here), specific cyanobacteria species or strains (by targeting more variable portions of the 16S rRNA gene or the intergenic-spacer sequence of the 16S and 23S rRNA genes), or cyanotoxin production (by targeting toxin production genes). Both ddPCR and HPLC require a high level of expertise to establish the assay on the user's system, and once set up a high level of laboratory skill is needed to ensure robust results. This study has demonstrated that both methods provide similar patterns in terms of difference among and within lakes in terms of cyanobacterial abundance. The pros and cons of each method (above and summarised in Table 3.3) should be considered when selecting which method is more appropriate for the specific aims and scopes of the paleolimnological study.

Table 3.3: Summary of the pros and cons of using droplet digital PCR (ddPCR) and high-performance liquid chromatography (HPLC) to infer historical abundance of cyanobacteria from lake sediment cores.

Method	Pros	Cons
HPLC	• Comparable with several decades of previously reported data.	• Medium capital investment • Long sample turnaround (1 week for extraction and analysis of a batch of approx. 20 samples). • Limited insight into cyanobacterial community composition. • High expertise level required. • Pigment degradation limits interpretation of more recent portions of sediment cores. • Potential inhibition due to co-extracted compounds.
ddPCR	• High throughput (up to 96 samples in one run). • Rapid sample turnaround (1 day to extract DNA and analyse samples). • Cost effective when multiple samples analysed simultaneously. • Can be targeted to specific genera or species and up to four assays can be assessed at the same time on one sample (tetraplexing). • Cost effective when a batch of samples is processed (about seven-times cheaper per sample than HPLC). • Not hindered by inhibitors in sample.	• Relatively new technique especially in paleolimnological research, so little comparative data. • High capital investment (twice the price of HPLC instrument). • Potential amplification of nontarget organisms. • Prior knowledge of sequences needed to design specific assays. • Potential multiple gene copies in one genome, therefore, not truly quantitative.

3.8 Conclusions

This study reported moderately strong correlations between cyanobacteria-specific 16S rRNA gene copies (determined by ddPCR) and four pigments (determined by HPLC) commonly used as cyanobacteria-specific markers in lake sediment cores. Positive relationships between these two analyses were detected, and the relationships were more consistent between lakes when all pigments were summed rather than considered separately. From an ecological perspective, and given that the ddPCR assay detects all cyanobacteria, using a sum of all pigments was more logical and increased the likelihood that a range of different species were detected. Variability of the relationship among lakes and down sediment core depths were evident, which are likely related to the composition of the cyanobacterial communities in each lake and the nuances of each analytical method. The two methods tested here provide proxies for two different cyanobacterial abundance measures: cell density (cyanobacterial 16S rRNA gene—ddPCR) and biomass (pigments—HPLC). It is important that this is acknowledged and that the caveats of each method are taken into consideration during method selection and interpretation of the results. When applied to sediment cores, ddPCR analyses would ideally be undertaken in parallel with cyanobacteria metabarcoding (e.g., Picard et al., 2022a) as this would provide a powerful approach to explore historic shifts in cyanobacterial communities alongside robust evaluation of cyanobacterial abundance through time.

3.9 Acknowledgments

We thank Ngāti Whātua o Kaipara, Te Arawa Lakes Trust, Ngāti Kahungunu ki Wairarapa, the landowners, Auckland Council, Bay of Plenty Regional Council, and Greater Wellington Regional Council for their assistance with sampling, accessing sites, and guidance throughout this work. The authors thank Mckayla Holloway, Katie Brasell, Sean Waters (Cawthron Institute), Rose Gregersen, and Andrew Rees (Victoria University of Wellington) for field assistance, and Lucy Thompson, Georgia

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Chapter 4

Optimal sample type and number vary in small shallow lakes when targeting non-native fish environmental DNA

4.1 Preliminary note

This paper under revision by the journal PeerJ (second round of revision).

The content of this chapter is therefore an exact copy of the manuscript submitted, except that (1) the lists of keywords and of references were omitted, (2) for consistency with the rest of the thesis, the citations were reformatted and the numbering of figures and tables adjusted.

4.2 Author list and author contributions

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M.P., A.T., G.T-L., S.W. performed the experiments; M.P. analysed the data, M.P. prepared figures and tables. All authors drafted and / or revised the manuscript for critically important content.

4.3 Abstract

Non-native fish have been shown to have deleterious impacts on freshwater ecosystems in New Zealand. Early detection is critical for their effective management. Traditional capture-based techniques may not detect newly introduced fish, especially if they are present in low abundance. Molecular techniques that target environmental DNA (eDNA) have been shown, in many instances, to be more sensitive, cost effective and require lower sampling effort. However, appropriate sampling strategies are needed to ensure robust and interpretable data are obtained. In this study we used droplet digital PCR assays to investigate the presence of two non-native fish in New Zealand, the European perch (*Perca fluviatilis*) and rudd (*Scardinius erythrophthalmus*) in three small lakes. Samples were collected from water and surface sediment at near-shore and mid-lake sites. Probabilistic modelling was used to assess the occupancy of fish eDNA and develop guidance on sampling strategies. Based on the detection probability measures from the present study, at least six sites and five replicates per site are needed to reliably detect fish eDNA in sediment samples, and twelve sites with eight replicates per site for water samples. The results highlight the potential of developing monitoring and surveillance programs adapted to lakes, that include the use of assays targeting eDNA. This study focused on small shallow lakes, and it is likely that these recommendations may vary in larger, deeper, and more geomorphologically complex lakes, and this requires further research.

4.4 Introduction

The introduction of non-native fish species into aquatic ecosystems can pose a major threat to local biodiversity. Once established they can disrupt food webs through

top-down trophic cascades (Dextrase et al., 2006; Bellard et al., 2016) and reduce water quality with adverse consequences for ecosystem services, human health, and the economy (Keller et al., 2009; Pejchar et al., 2009; Strayer, 2010; Ricciardi et al., 2013). A well-known example is the global spread of carp (*Cyprinus carpio*). In many lakes, their introduction has caused degradation in water quality with lakes flipping from clear water, macrophyte-dominated to turbid, phytoplankton-dominated, which has resulted in the loss of native biodiversity (Williams et al., 2002; Parkos III et al., 2003; Koehn, 2004; Miller et al., 2006; Kloskowski, 2011). Similarly, multiple non-native species such as the brown bullhead (*Ameiurus nebulosus*) and pumpkinseed (*Lepomis gibbosus*) have been introduced in North America, degrading waterways and changing food webs (Dextrase et al., 2006). To mitigate the impact of non-native fish, limiting their spread is paramount. The likelihood of successful control or eradication is enhanced if non-native species are detected soon after incursion and if their spread can be effectively monitored to measure the success of management response (Hulme, 2006; Britton et al., 2011; Rytwinski et al., 2018).

Newly introduced species are usually not very abundant, and early detection is a critical first step that may assist in effective management (Mehta et al., 2007; Roux et al., 2009; Britton et al., 2010). However, traditional approaches to detect and monitor fish (e.g., nets, electrical fishing, spotlighting) are time-consuming, costly, and can underestimate their spread or completely overlook their presence (Sigsgaard et al., 2015; Thomsen et al., 2015). Recent molecular approaches which target environmental DNA (eDNA) are promising tools for effective and non-intrusive surveying of fish (Darling et al., 2011). Environmental DNA refers to the genetic material cfrom whole cells or released by organisms in the form of tissue slough, shed or lysed cells, gametes, excretion and saliva, among other secretions (Taberlet et al., 2012; Taberlet et al., 2018; Pawłowska et al., 2020). Approaches that target eDNA are often more sensitive than traditional methods (Jerde et al., 2011; Ardura et al., 2015; Gantz et al., 2018), are not directly reliant on taxonomic expertise, and relatively simple, standardised sampling and analytical protocols can be developed. Quan-

titative PCR techniques also allow the precise quantification of target gene copies in environmental samples, and in some instances eDNA concentrations have been shown to be indicative of species biomass (Takahara et al., 2012; Doi et al., 2015; Eichmiller et al., 2016; Lacoursière-Roussel et al., 2016; Capo et al., 2019b; Rourke et al., 2022).

To date, most studies targeting eDNA to detect or track aquatic species have collected and analysed water samples (Rees et al., 2014), with a key rationale being that DNA degrades relatively rapidly (within days to weeks) therefore detection in these samples likely indicates recent presence (Buxton et al., 2017). However, given the mobility of fish and the labile nature of DNA, studies have shown that fish eDNA can be patchy in water samples (Eichmiller et al., 2014; Lawson Handley et al., 2019). Targeting surface sediment may allow for a more time-integrated approach, providing information on the presence of fish over the last few months to years. Some studies to date indicate higher levels and slower decay rates in sediment compared to water (Eichmiller et al., 2014; Turner et al., 2015; Sakata et al., 2020), while others have shown higher detection in water compared to sediment samples (Buxton et al., 2018; Valdez-Moreno et al., 2019). Further research is required to compare the detection of fish eDNA between water and sediment samples and to gain further insights into how this varies among species to assist with optimizing the use of eDNA approaches for non-native fish surveillance.

While many studies have been published on the application of eDNA monitoring for fish in rivers (e.g., Pont et al., 2018; Cantera et al., 2019; Rourke et al., 2022), there is less data on the spatial variation of eDNA within lakes or on how to develop appropriate sampling designs. Depending on the season, fish may occupy different habitats (e.g. pelagic or littoral), be more or less active and the water column may be stratified or fully mixed, all of which affects eDNA dispersal (Klobucar et al., 2017; Lawson Handley et al., 2019; Littlefair et al., 2021). Occupancy modelling has now been applied to data generated from eDNA surveys, to assist in understanding such spatial and temporal detectability variations (Schmelzle et al., 2016; Smith et al., 2020; McClenaghan et al., 2020). Occupancy modelling estimates the distribution of

a target (such as a species or its eDNA) in a given environment and the probability that it will be detected, while accounting for imperfect detection.

New Zealand is an island nation in the Southwest Pacific that has been isolated from other landmasses for about 85 million years (Daugherty et al., 1993). Isolation allowed unique flora and fauna to evolve, which has been heavily impacted by the introduction of non-native species over the last approximately 700 years (Towns et al., 1997). New Zealand's freshwater systems have been subjected to multiple introductions over this period, with a range of documented consequences including decreases in water quality and predation on native fish (Rowe, 2007; McIntosh et al., 2010). Two fish species of considerable concern are European perch (*Perca fluviatilis*, hereafter perch) and rudd (*Scardinius erythrophthalmus*). Perch are carnivorous coarse fish which were introduced for angling in the 1870s. This pelagic species is voraciously zooplanktivorous during its juvenile stage and becomes piscivorous as an adult. It is now well-established in many lowland lakes with causative links to declines in native fish and zooplankton communities (Romare et al., 1999; Rowe, 2007) and increases in cyanobacterial blooms (Smith et al., 2006; Smith et al., 2007). Rudd is a benthopelagic coarse fish which was introduced in 1967 (Department of Conservation, 2006).In New Zealand, the adults prefer to feed on native macrophytes, therefore their grazing impact has been linked to macrophyte collapse, with concomitant declines in water quality (Hicks, 2001; Lake et al., 2002). The feeding strategies of both rudd and perch differ from those of native fish, making New Zealand lake ecosystems particularly vulnerable to their introduction. It is now forbidden to sell or intentionally breed and propagate either species (Biosecurity Act 1993). However, perch is legally classified as a sport fish (Freshwater Fisheries Regulations 1983), while rudd is a noxious fish in all parts of the country except for the Auckland/Waikato region where it is considered a sport fish. Although the spread of perch and rudd between lakes is now limited, there continues to be intentional and accidental secondary introductions across the country (Mitchell, 2020).

The overall goal of this study was to optimize the use of assays targeting eDNA for the detection of non-native fish in small, shallow lakes. The aims of this study

were to determine; (1) which sample type, water or surface sediment, and which location, near-shore or mid-lake, would be best suited to detect perch and rudd in small shallow lakes, and (2) how many sites and replicates would be needed to reliably detect fish eDNA. Water and sediment samples were collected from fourteen sites in three lakes. Species-specific droplet digital PCR assays were used to detect eDNA and the results analysed using occupancy modelling. Sampling was designed to test three hypotheses, being 1) eDNA will be homogeneously distributed across lakes for both species but due to their life history (benthic rudd versus pelagic perch), 2) rudd eDNA will be better detected in sediment samples while 3) perch eDNA will be better detected by water samples.

4.5 Materials and Methods

4.5.1 Sampling sites

Three small, lowland lakes in the North Island of New Zealand were sampled: Pounui, Waitawa and Tomarata (Figure 4.1, Table 4.1). Fourteen sites were sampled in each lake using a small motorboat or canoe, seven near the shore (generally <3 m from the shoreline) and seven at mid-lake (>7 m from the lake edge, Figure 4.1, Table D.17). Field work was undertaken in spring, with cyanobacterial blooms observed in Lakes Pounui and Waitawa during sampling. Previous catch data indicate that Lake Waitawa has two to three times more perch than rudd, and Lake Waitawa has about three times more perch than Lake Pounui (Drake et al. (2011), Alton Perrie pers. comm.). Further lake characteristics and information on the presence of native and non-native fish are provided in Table 4.1. All samples were collected under the specifications of Special Permit 651 from the New Zealand government agency Ministry for Primary Industries.

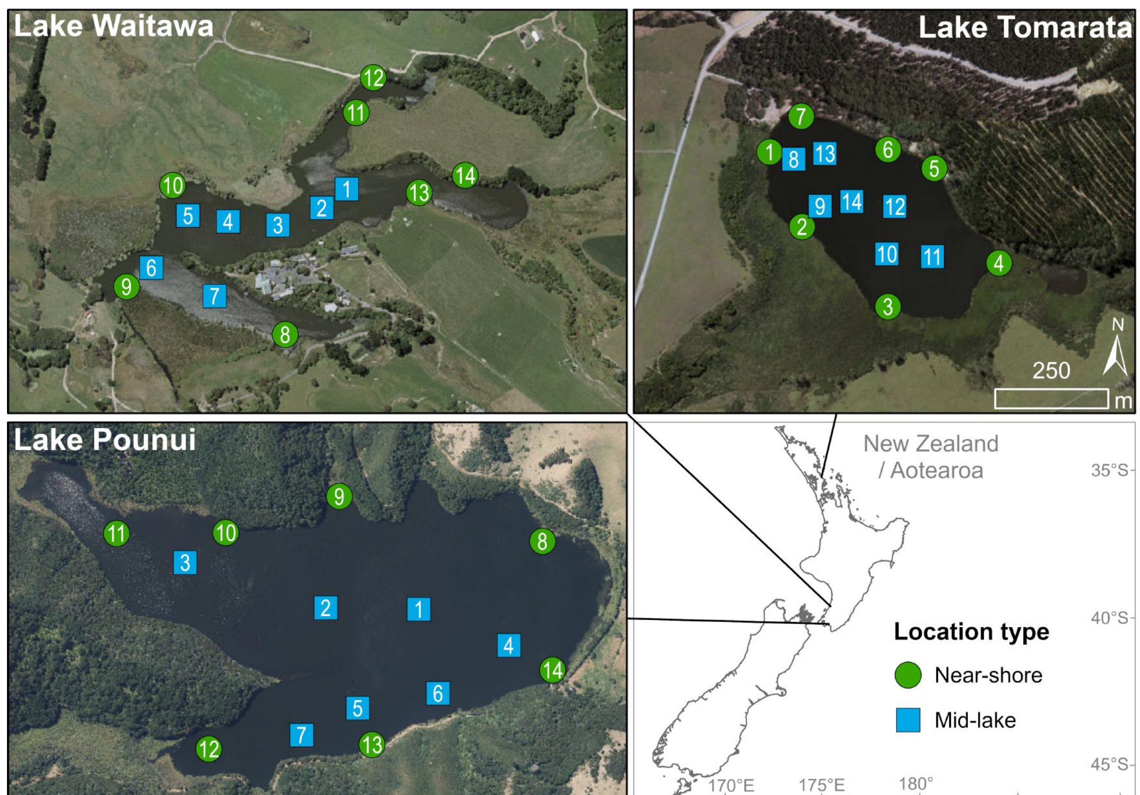

Figure 4.1: Sampling sites and location (near-shore vs mid-lake) in each lake. Created with ArcGIS 2.9 Desktop.

Table 4.1: Lake characteristics and fish community composition. Descriptions of the land cover in the catchments were derived from the Land Cover Database version 5 (Landcare Research New Zealand Ltd). Sources for fish catch data are: 1. Drake et al., 2011. 2. Greater Wellington Regional Council monitoring (Alton Perrie pers. comm).

	Pounui	**Tomarata**	**Waitawa**
Sampling date	4 December 2018	18 November 2018	13 October 2019
Location (Lat/Long)	41°20'41.3"S 175°06'47.7"E	36°11'36.0"S 174°39'00.3"E	40°43'28.3"S 175°10'21.6"E
Lake area (km^2)	0.46	0.144	0.158
Max depth (m)	9.6	5.6	5
Main catchment vegetation	Native vegetation (83%)	High producing grasslands (>50%)	High producing grasslands (>90%)
Non-native fish	European perch (*Perca fluviatilis*), brown trout (*Salmo trutta*)	Goldfish (*Carassius auratus*), Koi carp – may be eradicated (*Cyprinus rubrofiscus*), rudd (*Scardinius erythrophthalmus*), tench (*Tinca tinca*), rainbow trout – stocked (*Oncorhynchus mykiss*)	European perch (*P. fluviatilis*), goldfish (*C. auratus*), Koi carp (*C. rubrofiscus*), rudd (*S. erythrophthalmus*), tench (*T. tinca*)
Native fish	Longfin eel (*Anguilla dieffenbachii*) [1,2], shortfin eel (*Anguilla australis*) [1,2], common bully (*Gobiomorphus cotidianus*) [1,2], īnanga (*Galaxias maculatus*) [1]	Longfin eel (*A. dieffenbachii*) [1], shortfin eel (*A. australis*) [1], common bully (*G. cotidianus*) [1], īnanga (*G. maculatus*) [1]	Longfin eel (*A. dieffenbachii*) [1,2], shortfin eel (*A. australis*) [1,2], common bully (*G. cotidianus*) [1,2]

Table 4.2: Primer pairs used in this study. The amplicon length in base pairs (bp) are indicated for each marker gene. The rudd assay was tested on other fish DNA extracts and samples of known composition to check the specificity of the assay in-vivo (Table D.18 and D.19).

Target	**Marker**	**Primer name**	**Sequence**	**Source**
Perca fluviatilis	12S rRNA gene (92 bp)	P.flu_12S_F	Forward: 5'-GGGATTAGATACCCCACTATGCCT-3'	Furlan et al. (2016)
		P.flu_12S_R	Reverse: 5'-GGTTTCAAGCTGATGCTCGTAGTT-3'	
		P.flu_12S_P	Probe: 5'-(FAM)-CCATAAACATTGGTAGCACACT-(MGB)-3'	
Scardinius erythrophthalmus	16S rRNA gene (188 bp)	S.eu_16S_F	Forward: 5'-CACGTTAAACGACTCTGTAG-3'	This study
		S.eu_16S_R	Reverse: 5'-GTTCGTTGATCGGCTTTATCAGT-3'	
		S.eu_16S_F1	Probe: 5'-(HEX)-AGTGGCGAA-(ZEN)-TGAAGTTTTACCTTCG-(Iowa Black)-3'	

4.5.2 Sediment geochemistry

A single surface sediment sample was collected using a Ponar grab from the deepest part of each lake. The top 2 cm of the grab were collected using spatulas and placed in 500 mL containers. Sediment was homogenised, stored chilled (4°C) and shipped to the laboratory within 48 hrs for nutrient and elemental characterisation. Once in the laboratory, sediment was homogenised again, centrifuged (3,000×g, 40 min, 4°C), and the pore water decanted. Leftover sediment was dried and passed through a sieve (2 mm) for metal analysis using acid digestion followed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) analysis based on the US Environmental Protection Agency (EPA) method 200.8. The metals analysed were aluminum (Al), calcium (Ca), cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), phosphorus (P), lead (Pb), zinc (Zn) and sulfur (S). Reporting limits (mg kg-1) were: 12.5, 0.125, 2.5, 12.5, 0.05, 0.075, 0.05, 0.005, 10 and 250, respectively. Total Organic Carbon (TOC) and Total Nitrogen (TN) were analysed using catalytic combustion at 900°C (O2) and separation using a Thermal Conductivity Detector (reporting limit for both g/100g). Organic matter was measured using over drying, ashing (550°C), and gravimetric determination. Grain size distributions were determined using a laser diffraction particle size analyzer at the University of Waikato (Hamilton, New Zealand).

4.5.3 Rudd assay development

Two primers and a probe were designed to target rudd (*Scardinius erythrophthalmus*) without cross-amplifying native and other exotic fish that are present in New Zealand. Rudd sequences (16S mitochondrial DNA) were aligned with 87 fish sequences using the aphid R package (Wilkinson, 2019) , and primers and probe designed with the Geneious software (*Geneious* n.d.) (Supplementary Table D.17). The resulting amplicon was 101 bp (Table 4.2).

In-vivo, this primer set successfully amplified rudd DNA and did not cross-amplify the DNA of eleven other fish species (Supp. Tables D.18, D.19). Specifically,

this rudd assay did not amplify genomic extracts of brown bullhead catfish, goldfish, koi carp, *Gambusia*, European perch, and tench (Supplementary Table D.18). Environmental DNA samples were also tested with this rudd assay (qPCR), and then sequenced with an iSeq instrument (metabarcoding). This comparison revealed that the present rudd assay was able to detect rudd eDNA when it was present in the sample, and yielded no detection when rudd DNA was absent in the sample. Using this method, this assay did not cross-amplify the eDNA of brown bullhead catfish, short-finned eel, goldfish, *Gambusia*, skipjack tuna, rainbow trout, smelt, brown trout, and tench, which were present in the samples when rudd detection was negative (Supp. Tables D.18, D.19).

4.5.4 Environmental DNA sample collection

Two sample types were collected at each site: surface water (hereafter referred to as water samples) and lakebed surface sediment (hereafter referred to as sediment samples). Samples were taken in duplicate at each site, i.e., a total of 28 water and 28 sediment samples from each lake, for a total of fifty-six samples per lake. Water samples were collected in 1L plastic bottles from the lake surface. They were kept refrigerated during transport and filtered within 24 h (GF/C filters, pore size 1.6 µm, 47 mm dia., Whatman, UK). Two negative controls (tap water) were also included at the beginning and end of filtering for each lake. The filters were halved with sterilised scissors, the two half filters placed in separate Eppendorf tubes and immediately frozen (-20°C). Water samples were collected prior to sediment sampling for every site to avoid contaminating the water samples with sediment. Due to the presence of heavy cyanobacterial blooms in Lake Waitawa, water samples were prefiltered through a 50 µm mesh to remove particles likely to clog the GF/C filters. Sediment samples were collected using a Ponar grab which was dropped two times. Undisturbed surface sediment (c. 3 g) layer (max. 0.5 cm depth) was sampled using a sterilised spatula and placed in sterile 5 mL tubes. Samples were chilled during transport and frozen (-20°C) within 2 h. Sampling equipment was soaked in 2%

bleach solution (>2 min) and rinsed three times in lake water between sampling sites. Filtering equipment was also soaked in 2% bleach solution (>2 min) and rinsed three times with tap water between filtering different samples.

4.5.5 DNA extraction

Sediment samples up to 3 g were extracted using a combination of an alkaline lysis method with ethanol precipitation followed by the DNeasy PowerSoilTM kit (Qiagen, USA), as detailed in Thomson-Laing et al., 2022. For the lysis step, sodium hydroxide (6 mL, 0.33M) and Tris-EDTA (3 mL, pH 8) were added to the sediment samples in sterile 15 mL tubes. The tubes were thoroughly mixed by benchtop vortex (1 min), and incubated (65°C, 50 min). The samples were cooled to room temperature and centrifuged (3214 g, 1 h). Part of the supernatant (7.5 mL) was transferred to a sterile 50 mL tube and the lysis step neutralised with the same volume of Tris HCl (7.5 mL, 1 M, pH 6.7). For the precipitation step, sodium acetate (1.5 mL, 3M, pH 5.2) and ethanol (30 mL, molecular grade 100%) were added in the same tube and samples were stored at -20°C (> 12 h). Finally, samples were centrifuged (3,200 $\times$g, 1 h) and the supernatant discarded. These extraction steps were undertaken in batches of 20 including a blank control every second batch.

Following ethanol precipitation, the entire sediment pellet (up to 0.5 g) was transferred to the bead beating tube of a DNeasy PowerSoilTM kit (Qiagen, USA) using a sterilised spatula. Similarly, one half of the filters from the water samples was cut in smaller pieces and transferred to bead beating tubes. DNA was extracted from the filters and sediment pellet using the DNeasy PowerSoilTM kit (Qiagen, USA) and the automated QIAcube instrument (Qiagen, USA) following the manufacturer's protocol. Samples were extracted in batches of 10-12 samples, with extraction controls included every two batches (same controls as the pre-extraction method).

4.5.6 Single-species quantification with droplet digital PCR

Each following step was conducted in a separate sterile laboratory, with sequential workflow to ensure no cross-contamination. Rooms dedicated to PCR set-up and template addition were equipped with ultra-violet sterilisation which was switched on for a minimum of 15 min before and after each use. The ddPCR set-up and template addition were undertaken in laminar flow cabinets with HEPA filtration. Aerosols barrier tips (epT.I.P.S., Eppendorf, Hamburg, Germany) were used throughout.

Droplet digital PCR was used to quantify target mitochondrial genes from perch (12S rDNA) and rudd (16S rDNA; Table 4.2). Following the recommendations of BioRad when applying ddPCR probe assays on environmental samples, two restriction enzymes were used to help cleave non-target DNA: HindIII-HF and HaeII (New England Biolabs, Ipswich, Massachusetts, United States). All primers and probes were run in duplex ddPCR analyses, using a BioRad QX200 system. Each ddPCR reaction included 1 µL (10 µM) of each primer and probe, 10 µL of 1× ddPCR Supermix for probes with no dUTP (BioRad, Hercules, California, United States), 3 units of each restriction enzyme, and 6 µL of template DNA for a total reaction volume of 22.45 µL.

The BioRad QX200 droplet generator partitioned each reaction mixture into nanodroplets by combining 20 µL of the reaction mixture with 70 µL of BioRad probe droplet oil. After processing, this resulted in a total nanodroplet volume of 40 µL, which was transferred to a PCR plate for amplification using the following cycling protocol: hold at 95°C for 10 min, 39 cycles of 94°C for 30 s, 57°C for 1 min, and a final enzyme deactivation step at 98°C for 10 min. The plate was then analysed on the QX200 instrument. For each ddPCR plate run, at least one negative control (containing all reagents and RNA/DNA-free water) and one positive control (DNA extracted from perch and rudd tissue samples) were included.

To take into account the possibility of PCR inhibition, any sample with less than 10,000 total droplets was re-run. The minimum number of droplets in any sample in

this study was 18,244. Furthermore, following the methods from Dingle et al. (2013), a broad amplitude threshold was defined to account for inhibited positive droplets. In short, the threshold for positive droplets was set just above the cloud of negative droplets to account for variations in the amplitude of positive droplets. Last, if only one positive droplet was detected, the sample was re-run twice to help avoid false-positive detections. The sample was considered positive if two out of the three final technical replicates were positive, and the final concentration was calculated as the mean of the two positive technical replicates. Droplets counts were normalized with a Poisson distribution by the QuantaSoft Analysis software (BioRad), and are reported as the concentration of gene copies concentration (copies/µL) per ddPCR reaction.

Thresholds to differentiate positive vs negative droplets were determined for both assays by diluting the positive controls and running negative controls alongside. Amplitude thresholds were set at 2734 for perch and 1735 for rudd, as per methods from Capo et al. (2019b). The limit of quantification (LoQ) of the duplex assay was calculated as per methods used in Brys et al. (2021) and Picard et al. (2022a). The combined perch and rudd DNA extracts used as positive controls were measured on a nanofluorometer and diluted in 6 steps (1 ng/µL down to 10-5 ng/µL). These dilutions were measured on the ddPCR system using the duplex perch / rudd assay with three replicates per dilution step. The calculated concentrations (ng/µL) were then plotted against the measured concentrations (copies/µL) using a LOESS smoothing function, and the LoQ was determined as the threshold where the measured trend started to differ from the expected trend (Supp. Fig. D.18).

4.5.7 Data analysis

Data analysis and plots were performed with the R (R Core Team, 2021) and RStudio software (RStudio Team, 2022), using the Tidyverse and its associated packages (Wickham et al., 2019). Raw ddPCR concentrations as well as weight- or volume-normalised ddPCR concentrations were compared across lakes, species,

sample types, and locations using boxplots. To enable the comparison of eDNA levels across sample type, 1 mL of water was assumed to be equal 1 g so that both samples type could be plotted as gene copy numbers per gram (Sakata et al., 2020). The data were neither normally distributed nor homoscedastic and included null values, therefore it was transformed to log10 +1 before testing overall differences across locations and across sample type depending on the fish species, using the non-parametric Kruskal-Wallis test. Differences across locations for a given lake and sample type were also tested and these significant differences were displayed on the boxplots.

Occupancy modelling was used to estimate the probability of target eDNA being present at a given sampling site and detection probability for a given lake under a range of sampling strategies. Briefly, the models were used to test which combination of sampling method and location was best to detect fish eDNA. The environmental DNA levels were transformed into presence-absence for all biological replicates to run the occupancy models and sampling design simulations to optimize for the best detection probabilities for each lake and fish target. Since perch eDNA was only detected in one sample in Lake Tomarata, perch data from this lake were excluded from further analysis. Occupancy models were undertaken for perch eDNA in Lakes Pounui and Waitawa and rudd eDNA in Lakes Tomarata and Waitawa. Occupancy modelling was performed using the PRESENCE v12.31 software (MacKenzie et al., 2002; Nichols et al., 2008) to estimate the eDNA occupancy (Ψ) and the detection probability (p) for each target fish per lake (see details below). A single-season multi-method model variant was applied to compare sediment and water samples. The following parameters were estimated in each model:

ψnaïve = naïve estimate of occupancy probability or proportion of area occupied (PAO), calculated as the number of sites where fish eDNA was detected over all sites surveyed;

Ψ = large-scale occupancy, i.e., probability of sample unit being occupied by a target eDNA;

ϑ = small-scale occupancy or model-estimated occupancy, i.e., the probability

that the target eDNA is present in the direct vicinity of the sampler and available for collection at a sampling time, given that sample unit is occupied;

p = probability of target eDNA detection at a sampling time by method m, given that sample unit is occupied, and target eDNA is present at immediate sampling location.

Sampling sites (n = 14) were treated as sampling units and biological replicates (n = 2 for sediment and water, total n = 4) as repeated surveys. In the predefined models, detection probabilities were assessed for each sampling method (i.e., water vs. sediment) and location within the lake (i.e. mid-lake vs. near-shore, n = 7 each). All model combinations (hereafter models variants) and their rankings for each species and lake can be found in Supplementary Figures D.21 to D.24.

Model variants were ranked by the PRESENCE software according to their Akaike Information Criterion (AIC) values, and the lowest AIC was used to select the best model for each species per lake. Several parameters were extracted from the model summary (ψnaïve, Ψ, ϑ, p) to understand the distribution and detection probability of fish eDNA in each lake. Code from Guillera-Arroita et al., 2010 was then used to run simulations of detection histories in RStudio. Briefly, the simulations allowed us to specify several parameters (Ψ, p, number of sites sampled, number of replicates) to test whether the number of sites and replicates were adequate to detect the target given a specific occupancy (Ψ) and detection probability (p). We used these simulations to estimate how many sites and replicates were needed in a worst-case monitoring scenario (i.e., using the lowest probability detections) to detect fish eDNA using ddPCR in lakes of similar size. Simulations were run 10,000 times as described in Guillera-Arroita et al., 2010, and since all occupancy models showed constant occupancy, the Ψ parameter was set at 1 (100%). Avoiding false negative detection (i.e., complete lack of false negatives across all sites and replicates within the lake) was selected as the most important criterion (estimated empty histories = 0%), and the best simulation (per sample type) identified minimum meaningful sampling effort while reducing the potential bias, with less than 5% of standard error rates on the estimates and on false negative detections.

4.6 Results

4.6.1 Sediment geochemistry

The sediment in the mid-lake zone of Lake Waitawa had a fine gelatinous consistency which was not observed in the other two lakes. Waitawa sediments were very low density (21 kg.m-3 dry weight) compared to medium density for Lakes Tomarata and Pounui (respectively 205 kg.m^{-3} and 128 kg.m^{-3}). Grain size was skewed away from fine particulates with high surface area to mass. Only 28% of the Waitawa sediment had a grain size $\leq$ 63 µm compared to 58% in Tomarata (no results for Pounui). A relatively high organic to ash content was also measured for Waitawa and overall low concentrations of iron (Supp. Table D.20).

4.6.2 Overall detections

The eDNA of the target species were not detected in any of the negative control samples. Perch eDNA was detected in Lakes Pounui, Tomarata, Waitawa. Rudd eDNA was detected in Lakes Tomarata and Waitawa. Fish eDNA was rarely detected at all sites for a given sample type except in sediment samples (perch) in Lake Pounui and water samples for Lake Waitawa (perch and rudd, Figures 4.2 and 4.3). Fish eDNA levels varied greatly depending on the lake and sample type, from 0.05 to 1.9 gene copies/µL of ddPCR reaction in water samples and from 0.05 to 7.1 gene copies/µL of ddPCR reaction in surface sediment (Figure 4.2). The Limit of Quantification (LoQ) was calculated at 13 copies/uL for perch and 5 copies/uL for rudd (Supp. Fig. D.18), and the highest eDNA levels were in Lake Waitawa for both sample type and both species (Figure 4.2). Environmental DNA levels were normalised to gene copy numbers per liter or per gram in Supp. Fig. D.19.

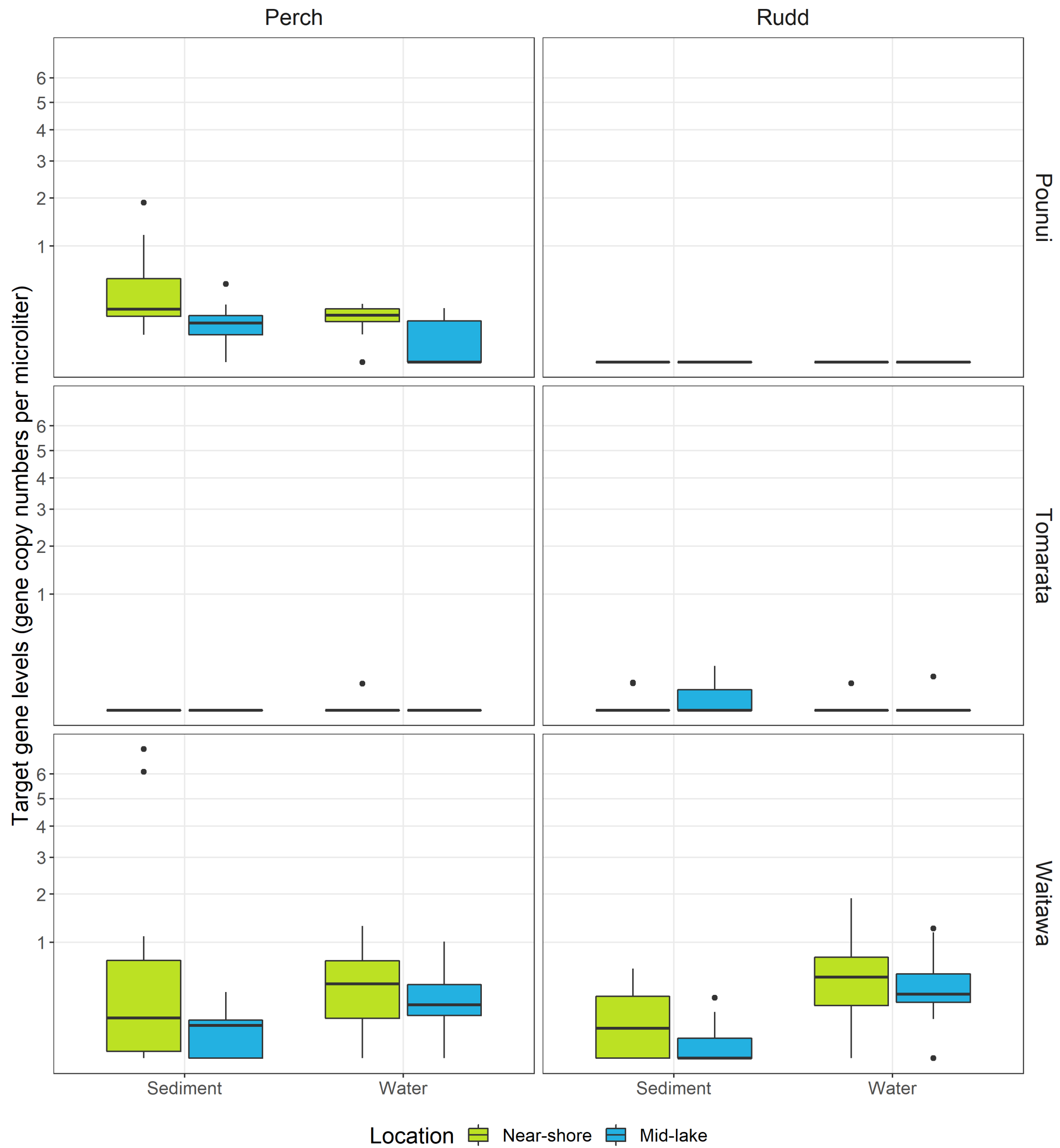

Figure 4.2: Target gene levels for each fish species per lake (12S rDNA for perch and 16S rDNA for rudd), separated by sampling method (sediment and water) and sampling location (near-shore and mid-lake). The concentrations displayed are the raw output from droplet digital PCR, in gene copy numbers / µL. Concentrations are plotted on a square root scale.

4.6.3 Detection comparison for Perch and Rudd

Perch eDNA was detected in 52% of all biological replicates (88 out of 168 samples) across all lakes and at 29 out of 42 sites (69%). This included all sites in Lakes Pounui and Waitawa, and one site in Lake Tomarata (although perch is not known to occur in this lake). Perch eDNA levels were significantly higher at near-shore sites versus mid-lakes sites in Lake Pounui (Kruskal-Wallis test H 15.296, df = 1, p < 0.001, Figure 4.2). Very high perch eDNA levels were detected in the sediment samples of Lake Waitawa (sites 9 and 12, 7 and 6 gene copies/uL, respectively) compared to other samples which were overall lower than 1 gene copy/uL (Figure 4.2). Once these outliers were removed, eDNA levels were significantly higher in the water samples of Lake Waitawa compared to its sediment samples (Kruskal-Wallis test H 10.067, df = 1, p-value = 0.002).

In general, detection patterns for perch eDNA, as presence-absence, were similar among sediment and water samples with 79% similarity among sample types (no detection at 13 sites, detection in 23 sites by both, four only in sediment, two only in water). However, the trends were different within each lake (Figure 4.3). In Lake Pounui, sediment samples yielded more detections than water (93% detection in sediment vs. 64% detection in water). Conversely, the opposite pattern was observed in Lake Waitawa (64% sediment, 89% water). Spatial patchiness, as evidenced by the number of sites where perch was not detected, varied by lake: eDNA was patchy in water for Lake Pounui and found at all sites by sediment, while the opposite pattern was observed for Lake Waitawa.

Rudd eDNA was detected in 27% of all biological replicates (46 out of 168 samples) and 21 of 42 sites (50%) across all lakes. The type of sample (sediment or water) yielding the highest detection varied between lakes, and there was only 52% similarity overall in detection among sample types (11 sites by both, five sediment only, five water only, Figure 4.3). Detection was highest in sediment in Lake Tomarata (21% sediment and 7% water) while water yielded better detection in Lake Waitawa (43% sediment and 93% water). Rudd eDNA was patchier in sediment samples for

Lake Waitawa with the opposite pattern observed at Lake Tomarata. Like for perch, rudd eDNA levels were significantly higher in water samples compared to sediment for Lake Waitawa (Kruskall-Wallis $H = 20.699$, $df = 1$, $p\text{-value} < 0.001$) while there was no significant difference in Lake Tomarata.

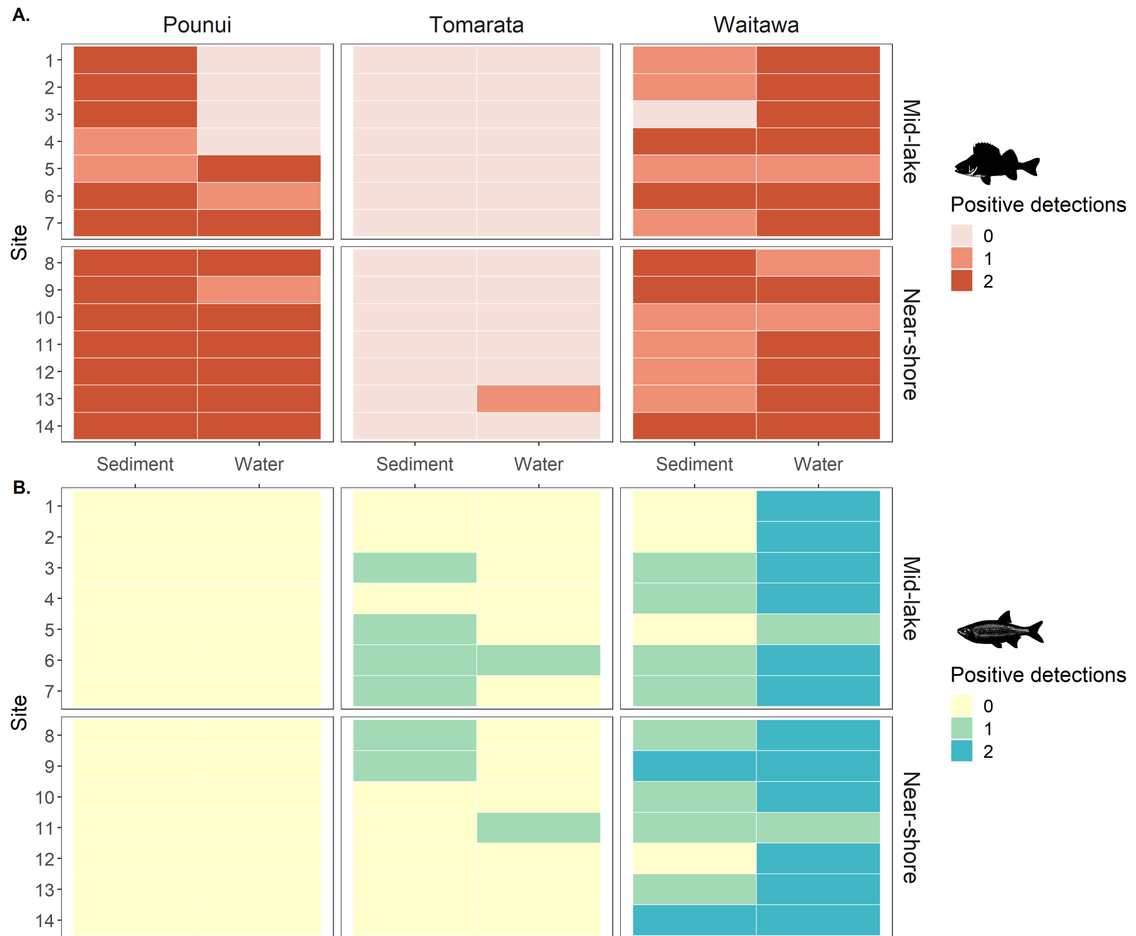

Figure 4.3: A. Perch (*Perca fluviatilis*) and B. Rudd (*Scardinius erythrophthalmus*) eDNA detection in each biological replicate across lakes, sites, location, and sample type. The genes targeted were 12S rDNA for perch and 16S rDNA for rudd.

4.6.4 Occupancy modelling

Sixteen model variants were run for each lake and species (Table D.21 to D.24), accounting for different combinations of sample type and location effect on target eDNA occupancy and probability of detection. The models indicated that, across all samples, perch and rudd eDNA was detected across all sites (ψnaïve = 100%) when the species was present in the lake, while rudd eDNA was only detected at 50% of

Table 4.3: Parameters derived from the best occupancy model for each fish's eDNA within each lake. The best model was selected by its Akaike Information Criterion, and the PRESENCE software also indicated the likelihood that it explained the data compared to the other models (see supplementary Table D.21 to D.24). The estimates for significant combinations of sample type and location identified by the model are shown, best combinations are in bold.

	Pounui	**Tomarata**	**Waitawa**	
Target	Perch	Rudd	Perch	Rudd
Likelihood compared to second-best model	96 %	19 %	61 %	7%
Proportion of sites occupied (Ψnaïve)	100%	50%	100%	100%
Large-scale occupancy (Ψ)	Constant distribution (1)	Constant distribution (1)	Constant distribution (1)	Constant distribution (1)
Small-scale occupancy (ϑ)	Constant distribution (1)	Constant distribution (1)	Constant distribution (1)	Constant distribution (1)
Detection probability	Method-dependent (sediment) and location-dependent (near-shore) Mid-lake sediment: p = 0.86 ± 0.09 **Near-shore sediment**: p = 0.99 ± 0.008 Mid-lake water: p = 0.35 ± 0.13 Near-shore water: p = 0.93 ± 0.06	Method-dependent **Sediment**: p = 0.21 ± 0.08 Water: p = 0.07 ± 0.05	Method-dependent Sediment: p = 0.64 ± 0.09 **Water**: p = 0.89 ± 0.06	Method-dependent Sediment: p = 0.43 ± 0.09 **Water**: p = 0.93 ± 0.05

the sites in Lake Tomarata (Table 4.3). All best-ranking models indicated constant distribution of eDNA across the lakes (both small- and large-scale occupancies), irrespective of the sampling location (near-shore or mid lake).

Detection probabilities varied depending on the sampling method and location. They were method-dependent for rudd eDNA in Lake Tomarata (sediment better than water in best and second-best models) and for perch and rudd eDNA in Lake Waitawa (water better than sediment). Perch eDNA in Lake Pounui was most likely to be detected in near-shore sediment samples (Table 4.3). The second-best model for rudd in Lake Waitawa ranked only 7% behind the first one and indicated that detection probabilities could be method and location-dependent, with near-shore water samples yielding the best detection. The probability of detecting fish eDNA was the highest (across the whole dataset) in near-shore sediment samples in Pounui ($p = 0.99 \pm 0.008$), and lowest in water samples in Lake Tomarata ($p = 0.07 \pm 0.05$).

To estimate the minimum required sampling effort, i.e., the number of sampling sites and replicates at each site, for different probabilities of detection, simulations were run assuming fish eDNA is present and consistent across the lakes ($\Psi = 1$), for two, three and four replicates per site, and for detection probabilities of 0.1 to 0.9, (Figure 4.4). Design estimates showed that adding more replicates decreased the number of sites needed for the same detection probability when $p < 0.9$, however for $0.3 \leq p \leq 0.9$ having three or four replicates made little difference.

The occupancy modelling calculated that the worst detection probability (p) for both methods were for rudd eDNA in Lake Tomarata (Table 4.3). Therefore, in a lake with low species abundance, to sample as few sites as possible and just by considering the detection probabilities of this study (at worst $p = 0.21$ for sediment and $p = 0.07$ for water, Lake Tomarata), the simulations indicated that at least six sites and five replicates (3 g) per site were needed to reliably detect fish using eDNA approaches in sediment samples (Table D.25) and twenty sites with eight replicates (500 mL) per site were needed for water samples (Table D.26).

Since the detection probabilities were very high for perch and rudd eDNA in Lake Pounui and Lake Waitawa, the simulations calculated that only two sites and

two replicates per site were sufficient for reliable detection (Table D.27 to D.29; illustrated by a star in Figure 4.4). The ideal sample type and location were suggested based on the occupancy model results (Table 4.3): in Lake Pounui, surface sediment from two sites near the shores would yield the best results (two replicates per site), while in Lake Waitawa, surface water from two sites anywhere in the lake would work better for both species (two replicates per site).

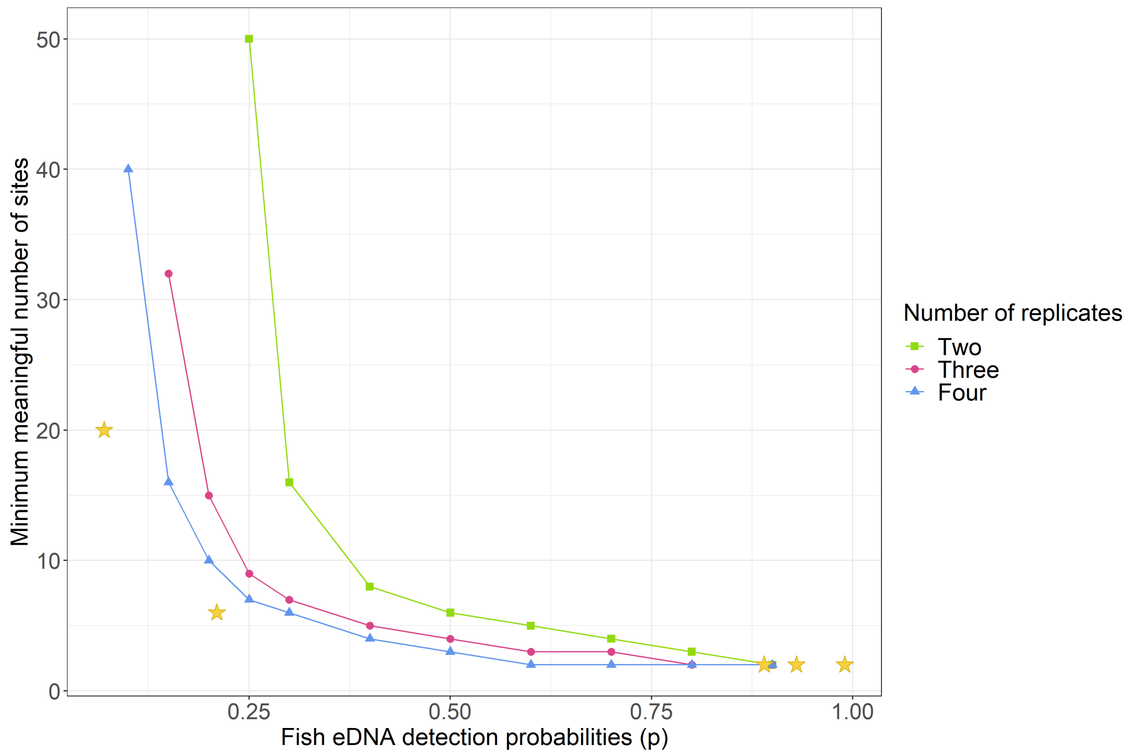

Figure 4.4: Comparison of the minimum number of sites needed to detect environmental DNA depending on detection probabilities and number of replicates, based on the assumption that eDNA is present throughout the lake ($\Psi = 1$). These statistics are applicable to any lake and sample type. The stars indicate where the study lakes fit according to the simulations: from left to right, Lake Tomarata water samples (p=0.07, 20 sites), Lake Tomarata sediment samples (p=0.21, 6 sites), Lake Waitawa perch eDNA water samples (p = 0.89, 2 sites), Lake Waitawa rudd eDNA water samples (p = 0.93, 2 sites), Lake Pounui sediment samples (p = 0.99, 2 sites).

4.7 Discussion

Designing robust sampling strategies is critical when using molecular approaches for monitoring lakes, especially when these are aimed at detecting organisms that are

present in low abundance. Our results demonstrate that perch and rudd eDNA is relatively homogeneously distributed in small shallow lakes, however, the optimal sample type (sediment versus water) and location (near shore or mid-lake) varies among lakes. It's likely that multiple factors, not all of which were explored in this study, account for this variability. These results, in concert with data from other studies, highlight the potential of using molecular techniques that target eDNA for detecting and monitoring fish in small shallow lakes.

4.7.1 Distribution of fish environmental DNA in lakes

As hypothesized, in the present study fish eDNA was mostly homogeneously distributed across lakes regardless of the species. Similar results were obtained in a study targeting eDNA of the great crested newt in water and sediment samples from ponds, where their DNA was present in all samples collected (Buxton et al., 2018). Given the motile nature of these species and the high likelihood that they are constantly shedding some DNA, a homogeneous distribution is expected in these small systems. Lake Tomarata was the only lake where rudd eDNA (16S rDNA) was not homogeneously distributed, and instead only found at half of the sites. This is likely attributable to low population density, with only small numbers of this species thought to occur in this lake (Drake et al., 2011). Previous studies have also found a relationship between low quantitative PCR signal and low population density (Weldon et al., 2020).

4.7.2 The effect of fish ecology on environmental DNA detection

The data contradicted our hypothesis that the best sample type would vary depending on fish ecology (benthic versus pelagic). The best sample type for perch and rudd in Lake Waitawa was water samples, while the best sample type for perch and rudd in Lakes Pounui and Tomarata was surface sediment. It was surprising that, despite being a pelagic fish, perch DNA was better detected in sediment samples.

This may be due to the small and shallow nature of the lakes sampled, leading to quick deposition of DNA in the sediment. The higher detection rates of rudd in water samples (Lake Waitawa) were also surprising and may be due to sediment geochemistry in this lake (more in the following subsection).

The similar habitat preferences of perch and rudd may also have impacted detection, since both species prefer warm waters and their juveniles are known to cluster near the shores. This suggests our sampling approach was already adapted to the target species, and the study lakes may not have been deep enough to see significant differences in near-shores versus mid-lake sites. Lake Waitawa and Tomarata are quite shallow (max. depth around 5 m) therefore it I unlikely that perch and rudd would avoid the deepest sites. In comparison, Lake Pounui is around 10 m deep at its maximum, and near-shore sites yielded higher detection of perch eDNA compared to mid-lake sites. More research is needed to separate the influence of fish ecology compared to lake hydrology, since most findings are unconclusive so far. For example, studies in shallow systems have found higher detection of the greater crested newt DNA in water compared to sediment samples (Buxton et al., 2018), and more vertebrate species in water samples of shallow sites in Lake Bacalar (Mexico) compared to sediment (Valdez-Moreno et al., 2019). Conversely, big headed carp eDNA detection was much higher in pond and river sediment compared to water samples (Turner et al., 2015). Studies in deeper systems have found more logical results, with a better detection of lake trout and char eDNA below the thermocline, which fits the ecology of these fish (Klobucar et al., 2017; Littlefair et al., 2021).

Perch eDNA was absent in the mid-lake water samples of Lake Pounui but was detected in all mid-lake sediment samples. The lack of perch DNA in the water samples does suggest that the populations of perch in Lake Pounui primarily inhabit the near-shore zone, which is corroborated by a previous study (Jellyman, 1980). The depocenter of lakes is known to be a site where sediment and other compounds accumulate and therefore selected for paleolimnology (Weisbrod et al., 2020). It is likely that this explains the detection of "cumulative" eDNA signal in these deeper samples. This could suggest positive results for future studies looking at fish sed-

imentary ancient DNA in shallow lakes, though for fish with a similar ecology to perch and rudd it may be preferable to take a sediment core in shallower waters.

4.7.3 Variations in environmental DNA levels and detection

Lake Waitawa was the only lake out of the three where water samples provided better detection rates, for both perch and rudd DNA. The low levels and low detection rates in the sediment samples from Lake Waitawa could be related to sediment properties. Different sediment substrates bind eDNA with varying degrees of efficiency (Buxton et al., 2017). The sediment geochemistry data suggest that Lake Waitawa sediments have less available mineral particulate surfaces, which may reduce the possibility of DNA binding to sediment compared to Lakes Pounui and Tomarata. Lake Waitawa is the most eutrophic lake included in this study and experiences heavy cyanobacterial blooms every summer. This likely leads to overall low redox conditions on the surface sediment-water interface, which may also accelerate DNA decay (Sassoubre et al., 2016; Wei et al., 2018). It is also the only lake of this study with houses on its shore, therefore contaminants from sewage waters may also alter sediment geochemistry. Unfortunately our analysis did not included tests for such contaminants.

Our results indicate that fish eDNA levels in shallow lakes may not always be related to population density. Catch data indicated perch density was higher than rudd in Lake Waitawa, and more perch were found in Lake Waitawa compared to Lake Pounui. However, overall the eDNA did not display the same trends. Weldon et al. (2020) found that eel eDNA levels in lakes (from water samples) only provide very coarse data on population density i.e., low vs medium to high density, which may also be the case in our study. Although some catch data were available for each lake considered here, the sampling approaches applied during those studies prevent an accurate assessment of population densities. Additionally, the studies were undertaken multiple years prior to the eDNA surveys, preventing any further analysis of the relationship between eDNA concentrations and actual biomass.

Our data showed that detection rates across sample type were similar, but that fish eDNA concentration varied, which highlights the advantage of using both sediment and water sampling to understand patterns of fish occurrence and distribution in lakes. The presence of eDNA in surface sediment provides an indication that the target organism has been in the system sometime in the last weeks to months (e.g., 132 days after carp removal - Turner et al. (2015)), whereas DNA in water is more sensitive to decay and indicative of recent presence (up to 20 days; Buxton et al. (2017) and Troth et al. (2021). When sampling a new environment, we recommend using both water and sediment to maximize detection rates and obtain insights into whether any positive detections are likely due to the recent presence of an organism. This approach will also overcome challenges, such as those observed in the Lake Waitawa sediment samples, where lake-specific conditions, may inhibit detection.

Avoiding false negatives and false positives is extremely important in surveys targeting a given species (Ficetola et al., 2015; Smith et al., 2020; McClenaghan et al., 2020; Langlois et al., 2021). To reduce the chances of false positives from ddPCR, we followed a strict process during the interpretation of low-level positives. If only a single droplet was observed in a sample, it was run twice more and only accepted as a positive if a droplet was observed in two of the three replicates. We cannot rule out the possibility that the detection of perch in a single sample from Lake Tomarata (following the above protocols) is not due to contamination, although all negatives were clear. In cases where confirming the reliability of a low-level detection, or unexpected results is important, we recommend further testing of all available replicates and then returning to the site to undertake additional sampling using both eDNA and traditional approaches to confirm (or rebut) the detection.

4.7.4 Considerations when designing a sampling program

Our findings may not be applicable to larger lakes. Studies on larger and deeper systems are relatively limited and given their more heterogeneous geomorphology, stronger currents, stratification, and greater dilution due to volume, they often

require more complex sampling strategies. For example, studies have shown that stratification may impact the vertical distribution of eDNA in deeper lakes (Klobucar et al., 2017; Littlefair et al., 2021).

The simulations used to estimate the minimum reasonable sampling effort when developing a monitoring program were based on the lowest detection probabilities. They indicated that six sites, with five replicates were sufficient when taking sediment samples ($p \geq 0.21$), and that a higher number of sites and replicates are suggested for water (since $p \geq 0.07$), twenty sites and eight replicates. The ideal type of sample and ideal sampling location may vary depending on the lake, therefore we recommend taking both sample types across the lake, so that occupancy modelling and design simulations may then help determine which is better and how many sites / replicates are needed for a specific lake. The number of samples and replicates could be reduced for lakes with high naive fish eDNA occupancy such as Pounui and Waitawa (max. two sites and two replicates per site according to the simulations), but not when targeting species with low abundances such as rudd in Lake Tomarata. The high number of replicates required for water samples may become cost-prohibitive. To reduce the cost of analysis, tiered approaches have been suggested (Sham et al., 2002). This could involve pooling samples (e.g., a portion of all water samples from a single lake) for an initial screening and if a positive signal is obtained then all replicates should be analysed. One limitation of this approach is that weak signals are diluted, which may result in false negatives.

A further consideration that influences detection is sample size. In this study we extracted eDNA from c. 3 g of sediment and 500 mL of water. We initially tried with 0.25 g of sediment and detection rates were very low (sediments of Lake Pounui, Table D.30). The DNA extraction method applied in this study has been shown to be effective on up to 10 g of sediment (Thomson-Laing et al., 2022). Extracting from larger sediment volumes may have improved detection rates, although it can also create other issues such as greater inhibition. Likewise, studies have found that increasing the volume of water sampled increases eDNA levels and detection rates (Sepulveda et al., 2019a). The type of filter used also impacts detection

(Hunter et al., 2019). During our study two of the three lakes were experiencing cyanobacterial blooms, which limited the volume of water that could be filtered, despite pre-filtration for the Lake Waitawa samples. In our study we filtered 1 liter of water and the filter was cut in half to avoid clogging the tubes used for extraction, which effectively meant that only 500 mL of water was analysed. Extracting both halves (therefore 1 L) and combining the DNA extracts could potentially increase the sensitivity of our assay without filtering more water. Furthermore, increasing the pore size of the filter (e.g., by using a nylon filter) would be another option (Zaiko et al., 2022) but would need optimisation to ensure that DNA is not lost through the pores. The development of new techniques such as passive sampling provide new avenues that may overcome some of the limitation with sampling water and sediment samples (Kirtane et al., 2020; Bessey et al., 2021; Verdier et al., 2022), but further research is required to determine their applicability for specific species.

4.8 Conclusions

The results of this study demonstrate that perch and rudd eDNA can be detected in lake water or surface sediment. In general surface sediment samples had higher detection rates but there were differences among lakes which we attribute to factors such as sediment geochemistry. When initiating a sampling program, we recommend initially including both water and sediment samples. The data generated from the two sample types also provide complementary information on fish dynamics. The sediment samples give information on fish presence integrated over a longer time frame, whereas the water samples provide contemporary insights. Occupancy modelling undertaken in this study indicates that for both perch and rudd, sampling near the shores of the lake is similar if not better than sampling in deeper parts of the lake – a valuable finding given that this reduced the need for boats and more complex sampling requirements. Using the detection rate data generated in the present study, we predicted that at least six sites and five replicates per site would be needed to reliably detect fish eDNA in sediment samples, and twenty sites with

eight replicates per site for water samples. The techniques used here could be applied to other fish species to aid in developing informed monitoring or surveillance programs.

4.9 Acknowledgements

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Chapter 5

Did the introduction of European perch (*Perca fluviatilis*) initiate cyanobacterial blooms in Lake Pounui? A multi-trophic level study

5.1 Authors and author contributions

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M.P. and S. W. conceived and designed the experiments; J.D.H, A.R, and M.J.V. provided the lake sediment cores; M.P., G.T-L., J.D.H., X.L. and C.M.M. analysed the samples; M.P. analysed the data; M.P. prepared figures and tables except supplementary figure D.20 which was prepared by J.D.H.; J.D.H, A.R., X.P., M.J.V, I.H., and S.A.W. provided advice leading to the completion of this chapter.

5.2 Introduction

Recent estimates suggest there are more than 117 million lakes on Earth (defined as greater than 0.002 km2; Verpoorter et al., 2014). These lakes often hold high cultural significance, and provide an array of ecosystem services such as water for drinking and irrigation, industrial applications, fisheries, and hydroelectricity generation (Inácio et al., 2022). Because lakes capture nutrients and environmental contaminants from the nearby landscape and the atmosphere, they are highly susceptible integrators of change (Hamilton et al., 2018). Lakes and their catchments have been used as a resource by humans for many hundreds or thousands of years, exposing them to multiple stressors including habitat modification, the introduction of non-native species, and diffuse and point-source pollution (Drake, Kelly, and Schallenberg 2011; McIntosh et al. 2010; McDowall 1968; Woodward 2013; Woodward et al. 2014). The current health of a lake is often a culmination of multiple recent and historic stressors, including climate change, that can be synergistic or additive (Paerl and Paul 2012; Paerl 2014; Monchamp et al. 2018; Ormerod et al. 2010).

A common symptom of severe degradation in lake health are cyanobacterial blooms. High concentrations of these photosynthetic organisms reduce the penetration of light into the lake, deplete the upper layers of lakes of nutrients, reduce in-lake oxygen levels when they decompose, and many bloom-forming species produce toxins that pose a health risk to humans and aquatic and terrestrial animals. The intensity, frequency and distribution of cyanobacterial blooms is increasing globally (Huisman et al., 2018) and this is primarily linked to cultural eutrophication (Huisman et al., 2018; Beaulieu et al., 2013; Gray et al., 2019; Kosten et al., 2012; Jöhnk et al., 2008; Paerl et al., 2016). There is also increasing evidence to show that climate change can be a major contributor to enhancing bloom frequency and persistence, through warmer water temperature and enhanced stratification (Paerl et al., 2008; Paerl et al., 2012; Jöhnk et al., 2008). A less well-studied mechanism that can cause cyanobacterial blooms are top-down stressors, for example, changes

in food-webs due to the introduction of non-native species (Smith et al., 2006; Smith et al., 2007).

New Zealand has been isolated from other major landmasses for about 85 million years (Daugherty et al., 1993). This has allowed unique freshwater flora and fauna to evolve, which have been highly susceptible to the introduction of non-native species, particularly where those introductions fill ecological niches that were vacant within the native biota (Towns et al., 1997). New Zealand's freshwater systems have been subjected to multiple introductions over the last 700 years, with declines in water quality and loss of native species documented in many cases (Rowe, 2007; McIntosh et al., 2010). The European perch (*Perca fluviatilis*, hereafter perch) is a carnivorous coarse fish introduced in the 1870s. Research suggests a link between their presence and cyanobacterial blooms in some New Zealand lakes (Smith et al., 2006; Smith et al., 2007). Perch are zooplanktivorous during their juvenile stage and becomes piscivorous as adults. Studies in Roto Kawau (Wellington) suggested that the presence of a stunted population dominated by juveniles (Hicks et al., 2007) was reducing the grazing pressure on cyanobacteria and allowing blooms to develop, since perch juvenile consume large-sized zooplankton (cladocerans) (Smith et al., 2006; Smith et al., 2007). However, historical data demonstrating the temporal relationship between perch introduction, modification of food webs, and the onset of cyanobacterial blooms is lacking for New Zealand lakes.

Although declining lake health has been occurring over many centuries, most lake monitoring records are short in duration, and are initiated after the onset of degradation. Thus, in many instances it is challenging to identify which stressors have, or still are, impacting lake ecosystems and distinguish their relative importance from one another in mediating these impacts. Paleolimnology provides a suite of approaches that can be used to reconstruct the history of a lake and its catchment over longer time frames. Traditionally, paleolimnological approaches have relied on fossil or biochemical remains such as diatoms, pollen and microfossils to characterise biological change (Douglas, 2013). Scanning techniques such as X-Ray fluorescence (XRF) are also commonly used to track mineralogic elements that can be used to

identify shifts in catchment erosion or within lake processes such as anoxia (Davies et al., 2015). The advent and application of sedimentary ancient DNA (sedaDNA) has now provided a mechanism to identify organisms, such as bacteria, fish and microeukaryotes that do not leave fossilised remains in sediment cores, paving the way for the reconstruction of multiple trophic levels (e.g., Smol (1985), Domaizon et al. (2017), and Capo et al. (2021b)).

Lake Pounui (North Island, New Zealand) is a small, eutrophic, lowland lake. As Māori (the indigenous people of New Zealand) arrived and settled in the surrounding region in the late 14th century (Schrader, 2017), there were changes in vegetation in the Lake Pounui catchment, which were further compounded with the settlement of Europeans (about 1840s) in the region (Cochrane, 2017). However, unlike most lowland lakes in New Zealand, Lake Pounui's catchment now primarily in native forest, albeit much of this is regenerating, and it hasn't been subjected to intense agricultural land use. In the mid-19th century, acclimatisation societies were formed in New Zealand and they facilitated the shipment and released of animals and plants from Britain for farming or hunting, or out of nostalgia (Walrond, 2008). There are documented releases by the local acclimatisation society of large number of rainbow trout fry into Lake Pounui in the 1940s and recent sedaDNA evidence suggests that perch may have been present in Lake Pounui soon after European settlement (1850's; Georgia Thomson-Laing, unpublished data). Water quality assessment in the 1980s categorised the trophic status of Lake Pounui as shifting from oligotrophic to mesotrophic (Lawless, 1983; Jellyman, 1990). The lake became eutrophic around 2010 (Perrie et al., 2012; Winton et al., 2011) and it now experiences severe summer cyanobacterial blooms.

In this study, a paleolimnological approach was coupled with historical data to explore the drivers of ecosystem change in Lake Pounui over the last approximately 1,000 years, with a focus on understanding the triggers of cyanobacterial blooms in recent decades. We hypothesised that; (1) the introduction of perch in Lake Pounui caused shifts in the foodweb that changed the trajectory of the lake, ultimately culminating in the formation of cyanobacterial blooms, and (2) that other additive

stressors, including climate change and nutrient enrichment accelerated changes in lake condition enhancing the intensity of cyanobacterial blooms in the last 20 to 30 years. To address these hypotheses a multi-proxy approach was applied to a sediment core from Lake Pounui, including metabarcoding of sedaDNA from bacteria, eukaryotes, and macrophytes, XRF scanning, and pollen and charcoal analysis.

5.3 Methods

5.3.1 Study lake

Lake Pounui is a small (c. 46 ha), shallow (max. depth 9.6 m), lowland coastal lake situated in the foothills of the Remutaka Range in South Wairarapa, about 30 km northeast of Wellington, New Zealand (41°20'34"S, 41°20'34"S). The lake was probably formed by a sedimentary obstruction of the valley (Irwin, 1975) and is fed by two streams draining into the north-western arm and one stream draining into the south-western arm of the lake. The 627 ha catchment is now dominated by native trees (96%), with the remainder in pastoral land cover (Perrie et al., 2012). Despite earlier reports that the lake did not stratify (Lawless, 1983; Jellyman, 1990), recent continuous logging data has shown that the lake is polymictic (Andrew Rees, unpub. data).

5.3.2 Historical information

To reconstruct the environmental history of Lake Pounui, a combination of published and unpublished reports, peer-reviewed publications, grey literature, aerial photographs, archaeological data, and personal accounts were compiled (Table D.31).

5.3.3 Sediment coring and subsamples

Two sediment cores were taken from Lake Pounui in March 2021. The primary core used for this study was from the approximate deepest point in the lake (total core length 115 cm) and a second core, used for pollen and charcoal analysis, was taken

from the lake edge (total core length 140 cm). The cores were collected using a Uwitech gravity corer (Mondsee, Austria) with a 2 m, 90 mm diameter polyvinyl chloride core barrel. The polyvinyl barrels were cleaned prior to coring with 2% bleach. Cores were kept at 4°C for 7 days until sedimentary DNA subsampling in a dedicated room at Te Pū Ao / GNS Science (Lower Hutt, NZ). The cores were split longitudinally and the surface of each half-core was scraped to remove cross-contaminated sediment. Each core was logged and plant material was subsampled if visible for radiocarbon analysis. One half of the depocenter core was completely subsampled for sedaDNA (30 subsamples from 0 to 66 cm depth) and half of the lake edge core for pollen (17 subsamples from 14 to 139 cm depth) using sterilised spatulas (Table D.32, Table D.33). Subsamples were kept in 50 mL Falcon tubes at -20°C for DNA and at 4°C for pollen until processing. The other half was used for scanning and radiocarbon.

5.3.4 Pollen and charcoal analysis

Seventeen 0.25 cm3 sediment samples were selected from the lake edge core, at sampling intervals of 5 to 10 cm (Table D.32). Pollen was extracted using methods from Fægri et al. (1989) and exotic *Lycopodium* tablets were added for pollen concentration calculations. Pollen and spores were identified using standard texts (Large, 1989; Moar, 1993; Pocknall, 1981b; Pocknall, 1981a) and New Zealand reference collections. To summarise ecological shifts, all terrestrial pollens were categorised: exotics (*Pinus, Salix, Rumex acetocella*), tall native trees, small native trees and shrubs, and grasses. The bracken fern Pteridium esculentum was included in the small trees and shrubs group due to its similar ecology (McGlone et al., 2005). Charcoal fragments were counted from the pollen slides. All charcoal fragments were summed (counts/cm^3) regardless of size.

While the complete composition of pollen samples was identified, selected species were relied on to indicate human land use practices. Specifically, bracken fern and an increase in charcoal served as an indicator of Māori settlement and the biggest

increase in charcoal as markers for European settlement.

5.3.5 Core chronology

Terrestrial plant macrofossils (leaves and twigs) were picked from the surface of all half-cores upon splitting. Carbon 14 (14C) activity was measured as described by Short et al. (2022), following the methods from Norris et al., (2020), Baisden et al., (2013), Hogg et al., (2020), and Turbull et al., (2017). Briefly, macrofossils were cleaned of carbonates, humic and fulvic compounds, converted to CO_2 and measured by accelerator mass spectrometry. The South Hemisphere ShCal20 calibration curve (Hogg et al., 2020) was used for conventional radiocarbon ages (prior to 1950), while dates more recent than 1950 were calibrated with the BHDCGO curve (Turnbull et al., 2017). The chronology for the two cores was established from ^{14}C dating only, ages were constrained using Bayesian age-depth modelling using the OXCAL 4.4 software (Bronk Ramsey 2009). Since there were no dated records of specific human impacts on the catchment, no other age constraints were used.

5.3.6 X-Ray fluorescence scanning

The full length of the depocenter core (115 cm) was scanned at a 2 mm resolution using an Itrax µ-XRF Core Scanner (Cox Analytics) at the University of Otago Repository for Core Analysis. The top of the sediment core (1.2 mm) was scraped to remove cross-contamination, then major and trace elements were measured using a molybdenum and a chromium X-ray tube configured at 30 kv, 55 ma and 10 s integration time. Raw measurements were transformed into ratios for normalisation. The incoherence/coherence scattering ratio (inc/coh) from the chromium tube provided an indication of historical levels of organic matter (Davies, Lamb, and Roberts 2015), while titanium and potassium normalised by incoherent scattering (Ti/inc and K/inc) were used to infer detrital input, clay content, and possibly tephra (Davies et al., 2015).

5.3.7 Sedimentary ancient DNA

5.3.7.1 Sample processing

DNA subsamples were processed at the Cawthron Institute (Nelson, New Zealand) in a laboratory dedicated to DNA analysis, with separate rooms for each step. PCR rooms were equipped with UV lighting on the ceiling, as well as laminar flows with HEPA filtration and additional UV sterilisation, and benches were sterilised with bleach and ethanol before and after each use.

DNA was extracted as described in Thomson-Laing et al. (2022). Briefly, about 10 g of sediment were mixed with sodium hydroxide and Tri-EDTA for a first lysis step, incubated and centrifuged, then lysis was neutralised with Tris-HCL. DNA was precipitated overnight using sodium acetate and ethanol. The resulting pellet was transferred to the first tube of a Qiagen PowerSoil kit, and DNA eluted in 100 µL following the manufacturer's instruction using Qiacube and Qiacube Connect robots.

Five primer sets were used to target various components of the food web. The V4 region of the 16S ribosomal RNA (rRNA) gene was used to target bacteria, the V7 region of the 18S rRNA gene to target micro-eukaryotes, the cytochrome c oxidase I (CO1) gene for other eukaryotes, and the p6 loop of the trnL gene and the rbcL gene to target the plant community. Primer details, PCR mix and cycling conditions are detailed in Table D.34. Bacteria and eukaryotes PCR amplifications were performed at the Cawthron Institute (Nelson, New Zealand) on 96-well plates in triplicate with a positive and a negative control in each batch. Amplicon products, including all negative extraction and PCR controls, were visualised on a 1.5% agarose gel electrophoresis stained with Red Safe DNA Loading Dye (iNtRON Biotechnology Inc, Kyungki-Do, Korea), and UV illumination to ensure amplification of specific amplicons. PCR products were purified using SequalPrep™ Normalization Plate Kit (ThermoFisher, Waltham, Massachusetts, United States) and submitted for sequencing to Genewiz (Suzhou, China). Sequencing adapters and sample-specific indices were added to each amplicon via a second short round of

PCR using the NexteraTM Index kit (Illumina, CA, USA). Amplicons were pooled into a single library and paired-end sequences (2 x 250 bp) were generated on a NovaSeqTM instrument using the TruSeqTM SBS kit (Illumina, CA, USA). Sequence data were automatically demultiplexed using MiSeq Reporter (v2), and forward and reverse reads were assigned to samples. Both plant markers were amplified by qPCR using a Stratagene MX300p instrument (Agilent, USA) at the WilderLab laboratory (Wellington, New Zealand) and sequenced on an Illumina iSeqTM platform.

5.3.7.2 Bioinformatics

The R software v4.2.1 (R Core Team, 2021) and RStudio software (RStudio Team, 2022) were used for all bioinformatic and statistical analyses. The Tidyverse v1.3.0 and its associated packages (Wickham et al., 2019) were used for data manipulation, exploration, and visualisation.

Primer sequences (16S rRNA, 18S rRNA, CO1) were removed using Cutadapt (Martin, 2011) (Anaconda environment adapted in R) allowing 1 bp of mismatch. The DADA2 package (Callahan et al., 2016) was used for general sequence quality assessment, quality profiles plots, and for the full amplicon workflow (quality filtering, merging of paired-end reads, dereplication, chimera identification, sample inference, and taxonomy assignment). Bioinformatic details followed methods from Picard et al. (2022a). The phyloseq package (McMurdie et al., 2013) was used to prepare the sequence table and associated information. Any ASVs found in the blanks sequenced (extraction, PCR, sequencing) were completely removed from the samples for bacteria and eukaryotes (16S rDNA, 18S rDNA and CO1). All non-bacterial reads (eukaryotes, mitochondria, chloroplasts) were removed from the bacteria dataset. Bacteria and plants (Streptophyta) were removed from the two eukaryotic datasets (18S rDNA and CO1). For the plant primers, the number of reads of specific ASVs found in the blanks were removed from all samples.

5.3.8 Data analysis

Multivariate analyses were undertaken on unrarefied metabarcoding samples, where reads counts were transformed to relative reads counts (%) per sample. Multivariate trends (biological communities) were summarised by a principal response curve (Van den Brink et al., 1999) using the prcurve function from the analogue package (Simpson, 2007; Simpson et al., 2021), using selected ASVs above 0.1% relative abundance per primer set. Principal response curves summarise the variance of the principal component in one dimension.

Changes in principal response curves (bacteria, eukaryotes, macrophytes) and in mineralogic signatures (XRF data) were then analysed by generalised additive (mixed) models (GA(M)Ms) following the methods of Simpson (2018). A continuous first-order autoregressive process (corCAR1) was applied for autocorrelation for all biological communities (GAMM), but this model was not flexible enough to accommodate the higher variability of the XRF data therefore a Generalised Cross Variance (GCV) model (GAM) was applied. Derivatives were calculated in all cases to visualise how much of the data the models were taking into account, and to highlight distinct changes.

5.4 Results

5.4.1 Environmental history

5.4.1.1 Catchment disturbances

From our review of historical records, two major earthquakes ($\geq$ magnitude 8, Wellington faultline) occurred within the timeframe of the study; 1460 and 1855. These events caused major tectonic uplifts and landslides in the wider region (Hancox, 2005), but there is little evidence from the literature that these impacted Lake Pounui significantly.

Charcoal remains in the Palliser Bay region, in the coast close to Lake Pounui, indicate that Māori were living there from approximately 1300 AD (Schrader, 2017)

and moved inland between 1460 and 1650 after the 1460 earthquake / tsunami (Cochrane, 2017). The remnants of two Māor pa (villages) have been found near Lake Pounui, one near the eastern shore and one 1.7 km to the east of the lake. The two pa are dated from the early Māori period (pre-1769; Leach (1981)).

No records of the British Crown buying the land around Lake Pounui were found, but records estimate that over three quarters of the Wairarapa region had been purchased from Māori by 1965 (Patterson, 1998). The vegetation in the catchment of Lake Pounui has undergone multiple changes since European settlement (McQueen, 1991). Significant parts of the catchment were burnt around the 1850s except the North-Western valley, with some areas likely burned up to four times prior to 1940 (McQueen, 1991). The first aerial photographs are available from 1944 and show a regenerating catchment, which then stayed mainly forested with repeated small-scale clearance of the forest until about 2000, especially in the Northern and Southern hills (Figure 5.1). Although there are no specific records for the Lake Pounui catchment, aerial topdressing (aerial application of fertiliser) began in 1949 in the Wairarapa where superphosphate fertiliser was applied on 101,000 hectares (Maber 2008). Studies also mention neighbouring farms (on the steep Northern and Southern Hills) using fertiliser to improve their pastures from the 1970s (Lawless, 1983; McQueen, 1969). Some of these pastures fall into the catchment of Lake Pounui (Figure 5.1). A survey in 2016 reported the absence of dense reeds and rushes at the lake's margin (Winton, 2016).

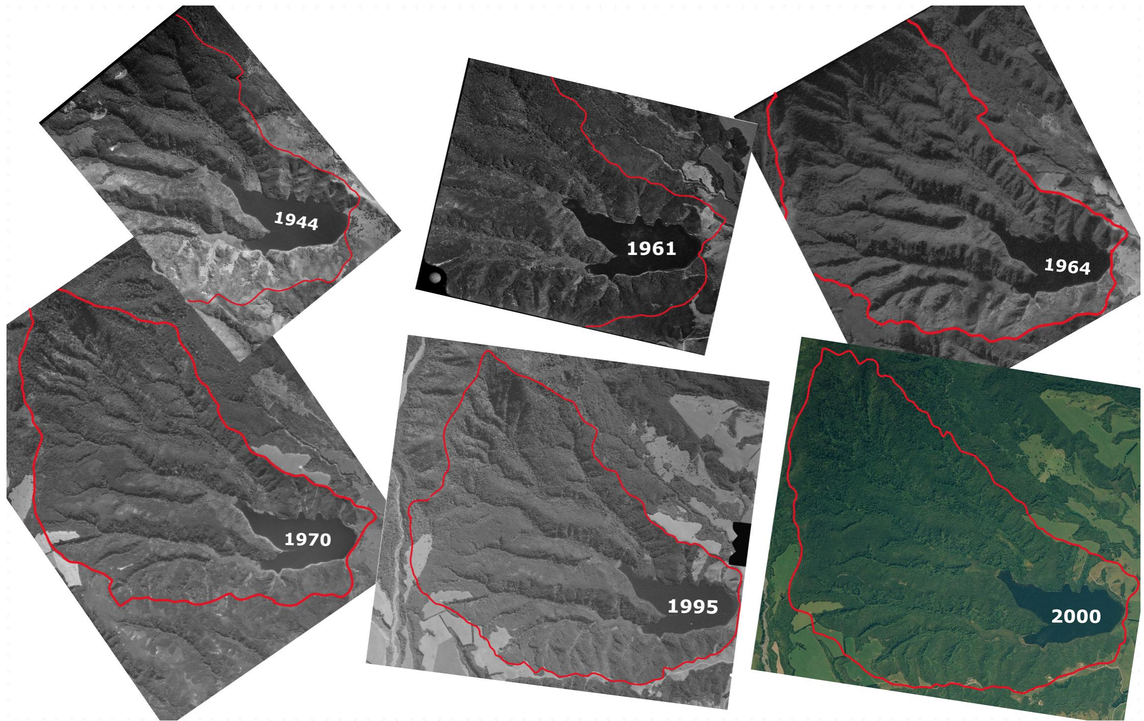

Figure 5.1: Overview of the main changes in the catchment of Lake Pounui from 1944 to 2000 from aerial pictures (*Retrolens - Historical Imagery Resource* 2021). Most of the changes in these 56 years occurred in the Northern and Southern part of the catchment while the Western area appears to have been cleared prior to 1944. Only one valley in the North-West had a native forest old enough to not have been cleared by Europeans (McQueen, 1991). Catchment limits (red line) were drawn from current catchment delimitations (*Takiwa NZ Lakes* 2018).

5.4.1.2 Climate

From 1909 to 2019, national average annual temperatures have increased by about 1.13°C, especially since the 1950s (*Our atmosphere and climate* 2020). Surface water temperature in Lake Pounui has been measured monthly over extended periods. Between 1977-1978, monthly temperatures ranged from 7.9°C to 19.6°C with an annual average of 14°C (Jellyman, 1990). Between 1980-1981, they ranged from 7 to 20°C with an annual average of 13.7°C and between 2012-2013 from 8.2 to 24°C (Cochrane, 2017). Continuous monitoring in 2013 and 2018 measured surface water temperature ranging from 8 to 24°C (annual average 15.5°C, Andrew Rees unpublished data). This represents an average annual increase of c. 2°C over 30 years.

Historical rainfall patterns differ depending on the region in New Zealand and

historical trends are inconsistent near Lake Pounui. There is an increase in rainfall in spring in the North of the Wairarapa, and decreasing rainfall in winter in Wellington (*Our atmosphere and climate* 2020). In Lake Pounui, studies observed that the lake level fluctuated, with some flooding between 1975-1978, but also periods where the outlet would be dry for three months (1980-1981, Lawless (1983)).

5.4.1.3 General ecology

Detailed studies from 1975-1980 observed a well-mixed water column for half of the year (April to October), with some weak stratification in summer but strong winds coming down the Remutaka range (Western hills of the catchment) usually prevented complete stratification (Jellyman, 1990; Lawless, 1983). Due to its proximity to the coast, the waters of Lake Pounui were slightly alkaline (sea spray observed 700 m from the lake; Lawless (1983). Studies identified that Lake Pounui shifted from oligotrophic to mesotrophic around 1980 (Lawless, 1983) and to eutrophic between 2007 and 2011 (Perrie et al., 2012; Winton et al., 2011). In 1980, total phosphorus levels were 0.022 g/m^3, nitrate was under the detection limit ($<$0.01 g/m^3) but ammoniacal-nitrogen was 0.28 g/m3. The lake was in good ecological condition, dominated by macrophytes, a diverse fish community (both eel species, common bullies, kokopu, and European perch). From 2013 to 2017, water samples were taken almost every month by Andrew Rees (Victoria University of Wellington). Average phosphorus levels increased from 0.03 g/m^3 in 2013 to 0.04 g/m^3 in 2017, while total nitrogen varied (highest average 1.04 g/m3 in 2016).

Lawless (1983) took monthly samples for a year and a half (1980-1981) and identified 25 zooplankton species with the dominant being a copepod, *Calamoecia lucasi*. Duggan (2022) took a single sample and found the complete dominance of the cladocera *Bosmina meridionalis*, which was also present in the 1980s but only peaked in December.

The macrophyte community in 1976-78 was diverse, both in terms of richness (12 species), with the main species being *Myriophyllum elatinoides* (syn. *M. triphyllum*, covering 8% of the lakebed), *Elodea canadensis* (17% lakebed), and generally beyond

4 meters deep to 7 meters deep, *Chara corallina* (15% lakebed). About 40% of the lakebed was unvegetated (Jellyman 1990). A macrophyte survey in 2011 showed that the maximum depth of macrophytes was only 5.6 m (Winton et al., 2011), and the native pondweed *Potamogeton ochreatus* (not identified in prior studies) dominated at mid-depths. The authors also indicated that some macrophytes were bioturbated by fish and grazed by swans (black swans were introduced in 1864 in New Zealand; Falla et al. (1981)). Another macrophyte survey in 2016 observed *Elodea canadensis* occupying most of the littoral zone (Winton, 2016). The same study also observed that reeds had been removed from most of the lake shore.

Cochrane (2017) studied diatom frustules from a sediment core and showed the community shifted at least five times in the last 3,000 years. The most recent shifts were c. 500 AD, c. 1560 AD, c. 1850 AD. One last shift not highlighted by the authors seemed to occur around 1950 AD, with an increase in eutraphentic species (*Aulacoseira granulate var. angustissima, Cyclostephanos dubius, Cocconeis placentula*). The green algae Volvox was observed in the mid- to late-1900s (Pocock 1951, Lawless 1983). The first occurrence of cyanobacterial blooms is unknown, though blooms of *Anabaena* (now *Dolichospermum*) *spiroides* was observed for up to 10 days (end of January) in 1976, 1977, and 1978 (Jellyman, 1990). Pigments and sedaDNA from sedimentary records showed that cyanobacteria abundances increased exponentially since European settlement, and that this increase was concomitant with the appearance and dominance of bloom-forming cyanobacteria species. The increases in abundance of bloom-forming species were especially apparent since c. 1900 (Picard et al., 2022a; Picard et al., 2022b). In 2013, a maximum of 183,000 cyanobacteria cells mL-1 in surface water samples was measured, with the dominant species being *Dolichospermum circinale* (Andrew Rees, unpublished).

5.4.1.4 Non-native species introduction

Records on the introduction of non-native fish are patchy, with the only official accounts being of rainbow trout (*Oncorhynchus mykiss*) stocking (1938-1958, from 20,000 to 120,000 fry per year; data from the Wellington Acclimatization Society).

This species is not found in the lake anymore. Introduction dates for brown trout (*Salmo trutta*) and European perch (*Perca fluviatilis*) are unknown, but they were documented in the lake in 1974, when Jellyman (1980) captured 1052 perch in the span of a year and half (July 1974 to December 1975). Perch sedaDNA was detected from the depocenter core used in this study at 1846 AD (1828-1855 95% HDPF range) and from the littoral core (used for pollen and charcoal analyses) at 1850 AD (1843-1857 95% HPDF range; Thomson-Laing unpublished).

Flood gates were constructed approximately 3-4 km downstream at Pounui Lagoon in the late 1960s which likely decreased native fish migration (Mitchell, 1996). Another study in 1975 indicated fish migration was limited to 12 h a day, however they were unlikely to limit eel recruitment (Jellyman et al., 1983). Jellyman (1989a) removed 992 eels (longfin and shortfin) from the lake in a three-months study (July 1974 to December 1975), studied their stomach content and showed that eels above >40 cm length for shortfin and >30 cm for longfin preyed on perch and other organisms including snails (*Potamorpygus*) and kōura (native crayfish, *Paranephrops*). This data associated with the strong recruitment of eels in Lake Pounui (Jellyman et al., 1983) indicated eels could be efficient predators of the perch community at the time of study. The introduction date of the non-native macrophytes *Elodea canadensis* and *Potamogeton crispus* is unknown, but they were first identified in 1976 (Jellyman, 1990).

The timeline reconstructed from historical records and studies is summarised in Figure 5.2.

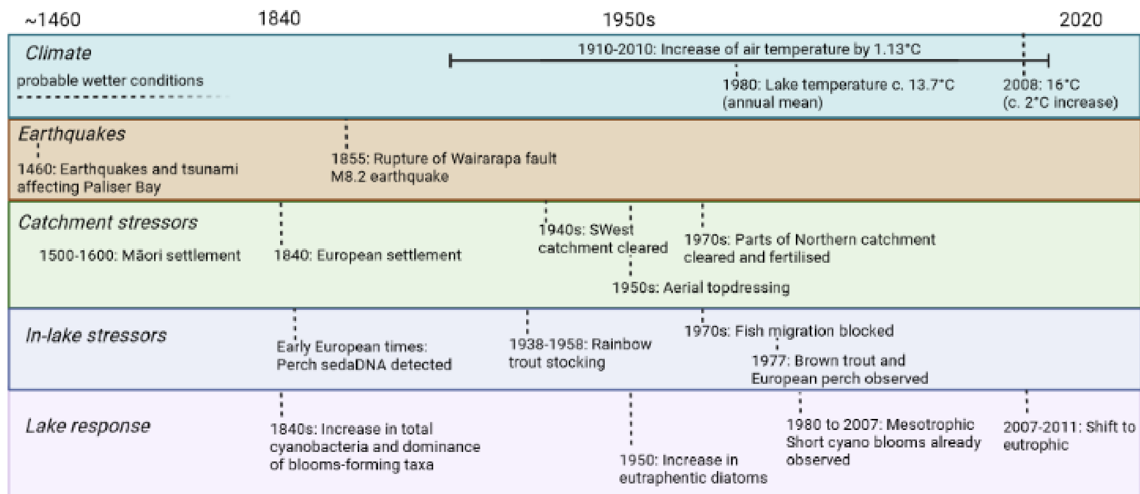

Figure 5.2: Timeline of key known external and in-lake stressors and lake response, derived from historical information, published sources, and monitoring data.

5.4.2 Sedimentary record

The radiocarbon dates provided a continuous age model from present-day to the bottom of the cores (Figure D.20, Table D.32, Table D.33). The depocenter core was dated at 58 AD (-37 – 1076 AD 95% high density probability [HDPF] range) at 115.7 cm, and the littoral core at 610 AD (477-739 AD 95% HPDF range) at 140.6 cm. The temporal precision was highest at the very top of the cores (1 year), however the age model indicated that the first 5.5 cm of the depocenter core could have been affected by bioturbation, since the higher bound of the 95% HPDF range was the year of sampling (2021). Age models indicated that the DNA records dated back to about 1250 AD (1220–1286 AD 95% HPDF range; Table D.33). Based on sedimentology in the depocenter core, Māori settlement was dated c. 1420 (1336–1518 AD 95% HPDF range). Charcoal signal from the littoral core indicated an increase in burning from c. 1400 (1335-1469 AD 95% HPDF range), and the highest peak was linked with European settlement in 1845 AD (1825-1856 AD 95% HPDF range; Figure 5.3).

5.4.3 External stressors

The pollen records indicated a catchment dominated by tall native trees (95-99%, primarily *Fuscospora* spp.) prior to Māori arrival (Figure 5.3). *Pteridium esculen-*

tum (bracken fern, often associated with vegetation disturbance) and an increase in charcoal concentrations (indicative of burning in the landscape) began about 1410 (1439-1484 AD 95% HDPF range), signalling the likely settlement of Māori in the region. Concomitant with this was a decrease in tall native trees and an increase in small trees and shrubs, including *Coprosma* and *Coriaria*. Charcoal concentrations peaked in 1845 (1825-1856 AD 95% HDPF range), correlating historical records of European settlement in the region (Figure 5.3). Low levels of exotic plants such as pine (*Pinus radiata*), willow (*Salix* spp.), and sheep's sorrel (*Rumex acetocella*) appeared from 1850 (1843-1857 AD 95% HDPF range), also signalling the settlement of Europeans in the wider region. The relative abundance of grass pollens (Poaceae) increased (<5% to 25%) between 1845 to the latest sample analysed (1990 AD).

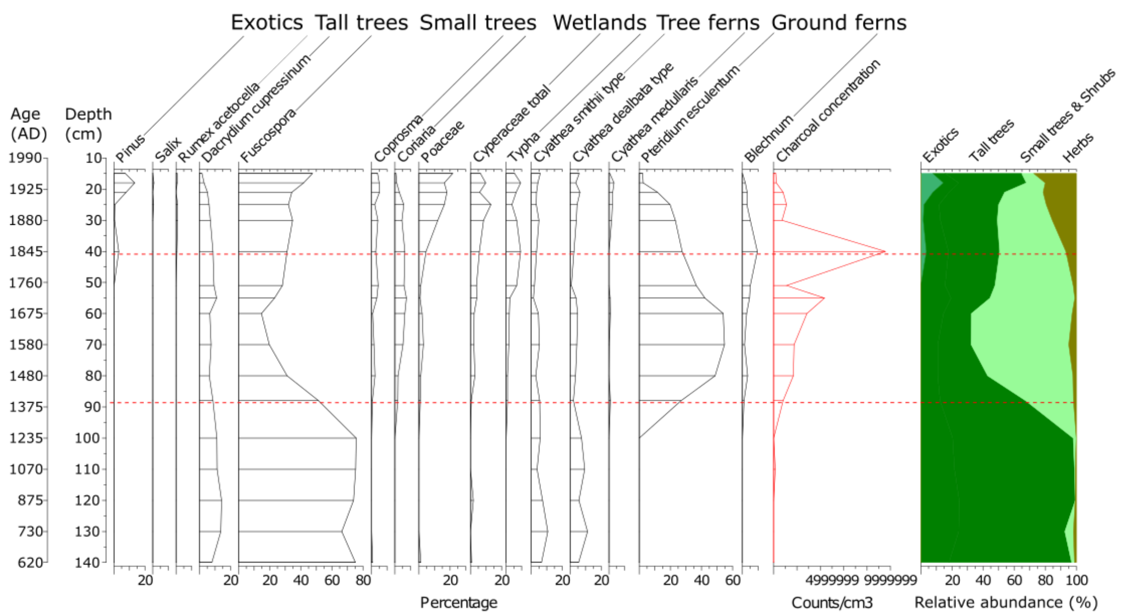

Figure 5.3: Pollen and charcoal profiles from the littoral core. Native species >5% relative abundance were selected for individual display, and all three non-native pollens are displayed. All terrestrial pollens are included in the final sum (far right). Dashed red lines indicate European (c. 1845) and Māori settlement (c. 1420). No samples more recent than c. 1988 AD (11 cm depth) were analysed.

The three selected mineralogic ratios fluctuated markedly over the c. 2,000-year records (Figure 5.4). A notable shift occurred around 1280 AD (60 cm depth) with an increase in K/inc, and Ti/inc, and a decrease in inc/coh. A marked shift in all ratios aligned with the estimated timing of Māori settlement (c. 1400), with a

peak in inc/coh and decrease in Ti/inc and K/inc coinciding with the first charcoal signal recovered from the littoral core. Overall, since European arrival (c. 1850s), the inc/coh ratios have increased and the Ti/inc and K/inc ratios decreased (Figure 5.4). The K/inc and Ti/inc showed very similar patterns and complete opposite trend from inc/coh, which provides evidence for their use as a proxy for clay content and detrital input and the role of inc/coh as an organic matter (in-lake productivity) proxy.

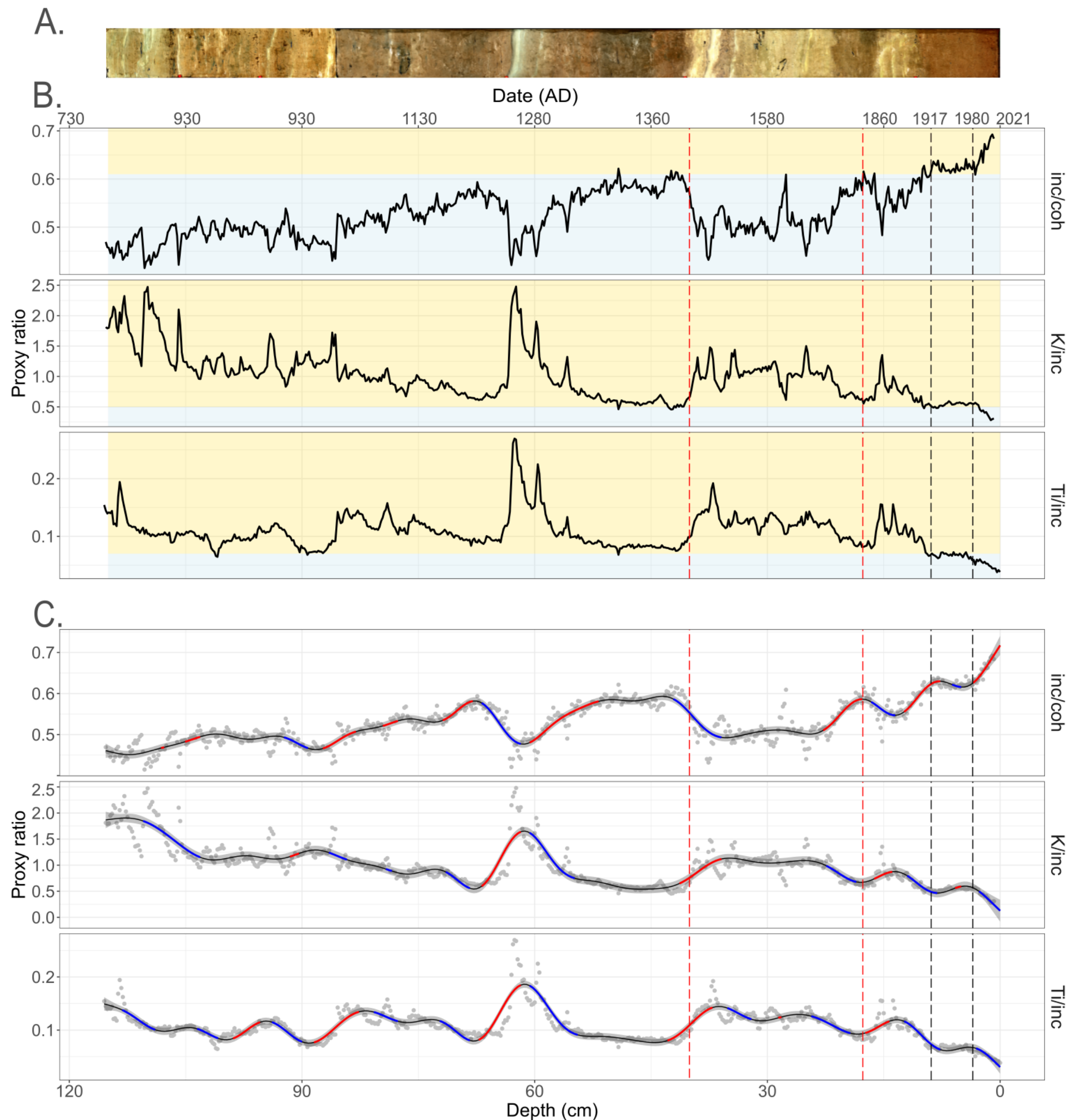

Figure 5.4: Core image (A.) and mineralogic trends (ratios) from XRF scanning (B. and C.). Incoherence/coherence (inc/coh) is a proxy for organic matter content, potassium (K) and titanium (Ti) normalised by incoherence are proxies for clay content, detrital inputs, and tephra. The two red dashed line indicate European arrival (c. 1840) and the latest estimate of Māori settlement (c. 1460). The two black dashed line indicate the start of the shift towards organic enrichment, away from historical levels (c. 1917) and the definite shift towards an alternate stable state (c. 1980). B. Blue and yellow shades highlight when the ratios shift to lower or higher values at the top of the core compared to historical trends. C. Generalised additive models (GAMs) were fitted to the XRF data to calculate significant shifts. The grey ribbon shows the derivative of each model, and significant changes are color-coded (red for increase and blue for decrease).

5.4.4 Biological responses

Total numbers of ASVs, minimum, maximum and average number of reads per sample varied depending on the primer set (Table 5.1). The plant communities (sequenced using the Illumina iSeq platform) yielded less sequences overall and per sample. Taxonomic resolution varied, with more sequences assigned to genus level in the CO1, trnL, and rbcL datasets compared to 16S rDNA and 18S rDNA.

Bacteria communities showed an overall shift from non-cyanobacterial photosynthetic taxa (Chloroflexi) and symbionts (Spirochaeota, Patescibacteria) to taxa preferring high nutrients and low oxygen conditions (Figure 5.5). Nitrospirota and Proteobacteria first appeared after the estimated timing of Māori settlement and increased progressively, representing about 20% of the bacterial community each in surface samples. Verrucomicrobiota increased about 300 years after Māori settlement (1716, 1627-1798 95% HPDF range), and MBNT15 increased after European arrival (Figure 5.5). The baseline bacteria composition underwent its biggest shift during the European era, around 1910 AD (1865-1971 95% HPDF range), when Chloroflexi ceased to be the dominant phyla and WOR-1 disappeared. Firmicutes, Proteobacteria, and Verrucomicrobiota increased over this period.

The eukaryotes amplified by the 18S rDNA primer set shifted from chlorophytes (green algae), amoebas (Apicomplexa), fungi and sponges (Porifera) to the dominance of worms (Annelida and Nematoda), different clades of fungi (Cryptomycotina), diatoms and other microeukaryotes (Gastrotricha, Cercozoa) over the study

Table 5.1: Sequencing details per primer set. Bacteria sequences were amplified by 16S rDNA, eukaryotes (euk) were amplified by targeting 18S rDNA and the cytochrome c oxidase 1 (CO1) gene, and macrophytes were selected from plant sequences amplified by the trnL and rbcL genes.

	Bacteria	Euk (18S)	Euk (CO1)	Plant (trnL)	Plant (rbcL)
Total #ASVs	66,862	13296	6,709	1,580	435
Mean read # per sample	22,805	20,648	33,465	143	27.4
Min read #	18,248	6,219	8,368	7	5
Max read #	27,289	33,305	163,104	581	160

period. Two shifts happened after European arrival. One single annelid ASV increased progressively after European arrival (10 to 50 reads in some samples prior, up to 11,865 reads after), and decreased c. 1975 (1910-2021 95% HPDF range). This was followed by a shift to a community dominated by nematodes. The second-most abundance annelid ASV was also present discreetly throughout the sedimentary record, peaking in 1935 (1877-2004 95% HDPF range) and in top-most samples. Both annelid ASVs belonged to oligochaetes (Clitellata, Haplotaxida, Naididae). The two most abundant nematode ASVs ($>$ 1,000 reads in some samples, max 3,612 reads) belonged to the family Tobrilidae (aquatic nematode family, not parasitic) and were not detected prior to c. 1910 (1865-1971 95% HPDF range).

The eukaryotes from CO1 dataset varied less and shifted from relative dominance of green algae (up to 75% to less than 10% per samples) to annelids (mainly *Branchiura*, 0 to 95% per sample, Figure D.22) shortly after European arrival (1860, 1844-1887 AD 95% HPDF range). The *Branchiura* ASV was present discreetly throughout the sedimentary record, first detected in 1330 (max 135,353 reads, min 1 read, 1299-1388 95% HPDF range). The second-most abundant annelid ASV was assigned to the *Limnodrilus* genus, and was not detected prior to 1990 (max 78,882 reads, 1929-2021 95% HPDF range). Both *Branchiura* and *Limnodrilus* belong to the Naididae family and could therefore match the main annelid ASVs observed in the 18S rRNA dataset. No nematode sequences were amplified (or at least not assigned as such). Some zooplankton sequences were successfully amplified by the CO1 gene. Rotifers (Phylum Rotifera) were also amplified (5 to 10% per sample) in most samples from 1872 onwards (Figure D.22). *Asplanchna* dominated the rotifer community, but only appeared post-European arrival, and decreased in relative abundance from 25% to 1% around 1950 (Figure D.22). Cladocerans (in the phylum Arthropoda) only had a higher relatively abundance than rotifers in the top-most sample. *Daphnia* and *Bosmina* were the only cladocerans identified in the dataset, with very low relative abundances (c. 1%, Figure D.22). Perch sequences were not identified in the 18S nor CO1 datasets.

The rbcL dataset showed the introduction of the Canadian pondweed (*Elodea*

canadensis), however, in general read numbers were very low or patchy (Figure D.21). Therefore, this data was excluded from further analysis and the trnL dataset was used as the main macrophyte proxy. Due to the high variability of the trnL amplicon (c. 20 to c. 90 bp), taxonomy could generally not be assigned at species level. *Potamogeton* was classified at family level due to the very short amplicon recovered (22 bp), therefore the historical diversity within this particular family is unknown.

The macrophytes amplified by the trnL primer set initially comprised of *Potamogeton* and *Glossostigma* (c. 50%) and shifted upon Māori arrival c. 1480 (1362-1589 95% HPDF range) to dominance of Potamogeton (c. 95%) with very small amounts of Glossostigma (c. 5%). The total number of *Potamogeton* reads over the Māori era doubled compared to total macrophytes read numbers in pre-human time (Figure D.21). *Myriophyllum* and *Isoetes* were present throughout the sedimentary record. *Ranunculus* was also present (but patchy) in the trnL dataset. Macrophytes in both plant datasets (rbcL and trnL) started to shift around 1890-1910 (1865-1971 95% HPDF range, Figure 5.5 and 5.6) characterised by a decrease in *Potamogeton* (25% to 50% depending on the primer set and reduction in total number of reads – Figure D.21) and an increase in *Myriophyllum* (40 to 70%) and other species known to grow in shallow to medium depth waters. At least two non-native species were detected: *Elodea canadensis* since European settlement (1846, 1828-1855 95% HPDF range) and *Ludwigia* (1910, 1865-1971 95% HPDF range). The non-native *Potamogeton crispus* could be part of the many Potamogetonaceae sequences recovered (e.g., the sequences attributed to the *Potamogeton* genus in 1992 and 2021, Figure 5.5), but the amplicon was too short and too general to differentiate native and non-native species.

Community compositions were summarised by Principal Response Curves (PRCs), giving a score between 0 and 4 to reflect differences in diversity between the top-most sample and the other samples (Figure 5.6). The biggest amplitude in scores (and therefore shifts in community) were observed in the bacteria and CO1 eukaryotes communities (0 to 4), with a smaller amplitude for the 18S eukaryotes (0 to 3) and

smallest for macrophytes (0 to 2).

Generalised Additive Mixed Models (GAMMs) analyses on PRC scores showed that biological communities were mostly stable throughout the pre-human and Māori settlement eras (Figure 5.6), except two short, low magnitude changes in the bacterial community prior to human arrival and one in bacteria and macrophytes concomitant with Māori settlement in the region. The continuous change in bacteria after Māori settlement (illustrated by a decrease in PRC scores) was not identified as a significant change by the GAMMs.

GAMMs showed significant changes in all communities after European arrival, but the timing was staggered (Figure 5.6). The eukaryotic community as characterised using the CO1 gene started changing significantly in c. 1860 (1844-1887 AD 95% HPDF range), followed by the community characterised by the 18S rRNA gene in c. 1870 (1847-1902 AD 95% HPDF range), and the 16S rRNA gene (bacteria) and the trnL gene (macrophyte) shifted c. 1890 (1860-1917 AD 95% HPDF range). GAMMS indicated these shifts were continuous until present day, except for the bacteria community. The first bacterial shift finished c. 1946 (1887-2014 95% HPDF range) and shifted significantly again in 1969 (1905-2021 95% HPDF range) until present day (Figure 5.6), which aligned with the dominance of nematodes from the 18S rDNA dataset (Figure 5.5).

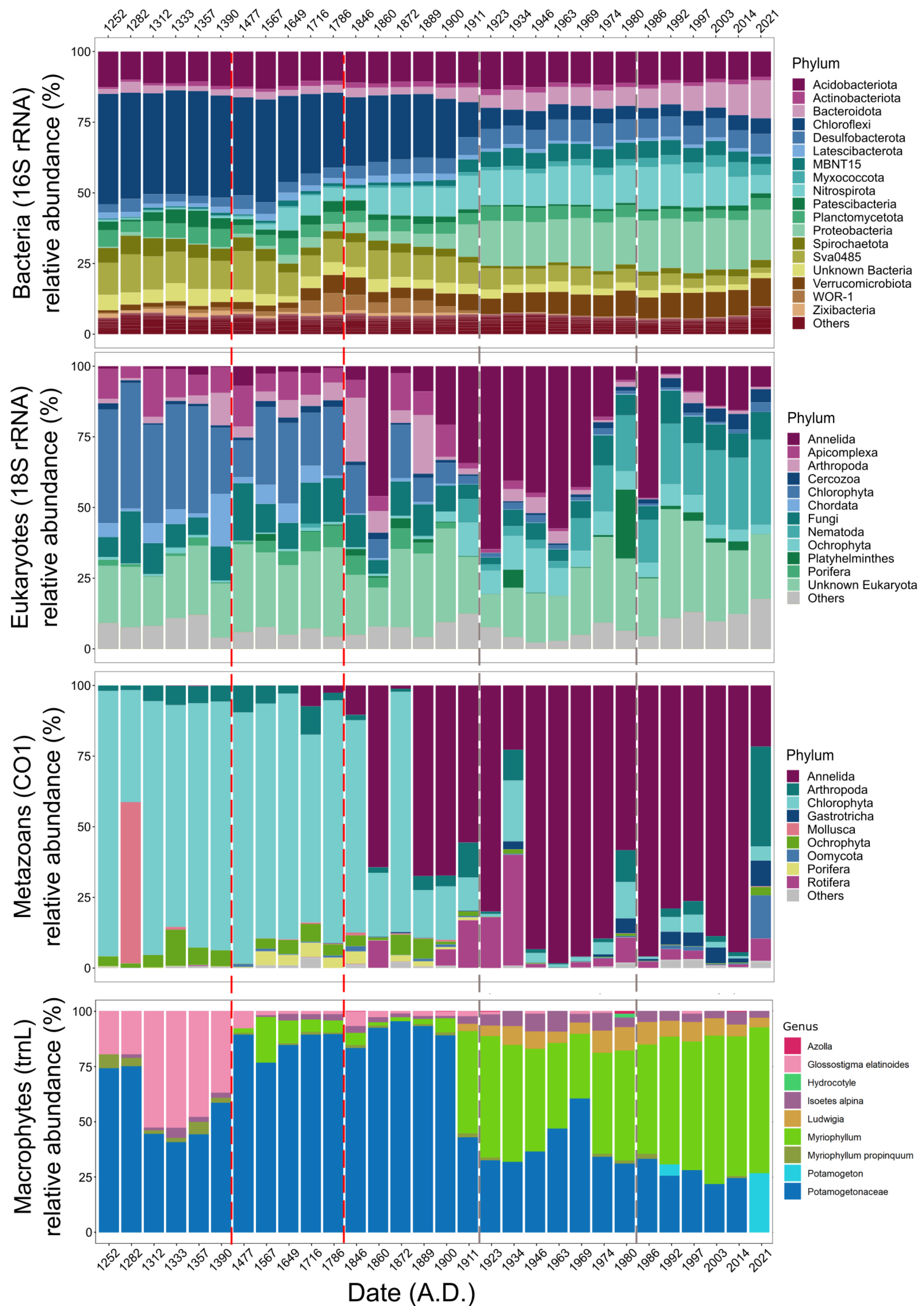

Figure 5.5: Community composition of bacteria (16S rDNA gene), eukaryotes (16S rDNA and cytochrome c oxidase 1 gene) at phylum level, and macrophytes (trnL gene) at Genus level. Red dashed lines indicate European arrival (c. 1850) and Māori settlement (c. 1460 AD). Black dashed lines mark the beginning and end of the organic enrichment transition as identified from mineralogic proxies (see Figure 5.4).

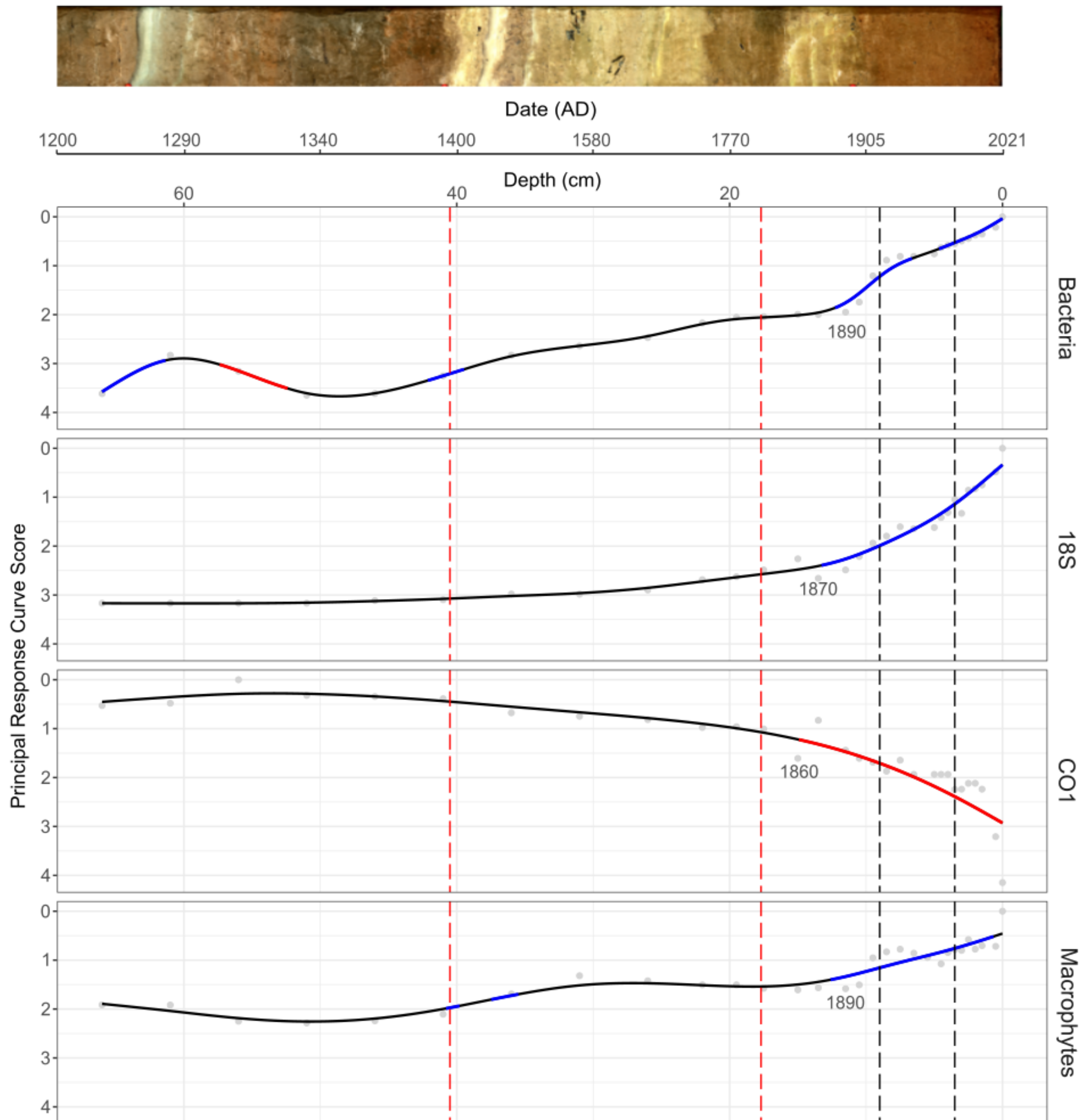

Figure 5.6: Generalised Additive Mixed Models (GAMMs) calculated on Principal Response Curves for all communities. The trnL gene was selected to represent macrophytes shifts. The grey ribbon shows the derivative of each model, and significant changes are color-coded (red for increase and blue for decrease). The red dashed lines indicate Māori and European arrival (c. 1420 and c. 1845 respectively), and the two black dashed line indicate a temporary stable state (c. 1917-1980) first identified from the mineralogic proxies (Figure 5.4). Plots are presented on a depth scale to match the core scan, with dates equivalences for specific depths.

5.4.5 Timing of stressors and responses

Historical data and the data produced in this study identified five anthropogenic stressors for Lake Pounui.

1. Terrestrial vegetation disturbance, which started around 700 years ago and varied in intensity: repeated small-scale clearance by Māori, followed by more extensive clearance upon European settlement, with repeated small-scale clearance until present day (charcoal data, Figure 5.3, Figure 5.7).
2. The introduction of non-native European perch around 1850-1870 AD (about 150 years ago, Thomson-Laing unpublished). Unlike other lakes in New Zealand, the perch population in Lake Pounui does not seem to be stunted (Jellyman, 1980) though no recent surveys have been undertaken to confirm this is still the case.
3. The known introductions of other non-native species (before 1975) were not linked with significant changes in the proxies measured in this study. Although 310,000 rainbow trout fingerlings were introduced between 1938 and 1958 (Wellington Acclimatization Society), their DNA could not be detected and none of the fish survey since then have observed them. This suggests they did not reproduce in the lake. Th introduction date of brown trout is unknown, they have been observed in fish surveys from 1975 at low abundances (Jellyman, 1990). Non-native macrophytes have also been detected (*Elodea canadensis, Ludwigia*, and *Potamogeton crispus* in macrophyte surveys). Any of these introductions could also have co-introduced aquatic nematodes in the early 1900s (Tobrilidae, Figure 5.5).
4. Artificial nutrient enrichment through the use of fertilisers, however their impact on Lake Pounui and its catchment is unknown. Earliest records mention aerial topdressing in the wider region from 1950 (*Early Flying in the Wairarapa / Masterton District Library* 2022).
5. Warming waters possibly due to climate change. Temperature data, albeit sporadic, indicate that between 1980 and 2012 Lake Pounui surface water

temperatures have likely increased (Jellyman, 1990; Lawless, 1983; Cochrane, 2017). Temperature trends at national level indicate an increase in air temperature since 1910.

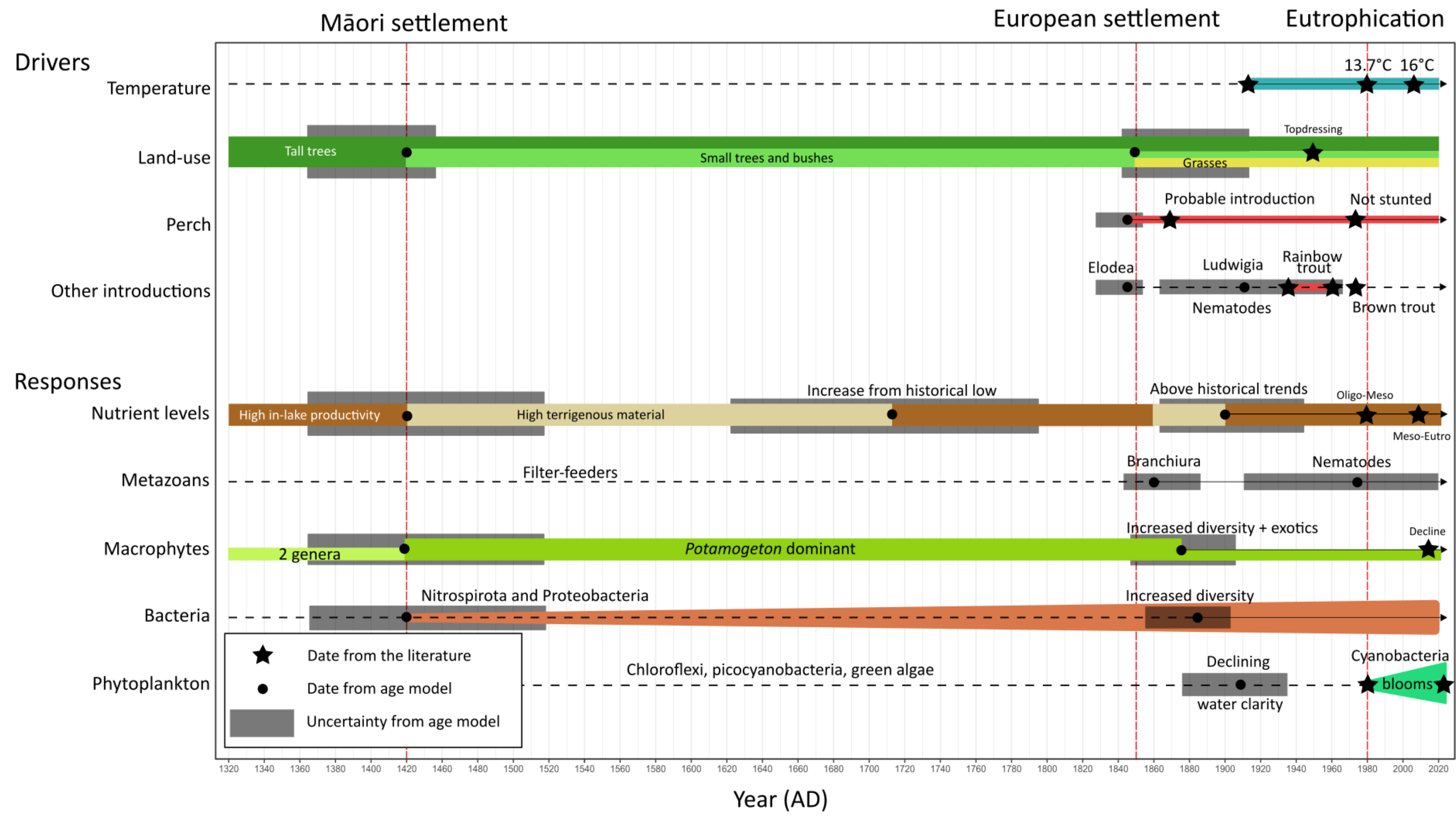

Figure 5.7: Main drivers and timing of biologic and mineralogic responses. Key dates from the start of shift are represented by points, the grey shades indicating uncertainties from the age model (95% high density probability range). Stars indicate key date from the literature. Temperature trends from historical data (1.13°C air temperature increase since 1910, lake surface temperatures in 1980 and 2008). Catchment composition from pollen data and start of topdressing c. 1950. Perch reconstruction from sedaDNA (this sediment core) and literature. Other introductions (fish and macrophytes) from sedaDNA and literature. Nutrient source fluctuated between in-lake productivity (inc/coh ratio) and terrigenous input (K/inc and Ti/inc) until the European era where in-lake productivity increased beyond historical trends, shifts in trophic levels from the literature (Oligo-Meso = oligotrophic to mesotrophic and Meso-Eutro = mesotrophic to eutrophic). Metazoan shifts reconstructed from 18S rDNA and CO1 gene, macrophyte shifts reconstructed from the trnL gene, bacteria shifts reconstructed from 16S rDNA. Phytoplankton shifts reconstructed from 16S rDNA and 18S rDNA, complemented by literature for cyanobacterial blooms intensifying.

Alignment of the stressors and responses indicates that terrestrial vegetation disturbance by Māori led to shifts in catchment cover, terrigenous inputs, slow increase of bacteria involved in nutrient cycling, and a shift in macrophytes (disappearance of *Glossostigma elatinoides*, a species of "turf" plant growing in the shallows and varial zone). However, no notable shifts in eukaryotes or phytoplankton were observed at that time. The largest responses occurred after European settlement, but before known agricultural intensification in the region (i.e., fertiliser). Extensive vegetation clearance in the catchment around 1845 was followed by shifts in all biological communities. The increase in oligochaete worms provided the first indication that the health of the lake was changing, accompanied by shift in bacteria, diversification of the macrophyte community (due to a decline in *Potamogeton*), and shifts in phytoplankton. The age model puts this shift in the late 1800s, about 100 years prior to the studies which showed a change from oligotrophic to mesotrophic status of Lake Pounui (Lawless, 1983; Jellyman, 1990). The most recent shift occurred c. 1980, which aligns with a significant shift in bacterial communities (Figure 5.5, 5.6). This also aligns with the period of marked increase in total cyanobacteria and the dominance of bloom-forming species (Picard et al., 2022a). Historical data and the sedimentary record show an increase in grasses (pollen data – Figure 5.3, aerial pictures – Figure 5.1) in the period leading up to this shift. The sediment also becoming increasing enrich in organic matter during this period, likely signalling an increase in lake productivity (Figure 5.4, 5.7).

5.5 Discussion

Sedimentary records suggested that the ecosystem of Lake Pounui was resilient and resistant to the natural disturbances that occurred prior to human arrival in New Zealand, and in the most part to the vegetation changes in the catchment and corresponding sediment inputs following Māori settlement in the region. This stability was illustrated by the stability of eukaryotes (phytoplankton and metazoans) over 800 years and contrasted with the marked shift that occurred in multiple proxies over

the last 170 years. This recent change occurred concomitant with European impact and potential climate warming. The results from the present study show ecosystem-level responses previously undocumented in New Zealand lacustrine ecosystems, such as shifts in native oligochaetes. This is also the first account of aquatic nematodes introduction in New Zealand lakes, raising awareness about these poorly studied components of lacustrine food webs.

5.5.1 Disentangling the drivers behind the change in trajectory

This study aimed to explore the relationship between perch introduction and the intensification of cyanobacterial blooms in Lake Pounui. In contrast to our hypothesis, the initial changes in the lake ecosystem did not appear to be solely linked to the introduction of perch, but more nuanced, with multiple factors likely contributing. This contrasts with findings from a nearby lake, Roto Kawau, where there is strong evidence that perch drive cyanobacterial blooms (Smith et al., 2006; Smith et al., 2007). This could be due to a number of factors, such as the difference between a natural system (Pounui) versus a man-made system (Roto Kawau) leading to different resistance and resilience mechanisms, for example, Roto Kawau does not have a well-established macrophyte community. Lake Pounui appeared to have a stronger predator community (1,000 eels captured in 3 months and up to c. 100,000 glass eels recruitment per year; Jellyman (1989b) and Jellyman et al. (1983)) compared to Roto Kawau which is dammed, so recruitment of elvers difficult, though there are a few resident eels present in the lake. This lack of predation could have resulted in the perch community becoming overcrowded and stunted in Roto Kawau (mostly juveniles, less than 100 mm which are zooplanktivorous; Hicks et al. (2007)) while the work of Jellyman in 1975 suggests that the perch community is not stunted in Pounui and includes adult piscivorous perch (Jellyman, 1980), possibly also preying on younger conspecifics. This would mean that the perch community in Lake Pounui may be less intense than in Roto Kawau and that the zooplankton commu-

nity could still efficiently graze down phytoplankton. However, no assessment of the population structure of perch has been made since Jellyman (1980), and it is possible the situation has changed since then, contributing to the cyanobacterial blooms observed in recent decades. The presence of macrophytes in Pounui for most of the lake's history as refuge habitat may have also contributed to alleviation of predation on zooplankton (Irvine et al., 1989) and the collapse of macrophytes may have allowed enhanced predation to have reduced the ability of zooplankton to control phytoplankton.

Furthermore, limnological studies by Burns and colleagues indicate that New Zealand zooplankton communities may be too simple to efficiently graze on cyanobacteria (Burns, 1987; Burns et al., 1987; Haney, 1987; Burns et al., 1991). Research on New Zealand *Daphnia* species suggest that they are unable to digest filamentous cyanobacteria such as *Dolichospermum* sp. (the genus blooming in Lake Pounui), which could ultimately lead to their deaths (Burns et al., 1987). In comparison, the cladoceran *Bosmina* avoids grazing on cyanobacteria altogether. These differences in diets (and fatality rates) could explain the changes in the zooplankton community in 1980, where cyanobacterial blooms lasted 10 days at most and *Bosmina* was not the dominant species (Lawless, 1983; Jellyman, 1990), compared to 2022 where cyanobacterial blooms last for extended periods during summer and *Bosmina* dominated the zooplankton community at 99% (Duggan, 2022). Unfortunately, the DNA approaches and/or primers used in this study did not adequately differentiate the key zooplankton species, making it impossible to determine historical changes in these taxa (discussed further below). In conclusion, the introduction of perch with voracious zooplanktivorous juveniles into a lake system with a zooplankton community already ill-equipped to restrain cyanobacterial blooms could have led to a slow decline in water quality by itself, however, when compounded with only stressors this is likely to have led to a more rapid decline in water quality.

Despite the lack of long-term historical data, differences in water temperature measured in 1980 and 2012 provided some initial evidence that climate change is also impacting Lake Pounui. Care needs to be taken with this interpretation as

the measurements taken in the 1970 and 1980s were from single points in time (albeit over many months) compared to the most recent data which comes from continuous loggers, which are more likely to capture periods of stratification in Lake Pounui which is subject to frequent wind mixing events. Climate events such as La Niña and El Niño can cause periodic differences in local climates, therefore because of the sporadic nature of the data we cannot be conclusive about whether the observed increase in temperature is a long-term pattern. However, increased temperatures enhance stratification which in turn influences bottom oxygen levels and potential release of nutrients stored in the sediment. An increasing number of studies have highlighted a competitive advantage of bloom-forming cyanobacteria over other phytoplankton clades in warmer waters (Paerl et al., 2012; Paerl et al., 2008; Erratt et al., 2021). Increased internal nutrient cycling and warmer waters may therefore be a strong secondary driver of the intensification of cyanobacterial blooms in Lake Pounui.

The link between land-use change in lake catchments and eutrophication is well documented (Banks et al., 1953; Harper, 1992; Callisto et al., 2014). In Lake Pounui, the changes in vegetation cover associated with human transitions were associated with increases in sediment inputs into the lakes (as indicated by the erosion proxies from the XRF) which aligned with several relatively minor shifts in the bacteria and macrophyte communities. The decline in native tall trees after Māori settlement is aligned with an increase in wetland species such as *Typha* and Cyperacae, and it is plausible that the removal of trees around the lake allowed more light to reach the lake edge, allowing the expansion of these species. European settlement appears to have magnified the change in vegetation around Lake Pounui, with an increase in grasses and a decrease in small trees and shrubs trees in the pollen data indicating more open and likely farmed land. A relative increase in oligochaete worms starting in 1860-1870 and in Proteobacteria in 1890 suggest an increase in nutrients that is most likely related to this land-use change. The last shift in bacterial communities around 1970 (1905-2021 95% HPDF range) may also be due to land-use change, specifically to the addition of fertiliser. Aerial topdressing began in 1950 in the

wider region, and such artificial nutrient enrichment would have further intensified the impact of all other drivers.

The most recent community shifts may be the results of additive effects of the drivers listed above, and others that we were not able to characterise. Rotifer sequences belonging to the genus *Asplanchna* were not detected prior to European settlement (first detection of 7.5% relative abundance in 1872, 1847-1902 95% HPDF range). Fungi sequences (Order Cryptomycotina) were also observed starting in the same sample (from c. 1% to c. 10% relative abundance in the 18S rDNA dataset). It is possible that these organisms were introduced at the same time as perch or trout stocking, and there is not enough data to ascertain whether these (voluntary and unvoluntary) introductions have had an impact on the food web of Lake Pounui. Nematode (Tobrilidae) sequences were found later, starting from 1910, suggesting they may also have been introduced into the lake through another vector. Overseas, members of the family Tobrilidae have been found to prey on detritus, small invertebrates and unicellular phytoplankton (Abebe et al., 2006). Non-native macrophytes were also detected, *Elodea canadensis* since 1845 AD (1828-1855 95% HPDF range) and *Ludwigia* at the same time as nematodes (1910, 1865-1971 95% HPDF range), but the sedaDNA data suggests these species did not replace native macrophytes.

The shifts in macrophytes reconstructed by sedaDNA were interesting and some were unexpected. The disappearance of *G. elatinoides* and increase in *Potamogeton*, concomitant with the expansion of emergent reeds and sedges after Māori settlement may also be related to the nationwide extinction of the New Zealand black swan / poūwa (*Cygnus sumnerensis*) around 1450 AD (Rawlence et al., 2017). Poūwa were once found throughout New Zealand and are assumed to have had a diet similar to other *Cygnus* species, disturbing sediment in shallow waters to eat aquatic invertebrates as young and eating macrophytes shoots as adults. The Australian Black Swan can dive down to 1 m to feed (Choney, 2012), and the New Zealand poūwa was both larger and heavier (Rawlence et al., 2017) therefore it is possible it could have had significant impact on plants from both the shoreline and shallow

waters until its extinction. However, Poūwa remains have not yet been found near Lake Pounui and the CO1 dataset did not detect *Cygnus* sequences this far back in the sediment core, therefore it is not possible to draw robust conclusions using the present data.

5.5.2 Variation in the historical stability of Lake Pounui

Shallow lakes are often described as being in either a clear-water, macrophyte dominated or turbid, phytoplankton-dominated state (Phillips et al., 2016). Between these two categories, alternative stable states also exist due to intermediate nutrient levels and food-web interactions (Scheffer, 1989; Hobbs et al., 2012). A current limnological paradigm, the flipping lakes concept, argues that while the clearwater state is favoured by low nutrient status, and the turbid by high nutrient concentrations, between these two extremes, either of the two alternative states can persist, each sustained by internal feedback mechanisms (Scheffer, 1989; Schallenberg et al., 2009). Models of alternate stable states have shown that lakes can stay in a given state due to their resilience and resistance mechanisms, which prevent them from tipping towards other stable states. Major disturbances can bypass these mechanisms and tip lakes into alternate stable states. Based on these theories, I propose that Lake Pounui has been in four stable states since 1200 AD (beginning of the sedaDNA reconstruction), with the latest change around 1980, when the lake shifted towards a nutrient-enriched state with frequent cyanobacterial blooms (Figure 5.8). Over the timeline studied, XRF scanning and biological communities (especially bacteria) showed shifts that aligned with early land-use change around 1400s, more significant land-use change and the introduction of perch around 1850, and a stable state from c. 1917 to c. 1980 after which the water quality started to degrade (Jellyman, 1990; Lawless, 1983; Perrie et al., 2012; Winton et al., 2011; Winton, 2016). Collectively, the historical records and data generated in this study suggest that Lake Pounui was in a clear water state prior to 1980, as indicated by the dominance of macrophytes (mainly *Potamogeton* sp.) and of some phytoplankton

species (Chloroflexi, picocyanobacteria). Short cyanobacterial blooms were reported in 1975-1977, with studies suggesting these intensified following this, aligning with a shift to a lake where cyanobacteria are abundant. However, we note that macrophytes are still present in the lake, and it is not completely turbid, therefore it may not conform to the classic regime shift describe in the literature (Scheffer, 1989; Phillips et al., 2016).

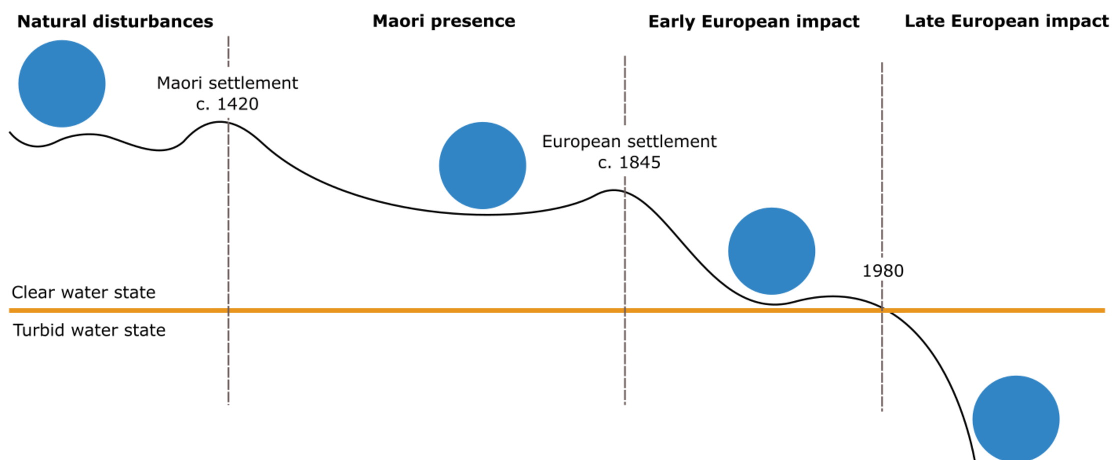

Figure 5.8: Model representing the possible stable states of Lake Pounui since 1200 AD. The blue ball represents the ecosystem of Lake Pounui, and the black line represents its resilience and resistance mechanisms keeping it in a specific state. The data showed that Māori settlement led to shifts but the lake was mostly stable. European settlement led to major shifts away from the baseline condition, but the lake stayed in a stable state for c. 60 years before shifting again in 1980 towards a phytoplankton-dominated state.

5.5.3 Methodological considerations and next steps

The age model was less reliable at the top of the sediment core. The depocenter core used in this study and in Thomson-Laing et al. (unpublished) showed evidence of bioturbation, and the presence of perch was first detected 25 years earlier than possible based on historical accounts of when this fish was introduced to waterways across New Zealand. The age model dated the sedimentary layer in which perch DNA was detected to 1848 (1828-1855 95% HPDF range), but perch were only introduced in 1869-1870 in New Zealand (McDowall, 1990). A kākahi (freshwater mussel) was present in the sediment core and was thought to be the main cause of

the bioturbation, however, perch DNA was also detected in the littoral core at a date of 1850 (1843-1857 95% HPDF range). There was no evidence of significant bioturbation in this core, albeit is possible that the increase in oligochaete worms may have caused some mixing. The final shift towards eutrophication was dated c. 1980, which matches historical studies documenting the water quality switching from oligotrophic to mesotrophic (Jellyman, 1990; Lawless, 1983) suggesting that the integrity of the sediment core is relatively robust.

The bacteria and macrophyte datasets used in this study provided robust indicators of water quality changes, with both communities shifting in concert with the first land clearance (Māori settlement, c. 1400 AD), and again as precursors to eutrophication. Sedimentary bacteria communities have been documented as reliable indicators of water quality in New Zealand (Pearman et al., 2022), and shifts in macrophyte communities have long been markers of a clear-water state (macrophyte dominated) versus a turbid water state (phytoplankton dominated, usually eutrophic) (Clayton et al., 2006). However, the shift in macrophytes reconstructed in the present study is mainly based on species diversity and total number of reads. A quantitative analysis (e.g., droplet digital PCR) would provide a better proof of the timing and extent of the shift in *Potamogeton* spp.

Additionally, the macrophyte community composition from sedaDNA showed differences from survey results. *Potamogeton* sp. sedaDNA was recovered throughout the sedimentary record, though it is not possible to distinguish between native versus non-native species. Jellyman (1990) did not report any native *Potamogeton* species in 1975, while later macrophyte surveys report a high portion of the lake bed being covered in native *Potamogeton ochreatus* (Winton et al., 2011; Winton, 2016). A misidentification from Jellyman is possible, but it is also possible that native Potamogeton was only present in very low abundance in 1975. The lack of taxonomical precision from the trnL and rbcL amplicons also hampers further interpretation within the *Myriophyllum* genus, since native species include short, stumpy taxa (e.g., *M. pedunculatum*) as well as tall, deeper growing ones (e.g., *M. triphyllum*).

All macrophyte surveys reported an abundance of characeans (one or several species) which were not identified by the trnL or rbcL primer sets. Green algae (Chlorophyta) were amplified both by the 18S rDNA gene (Class Trebouxiophyceae) and CO1 gene (Unknown Class and Class Chloropicophyceae), but due to the lack of taxonomic resolution of these amplicons it was not possible to identify specific species and determine their ecology (benthic / epiphytic or pelagic). The complete lack of Characeae sequences was surprising, despite their important coverage of the lakebed in recent years. The trnL primers should be able to amplify New Zealand species as well, therefore some methodological checks should be done to understand why Characeae are missing, and if other key historical species could be missing as well.

The bacteria community of Lake Pounui showed similar trends with other New Zealand lakes. Chloroflexi were also a large part of the community in the pre-human times of the nearby Lake Nganoke (Parrish, 2020), lakes of the Tasman region (Appendix A), and of Lake Paringa (West Coast of the South Island; Brasell et al. (2021)). Proteobacteria also increased progressively over time in most lakes (Appendix A). However, this may not be the norm for New Zealand lakes, since Proteobacteria already represented half of the bacterial community in Lake Okataina (Taupo Volcanic Zone, North Island; Caird (2021)) prior to European arrival. More New Zealand lakes need to be studied to be able to disentangle the impact of regional drivers and lake type (river, glacial, volcanic) from national and historical trends. Caution also needs to be taken when interpreting bacterial communities from sedaDNA, since some of the taxa detected could actually be living in the sediment.

Shifts in oligochaete worms have been less documented, but like bacteria there seem to be species adapted to specific water quality conditions (Lang, 1997; Lang et al., 1996). Due to the low taxonomic resolution of the 18S rRNA gene, the worms could only be identified to Class level (Naididae) while the CO1 gene enabled some taxa to be assigned to the *Branchiura* and *Limnodrilus* genera. More specific primer sets could shed some light on which species are present, whether they are native or

not, and therefore help understand what led to the dominance of these worms in the benthos as reconstructed by sedaDNA.

Other taxa would also benefit from targeted methods, whether molecular or microfossils. For example, sedaDNA showed the historical presence of interesting aquatic native species, such as sponges (1 ASV), freshwater bryozoans (4 ASVs), and freshwater mussels (2 ASVs with high abundance, 36 ASVs total). Metabarcoding also confirmed the presence of several species introduced by Europeans in and around Lake Pounui (*Elodea canadensis*, *Ludwigia*, pig), but the method was not sensitive enough to reconstruct their continuous presence. The zooplankton community was not well reconstructed with a lack of key species found in historical studies and those that were detected were in low abundance and patchy. The historical presence and disappearance of the New Zealand black swan / poūwa (*Cygnus sumnerensis*) upon Māori settlement could be further investigated to further interpret shifts in macrophyte communities. Techniques such target-capture (baits) could aid in detecting these species in sediment cores (Armbrecht et al., 2021). Assessing Cladocera remains in the sediment cores using traditional approached (e.g., Jeppesen et al. (2001) and Monchamp et al. (2021)) would also be valuable and allow assessment of whether there were early shifts in these communities.

Finally, XRF scanning provided novel insights on mineralogic trends, with good correlation between titanium, potassium, and in-lake productivity (incoherent/coherent XRF scattering). Other mineralogic ratios still need more refinement and comparison with independent data: Calcium / Silica and Manganese / Iron have been reported as interesting proxies for water temperature and oxygen levels, respectively, but their trends in the sedimentary record of Lake Pounui were not conclusive.

5.6 Conclusion

The combination of historical data and the results from this study provide a robust reconstruction of the environmental history of Lake Pounui. The period from 1200 AD to 1400 AD is characterised by relative stability with the lake in a clear-water

state dominated by macrophytes and very small bacterial autotrophs (Chloroflexi, picocyanobacteria). There were some shifts after initial changes in the vegetation about 1400 AD but the most pronounced changes occurred as European settled in the region, further modifying the landscape. Unfortunately, in this dataset we were unable to separate the impact of landscape change and the introduction of perch. It is likely that these had additive effects, with evidence of nutrient enrichment through the early changes indicated by increases in oligochaete worms. Climate change is also likely to be impacting the lake, with evidence of temperature increases and possible longer periods of stratification. Further studies on the current size structure of perch and the composition of zooplankton populations are recommended.

Chapter 6

General discussion

Written accounts of New Zealand ecosystems are only a few hundred years old at most, but it is clear from oral traditions and palaeoecological observations that dramatic changes have occurred over the past 800 years, coinciding with waves of human settlement. The impacts of human arrivals are seen through all ecosystems, and lakes are particularly vulnerable as receiving points for catchment processes. Science only started to focus on lakes from the 1950s and many gaps in our understanding of the progression of impact remain, as outlined in Chapter 1. In particular, there are still significant knowledge gaps regarding the timing of introduction of zooplanktivorous fish such as perch and how lakes responded to these food-web changes. Lake Pounui was an ideal study lake for such a question: low agricultural land-use in the current catchment, European perch are the main non-native fish species, and the lake is undergoing heavy cyanobacterial blooms in recent decades.

Molecular tools coupled with paleolimnology offer unprecedented opportunities to reconstruct past aquatic communities and complement known records of change. Studying sedimentary ancient DNA (sedaDNA) allows the reconstruction of aquatic communities that are difficult to target otherwise, and a growing body of literature illustrates the multiple applications that can be derived from these methods (Chapter 1 section 1.6.2).

The overarching goal of this thesis was to demonstrate how eDNA could be used to detect cyanobacterial blooms and non-native fish and then combine these to link

perch introduction and cyanobacterial blooms in Lake Pounui, as an example of the impact of non-native fish on New Zealand lacustrine ecosystems. The motivation behind this was that previous studies in a man-made lake indicated that the controlling pressure of juvenile perch (a zooplanktivorous part of the lifecycle) on zooplankton grazing could be strong enough to allow cyanobacterial boom formation (a top-down control), and that this impact was superior to nutrient addition (a bottom-up control) (Smith et al., 2007). The paradigm that food web processes could be as or more important than nutrient enrichment has otherwise received relatively little attention in the limnological literature.

6.1 Synthesis

My second chapter proved that it was possible to amplify sedimentary ancient DNA (sedaDNA) left behind by cyanobacteria in a range of lakes throughout New Zealand. This chapter contributed to a growing body of literature on cyanobacterial sedaDNA, including reporting on the composition of cyanobacteria assemblages in the absence of human populations. All baseline communities were dominated by picocyanobacteria, while bloom-forming species later became abundant in lakes impacted by human activities. This was a novel finding for New Zealand lakes, and since then studies have further investigated the poorly-known picocyanobacteria compartment in New Zealand lakes (Schallenberg et al., 2021b; Schallenberg et al., 2021a).

Based on the cyanobacterial eDNA data, total cyanobacteria abundances (and bacterial abundances, Appendix B) increased in most lakes in concert with increasing European impact, with the relative abundance of bloom-forming species eventually dominating in lakes undergoing cyanobacterial blooms. Sediment records suggested that most lakes remained relatively pristine prior to European arrival, and it was interesting to see that even in lakes where Māori had settled nearby (Pounui, Wairarapa, Rotoehu) cyanobacteria assemblages did not become dominated by bloom-forming species until after European settlement. This could indicate a certain threshold of disturbance needed to lead to cyanobacterial blooms, perhaps

also linked to changes in aquatic foodwebs and climate warming, as discussed in Chapter 5. In the context of Lake Pounui, this chapter identified the main bloom-forming species as *Dolichospermum* sp., matching recent phytoplankton counts from the water column.

My third chapter demonstrated that total cyanobacterial abundances were similar when measured from ddPCR (sedaDNA) and HPLC (a range of pigments). This was an important methodological check, since few sedaDNA paleolimnological studies had done comparisons with pigments and where that had been done their findings were contrary. Cyanobacterial sedaDNA related better to a sum of cyanobacterial pigments (canthaxanthin, echinenone, myxoxanthophyll, zeaxanthin) compared to individual pigments, which was a logical conclusion. Since earlier studies have proved that different cyanobacteria species produce different pigments, this showed that the sedaDNA amplified by ddPCR came from a wide range of cyanobacterial species (when the appropriate primer set was used). Furthermore, it indicated that degradation rates and real historical increases were reconstructed in the same way by these two proxies. This chapter provided further evidence to support an increase in total cyanobacteria in Lake Pounui, as both sedaDNA and cyanobacteria-specific pigments (especially echinenone) increased at similar rates over time.

My fourth chapter focused on detecting fish eDNA in contemporary samples (lake water and surface sediment). This was another crucial methodological check - because my thesis aimed at linking non-native fish (European perch) with historical changes in lake foodweb. It was paramount to be able to know when these fish were first introduced in the system and as records of introductions were patchy, and altogether lacking for some species, my next option was to detect their sedaDNA. I started with contemporary samples, where we knew we would find fish sedDNA if the technique were to be useful, since the target fish was present in the lake. My goal was also to show that fish DNA was present in high abundance in lake sediments, hypothesised to be because DNA binds to sediment particles and is therefore more

stable. The findings from previous studies provided contrasting evidence on this point. Selecting perch and another non-native coarse fish (rudd) ensured the results would not be too species-specific.

My early attempts at detecting fish from DNA in sediments hinted that we were missing some fish sedDNA. We knew that some lakes (Pounui and Waitawa) had high levels of the target species, yet we detected very low sedDNA detections, suggesting that sedDNA was a first not a good proxy for fish distribution. The hard work from Georgia Thomson-Laing (concurrent PhD thesis on fish sedaDNA) developed an extraction method that was tailored to bigger sediment volumes and worked well with most sediment types (Thomson-Laing et al., 2022). I was therefore able to use this extraction method on my surface sediment samples, and it worked better. I found that fish sedDNA was generally well distributed across the lake bed and also found in the depocenter of shallow lakes. This was good news, since my sediment cores were taken from the same spot and it indicated that depocenters could be a good location for historical fish sedaDNA trends. However, shallower parts of the lakes generally had higher fish sedDNA levels (or higher distribution), not surprising since they were the areas where the targeted coarse fish lived. Therefore, my findings meant a littoral core should yield more coarse fish sedaDNA. This was later investigated and proved by Georgia Thomson-Laing in actual sediment cores from Lake Pounui (Thomson-Laing unpublished). In my chapter, it was interesting to be able to compare results across several lakes, since fish sedDNA distribution was higher than water in Lake Pounui (and Tomarata) but lower in Lake Waitawa. This was a good example that due to differences in sediment biogeochemistry, some lakes would not be good candidate for fish sedDNA studies.

Furthermore, my study was an interesting demonstration of how molecular methods could be used in a biosecurity context in New Zealand. Traditional capture-based fish surveys are consuming in terms of time and manpower and can miss the presence of cryptic species. In one afternoon of sampling per lake, my data provided spatial information derived from 14 sites in medium-sized shallow lakes, and the samples could be analysed for specific species (Chapter 4) or community-wide metabarcod-

ing (Appendix C). This data provided me with an opportunity to apply occupancy modelling, to statistically assess if we had enough sites and samples compared to the fish community targeted (influencing their eDNA distribution). It was important to be able to detect fish sedDNA and provide statistically sound and repeatable findings as well, since a lot of studies have struggled with this subject. I found that, in small and shallow lakes, at least six sites and five replicates per site would be needed to reliably detect fish eDNA in sediment samples, and twenty sites with eight replicates per site for water. For small coarse fish (perch and rudd), sampling location did not matter in a very shallow lake (c. 5m max depth), while for a lake a bit deeper (c. 10 m max depth) it was preferable to sample shallower parts of the lakebed.

My fifth and final data chapter built on these preliminary checks and the perch sedaDNA reconstruction from Georgia Thomson-Laing to reconstruct the drivers behind the long and heavy cyanobacterial blooms in Lake Pounui in recent times. The answer was not straightforward, and seemingly a combination of several factors - land-use, perch introduction, and climate warming – were implicated. These drivers created negative feedback loops which, over time, led to the degradation of the water quality in Lake Pounui. The bacteria community was dominated by Chloroflexi in prehuman times, shifting once after Māori settlement and twice after European settlement to become dominated by Proteobacteria and Nitrospirota. The water quality went from clear (macrophyte-dominated) to turbid (bloom-forming cyanobacteria), became enriched in nutrients, correlated with an increase in oligochaete worms and freshwater nematodes. The main biological shifts were observed with a lag-time, after European forest clearance and perch introduction. Interestingly, there was a period of stability before more intensification land-use (fertiliser) which, with climate change, led to the final shift to the current substantially degraded state of Lake Pounui. The impact of perch is not clear, however it is probable that they contributed to destabilise the ecosystem prior land-use intensification.

This thesis thus showed that bloom-forming cyanobacteria are becoming more

dominant in New Zealand lakes due to a variety of factors – not just eutrophication. This conclusion should alert anyone relying on the ecosystem services these lakes provide, since an increase in blooms will affect water use and native ecosystems. Land-use impact can be long-lasting, non-native species impact can be insidious due to a possible lag-time, and climate change is affecting every lake in both similar (warmer temperatures) and different (drier or wetter) manners.

6.2 Study limitations

Though molecular methods are very powerful, they have several drawbacks which are generally well highlighted in the literature (Sidstedt et al., 2020; Ruppert et al., 2019; Capo et al., 2021a). The main flaw of sedaDNA as analysed in this thesis is the use of PCR, which can induce bias in community composition as it preferentially amplifies the most abundant sequences and the most linear ones (secondary structures can prevent polymerase activity). Therefore, rare sequences and regions physically convoluted may not be amplified at all. The annealing temperature also determines how stringent the amplification is, thus primers with low annealing temperatures (c. 50-52°C) will amplify less specific sequences, and possibly outside of the desired targets. Restriction enzymes can be used to cut secondary structures and avoid amplifying non-targets, like for perch and rudd detection in Chapter 4, however these work best when targeting short amplicons of specific species. In the case of metabarcoding, the amplicons are longer and many different, possibly unknown sequences need to be amplified, which makes the use of restriction enzymes too complicated. Finally, one long-standing struggle of eDNA studies is to have adequate primer sets (for PCR) or baits (for in-situ hybridisation) and adequate references databases (all methods). This was well illustrated by Chapter 3, where the cyanobacteria primer set needed to be adapted, and Chapter 5, where the CO1 'zooplankton' primer set amplified other targets but very little zooplankton, perhaps indicating little specificity for New Zealand zooplankton taxa.

The impact of physical and chemical characteristics of the environment on eDNA

samples is still poorly understood. Studies have investigated how oxygen levels, light levels, pH, temperature affects DNA degradation in water samples, but similar studies have not yet been undertaken on sediment samples. Chapter 4 provided some insights into how sediment geochemistry could affect fish sedDNA detection. This could have drastic consequences when recovering sedDNA from some lake sediment core, or when encountering a difficult layer, where the biological trends recovered could be markedly different from what they 'really were'.

Due to the field of sedaDNA being very recent, studies have generally put more emphasis on being able to detect their target, rather than being robust and providing repeatable results. In Chapter 2, due to different core lengths, non-linear sedimentation rates and unknown timings of change prior to DNA analysis, the number of samples in each era (pre-human, evidence of Māori settlement, post-European settlement) differed in each core and across lakes. The pre-human era was not even recovered in the Lake Hayes core. This makes conclusions on changes across eras a little less robust than planned. Furthermore, the number of lakes sampled (six) does not compare to all New Zealand lakes (c. 3,800), therefore conclusions on the general impact of Māori settlement and European settlement may not be representative of overall changes.

Including other proxies (pigments, pollen and charcoal, XRF scanning, sediment dating) was necessary for a more robust interpretation of sedaDNA amplified by ddPCR and metabarcoding. Most of these proxies helped to complement sedaDNA, but they did not always confirm or contradict sedaDNA findings. Using proxies reconstructing the same target would be very useful. This was done in Chapter 3, by comparing sedaDNA (ddPCR) to pigments (HPLC), but in Chapter 5 there were no other proxies to complement within-lake biological trends, such as microfossils analyses. The unexpected biological results from sedaDNA (early macrophyte collapse, early benthos shift to worms) can therefore not be confirmed at this point.

Molecular methods aside, the heavy reliance of this thesis on charcoal and pollen analysis may be a weakness to robust reconstructions. Whenever possible, charcoal trends were complemented with age models, sedimentology, and cross-checked with

shifts in sedaDNA to verify that they were most probably indicating human settlement and not to natural fires. However, due to the discrete subsampling used by most analyses, it is possible that human settlement happened earlier than indicated, especially if the landscape was not cleared through burning. Furthermore, changes in catchment vegetation were solely reconstructed by pollen analysis. This is quite common in traditional paleolimnology, but it means that key changes might be incorrectly reconstructed. Pollens can come from the wider region rather than a local signature, and for example in Lake Pounui, any pollens coming from the East of the lake (downstream) would not relate to changes within the catchment itself. Other proxies could, and perhaps should, be used to make sure that these reconstructions accurately reflect past events. XRF scanning is promising since it provides continuous measurements (therefore specific timings of change are unlikely to be missed), and it can detect a wide range of elements including some related to catchment changes (e.g., titanium, potassium, aluminium). Sedimentary ancient DNA could also complement pollen by detected taxa more locally, whether they produce pollen / spores or not.

6.3 Future directions

The multi-trophic response of Lake Pounui to human impact opens up a multitude of avenues for further investigation. Though traditional paleolimnology can reconstitute some communities (e.g., diatoms, cladocerans, chironomids, phytoplankton abundance), it cannot look into the whole lacustrine foodweb. Sedimentary ancient DNA provides many new opportunities, but it is sometimes difficult to link DNA data with ecological trends. More studies are needed on specific components of lake foodwebs and historical shifts (benthic filter-feeders vs oligochaetes, impact of freshwater nematode predation), but also multi-trophic studies to shed light on lake responses to disturbances. Investigating how biodiversity contributes to the resilience and resistance of New Zealand lakes will provide a more complete picture of lake health. Further research topics could include whether restoring native food-

webs might reduce blooms, such as grazing impact from zooplankton or filtering from kākahi / freshwater mussels. Oligochaete worms are already used as indicators of trophic level (Manaaki Whenua / Landcare Research), but perhaps it is time to consider the ecological impact of specific species (e.g., in internal nutrient cycling) on top of their simple presence or absence. Further, if freshwater nematodes (Tobrilidae) were indeed introduced to Lake Pounui c. 1910, did this introduction happen in other lakes, and how did this new and tiny predator affect the food-web? Finally, reconstructing historical macrophyte communities when historical data is lacking will complement contemporary monitoring. Informing on baseline conditions, providing a (non-exhaustive) list of the species that were once present, and indicating whether the macrophyte community has already shifted away from its previous state will yield precious insights for management practices. The matter of perch impact has not been completely solved. A continuation of the mesocosm study from Smith et al. (2007) could yield more results on which zooplankton species are targeted by juvenile perch in New Zealand freshwaters, and what would be the ratio of juvenile and predators to see an significant impact (or not) on zooplankton densities. Comparing juvenile perch to other native zooplanktivorous species (e.g., bullies) would further highlight the differences in impact native ecosystems.

6.4 Going further - the need for community-wide discussion around non-native fish

Chapter 5 provides leads that non-native fish such as perch are one of the drivers contributing to declines in lake health. Non-native fish were often introduced because people wanted to enjoy fishing, and native fish are generally not good sports fish. Campaigns have been led to sensitise lake users to the impact of benthic herbivorous fish such as koi carps, goldfish, and rudd, with many councils now taking actions to reduce or eradicate their populations. However, despite the decades of research on the impact of trout, these same actions have not been taken where trout

threaten the survival of native / endemic fish species. Perch is treated even more ambiguously since it is classified as a noxious fish in most regions and there is some proof of its impact (Smith et al. (2006) and Smith et al. (2007); Chapter 5), but no measures are taken to reduce its population or prevent its spread.

This is an example of colliding opinions between i) scientists, indigenous communities and lake users focused on native biodiversity and environmental issues, and ii) lake users who may have very different priorities in their daily lives and therefore a different perception of non-native fish species. It may be hard for lake users to consider supporting and contributing to the removal of non-native species, whether due to being used to seeing these species around (thus considering their presence as the norm), enjoying fishing them since they give different fishing experiences compared to native species (especially carnivorous species such as trout and perch), relying on these species as an economic gain or food source, or other reasons. This situation is well explained by Tadaki et al. (2022) for trout: "*[...] it is not just the fish themselves that are of moral concern, but the relationships between people and fish, and between different groups of people that are at stake in current conflicts over trout*." Drawing from discussions with colleagues, fishermen and other lake users during my thesis, I join others in suggesting to start discussing the fate of non-native fish in New Zealand on a community-wide basis, to find the best solutions which merge local needs and the protection of native food-webs. This would ensure the continuity of healthy New Zealand ecosystems and communities for generations to come.

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Appendix A

Historical bacteria community composition in lakes within the same region

A.1 Context

With the creation of molecular databases and the rise of eDNA metabarcoding, studies have been able to identify bacteria present in the environment without the need to cultivate them. Although the ecology and morphology of most species are still not known, they can still be classified and grouped at higher levels (Family, Order, Class). Bacteria metabarcoding from environmental samples has been undertaken in several water bodies of the Northern Hemisphere (Chen et al., 2018; Llirós et al., 2014; Bertilsson et al., 2021), but less so in the Southern Hemisphere. In New Zealand, there is a lack of knowledge regarding which bacteria are found in lakes, how they change depending on trophic levels, and how they have changed over time. The Lakes380 project (lakes380.com) has collected sediment cores and water samples from over 300 lakes throughout New Zealand over the past five years, providing a unique sample set for palaeoecological research. In collaboration with a student doing an undergraduate placement (Leon Walther, University of Konstanze), we selected some lake sediment cores sampled by the Lakes380 project to study their

bacterial communities over time. Leon wrote his thesis on a set of lowland lakes from various regions, and he focused on human-driven impacts.

My goal was to focus on a single region and see if there was a core community of bacteria shared by all lakes, whether altitude and trophic levels affected bacterial composition, and if bacterial communities changed upon Māori and European settlement in the region. I selected seven lakes from the Tasman region with a range of altitudes and catchment land uses. This work served as an initial check of methods and bioinformatics for bacteria in sediment cores within the Lakes380 team, and for me to see what kind of data I could expect in my last data chapter (Chapter 5).

A.2 Methods

The Tasman region represents the North-West tip of the South Island of New Zealand and is comprised of coastal landscapes and high alpine landscapes. The western coast faces the Tasman Sea (towards Australia), the eastern coasts (Golden Bay / Mohua and part of Tasman Bay) are more sheltered and face the beginning of the Cook Strait. The history of human settlement in this region is diverse, with Māori settlement from c. 1450 AD, followed by early European settlement in 1842 in response to coal and then gold rush. Currently, most settlements are concentrated in the Golden Bay region, while the western coast is less populated. Many lowland areas have been deforested for pastures and pine plantations. Most of the mountains have been protected since 1970 and are now within the Kahurangi National Park.

The seven lakes were cored in October 2018. Catchments ranged from near-pristine to high human presence, and altitude from sea-level to c. 1,300 m above sea level (Table A.1). Lake Killarney sits in the middle of Takaka township (Golden Bay) surrounded by houses on three sides and pastures on one side. It has degraded water quality and was experiencing cyanobacterial blooms at the time of sampling. The locals informed us that pastures were previously coming down to the lake and now stopped a few hundred meters from the lake shore. The local council also informed us that the lake level fluctuated a lot and could be linked to underground water,

Table A.1: Summary of lake details. Depth categories are as follow for the maximum depth: shallow <10 m, medium < 50 m, deep >50 m. Size categories are as follow for the longest axis: small < 100 m across, medium < 700 m across, rather large > 700 m across.

	Altitude	Catchment	Depth	Size
Killarney	Sea-level	Urban, pasture	Shallow	Small
Kaihoka1	Lowland	Pasture, native forest	Medium	Medium
Kaihoka2	Lowland	Pasture, native forest	Medium	Medium
Mangarakau	Lowland	Regenerating native forest	Shallow	Medium
Otuhie	Lowland	Pasture, native forest	Shallow	Rather large
Peel	Alpine	Native bush (tussock)	Shallow	Small
Lockett	Alpine	Native forest	Deep	Medium

possible seawater (Lake Killarney is situated c. 4 km from the coast). The Kaihoka lakes are situated within 100 meters of each other, and c. 1 km of the West Coast. The lakes themselves are surrounded by native forests but the wider catchment is comprised of pastures with sand dunes in the North-West. Their waters were dark, stained by tannins from the surrounding forests. Lake Kaihoka 1 is the western lake, composed of two basins which are connected through a shallow (<1 m) channel. The eastern basin was cored at its depocenter. The headwaters of Lake Kaihoka 2 sit in pasture. Lake Kaihoka 1 showed fluctuations in lake level (shores could be walked upon at the time of sampling) while Lake Kaihoka 2 had swampy margins. Lake Mangarakau is presently surrounded by swampy regenerating manuka/kanuka native forest and drains into extensive wetlands. Lake Otuhie is a very long and shallow lake, dominated by weeds (not identified, probably non-native) in its eastern part at the time of sampling. It is surrounded by very steep hills covered by native forest, pastures, and pine plantations in the east. It drains through paddocks, c. 1 km from the sea (West Coast). The waters of Lakes Mangarakau and Otuhie were also stained dark by tannins from their catchment. Lakes Lockett and Peel are situated near the Cobb reservoir (Kahurangi National Park). Lake Lockett is situated at 1,300 m above sea level, at the treeline, with high water quality and clarity. Lake Peel is situated at c. 1350 m above sea level, about 100 meters above the treeline, surrounded by native tussocks. It has high water quality and clarity with the deepest

point (8 m) visible during sampling. No obvious signs of human impacts could be seen around these alpine lakes, though they are both accessible on foot and therefore also by non-native wildlife (e.g., deer, rabbits). Historically, some very low intensity stock grazing occurred in this region.

Pollen laboratory techniques were identical to the methods of Chapter 2 and 5. Pollen and charcoal were counted as relative abundances, using exotic *Lycopodium* tablets as an internal control.

SedaDNA laboratory practices and bioinformatics for the bacteria marker (16S rRNA gene) were very similar to the methods of Chapter 2 and 5. Briefly, c. 0.25 g of sediment were extracted from specific depths of the sediment cores. DNA was extracted using the PowerSoil kit (Qiagen, see Chapter 2) and bacteria were metabarcoding by amplified the 16S rDNA gene, following the methods used in Chapter 5. PCR products were cleaned with Agencourt AMPure XP Kit, Beckman Coulter, CA, USA and sequenced by Auckland Genomics, University of Auckland (see Chapter 2). Bioinformatics are identical to the ones of Chapter 5.

Samples were not rarefied, and instead transformed to relative abundances. Bacterial communities were visualised for each lake using barplots, at phylum and family level. A threshold of relative abundance was set (different for each taxonomical level) to visualise the most abundant and most relevant groups. Using pollen data (similarly to Chapter 2, two to three cultural eras were distinguished for each lake (pre-human - PH, evidence of Māori settlement - EMS, post-European settlement - PES). Eras were defined based on increases of charcoal deposits within the core and of the appearance of non-native, European plants in the pollen analyses.

Multivariate trends were summarised by a principal response curve (PRC, see chapter 5). Principal response curves summarise the variance of the principal component in one dimension, and therefore reflect changes in species diversity.

A.3 Results

A.3.1 Defining eras

Charcoal signals were found throughout the sedimentary record of Lake Lockett, therefore it was not possible to assess whether these were natural fires or if the sediment core did not capture pre-human times. Charcoal signals were quite high in lakes Kaihoka2, Lockett and Peel (peaks >1,000 pieces/cm ^{3}). Inflections in the PRC plots that coincided with these markers were viewed as confirmation of relatively abrupt changes in lake condition.

Depths within cores for DNA samples were selected before charcoal analysis was complete, and consequently bacteria samples that captured pre-human times were not always taken (lakes Kaihoka2 and Mangarakau). This was an important learning for subsequent studies.

A.3.2 Bacterial univariate trends

Overall, 41,647 ASVs were amplified from 134 samples. The sequencing depths varied across lakes and within lakes (Figure A.1, A.2), from a few hundred reads (especially in Lakes Kaihokoa 1 and Lockett) to more than 80,000 reads (Lake Killarney). The principal response curves (PRCs) showed that all bacteria communities changed over time, and for most lakes this was illustrated by a smooth continuous decline in PRC score (Figure A.2). Scores ranged from 0 to 4 except in Lakes Mangarakau and Peel where they ranged from 0 to 3. Some shifts in PRCs could be linked to sequencing depths, e.g., three samples beyond 60 cm in Lake Lockett and the top-most sample in Lake Otuhie. Lakes Killarney, Mangarakau, Peel exhibited shifts in PRC scores that may be linked to human impacts, right after Māori settlement for Lake Killarney and right after European impact for Lakes Mangarakau and Peel (Figure A.2).

A.3.3 Bacterial composition trends

The three most abundant phyla were Chloroflexi (24.4% of all reads), Proteobacteria (22.8% of all reads), and Spirochaetes (15.4% of all reads). Across all lakes, Chloroflexi and Spirochaetes dominated in older samples while Proteobacteria generally dominated in recent samples (Figure A.3). Firmicutes and Rokubacteria were sometimes abundant in older samples (Firmicutes in Lakes Killarney and Lockett, Rokubacteria in Lakes Peel and Lockett). Cyanobacteria appeared in the most recent samples of Lakes Kaihoka 1, Killarney, and Peel, dominating the top samples in Lake Killarney (Figure A.3). Verrucomicrobia were found in all lakes, appearing in the middle or top of the sedimentary records. Lake Killarney was the only lakes where Caldiserica dominated the community (3/4th of the sedimentary record), right after a sudden dominance of Firmicutes (Figure A.3). Actinobacteria were only a significant part of the community (up to c. 10%) in the top of the sedimentary records of Lakes Kaihoka 1 and 2 and Lake Peel (Figure A.3).

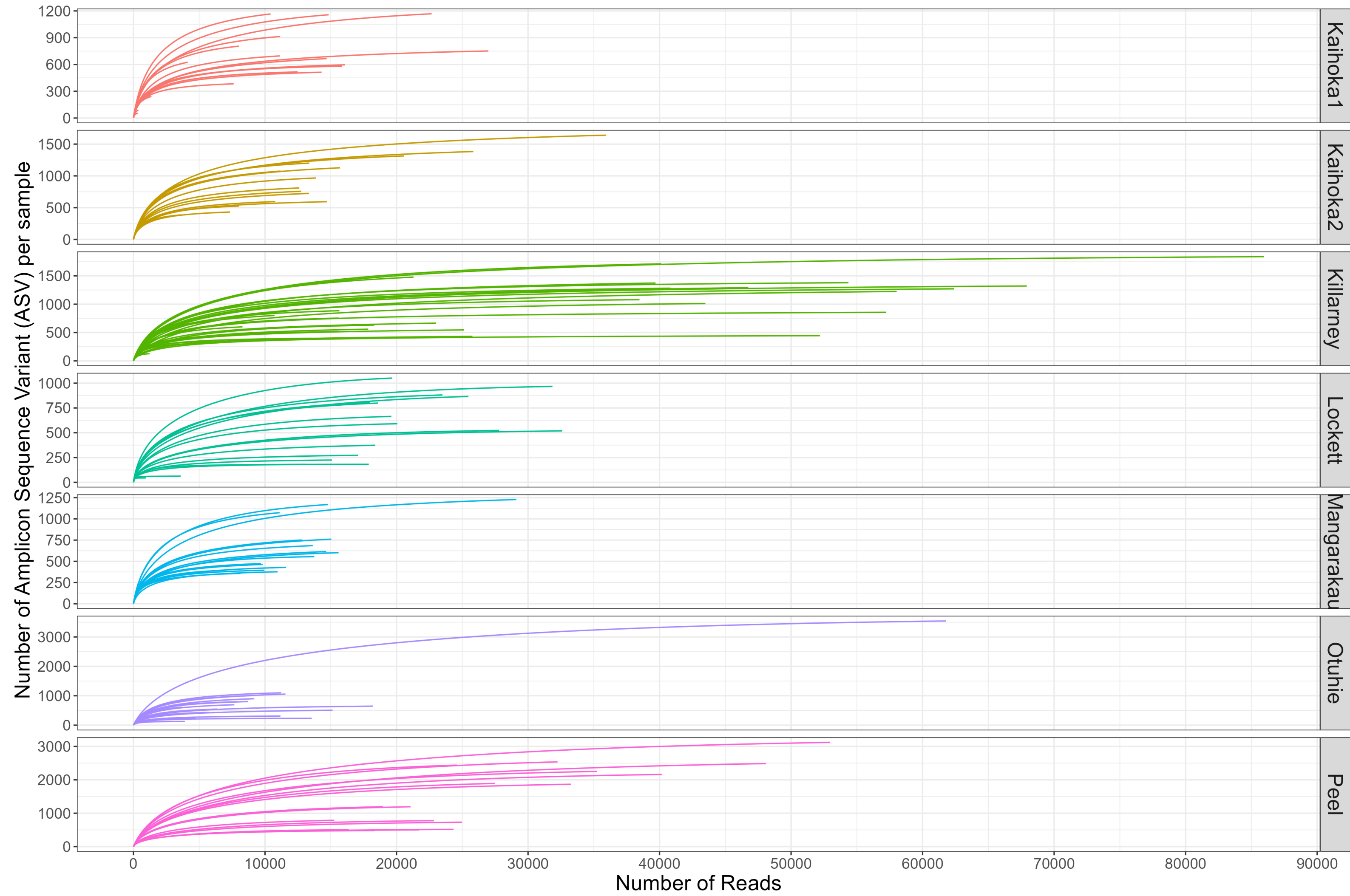


Figure A.1: Sequencing depth for each sample per lake. Note the difference in scale for the y-axis and the very low sequencing depth for some samples of Lakes Kaihoka , Killarney, Lockett.

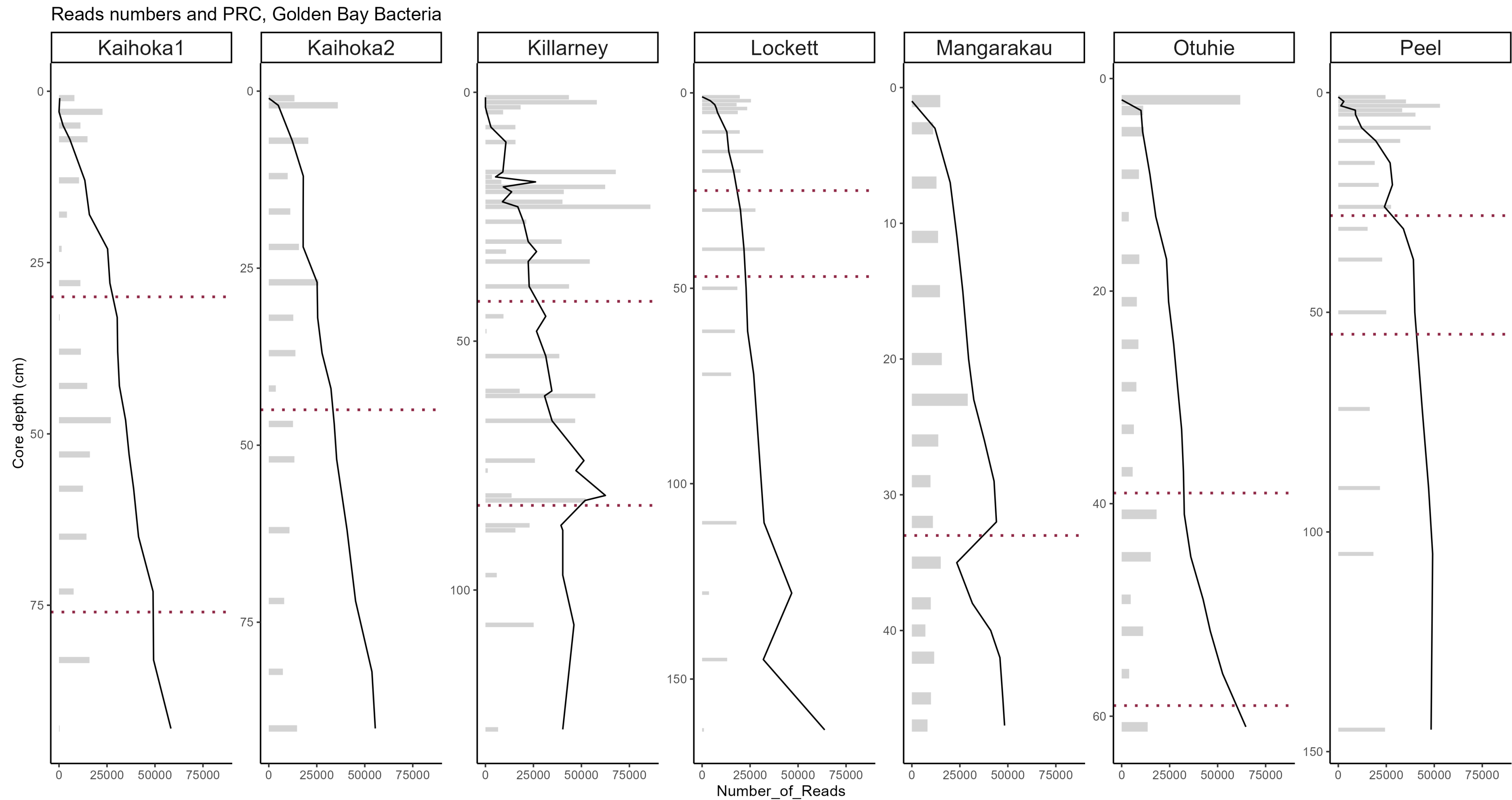


Figure A.2: Total bacterial read numbers (grey bars) and Principal Response curves (black lines) for all sedimentary records. The Principal Response Curve scores were multiplied (x 1500) to fit on the plot.

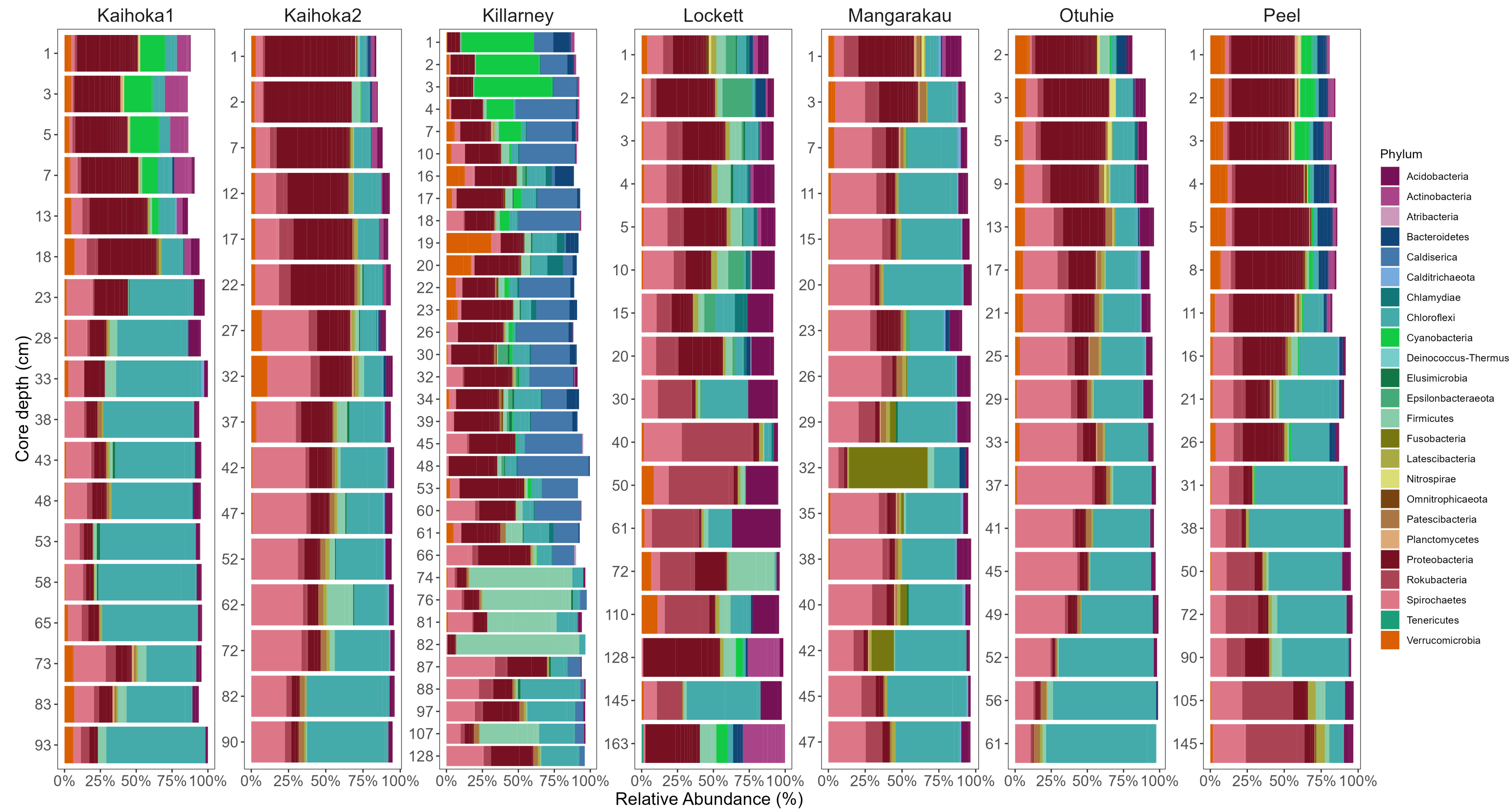


Figure A.3: Bacteria composition (16S rRNA gene) at phyla level. Amplicon sequence variants <0.05% abundance were filtered out. The horizontal dashed lines separate the three phases, pre-human (PH; bottom), evidence of Māori settlement (EMS; middle), and post-European settlement (PES; top). Pre-human times were not recovered in Lakes Kaihoka 2 and Mangarakau.

A.4 Discussion

A.4.1 Bacteria found in Tasman lakes

Many more analyses should be undertaken on this interesting dataset, but first some samples would need to be re-sequenced to ensure all species present in the sediment were amplified. Furthermore, some sediment cores would need to be sub-sampled again since the sampling design was not balanced enough to yield robust comparisons depending on the era, with some lakes having zero, or only one or two samples for a given phase.

Without age models, not one of the three analyses used (charcoal/pollen, PRCs in bacteria, bacteria composition) provided a robust method for determining Māori and European settlement. Pollen from non-native plants introduced by Europeans was the more robust indication of European settlement, unfortunately pollens can come from the wider region and are not necessarily local signals. This was well illustrated by the pollen data from Lake Peel, which was dominated by beech (Fuscospora) while the lake is situated above the treeline and surrounded by tussock. In the future, age models complemented by pollen/charcoal data seem therefore like the most robust method to estimate settlement times.

Bacteria communities offered interesting insights into the historical ecology of the seven study lakes. There seemed to be a marked difference between alpine and lowland lakes, with the presence of Rokubacteria in alpine lakes (>25% relative abundance – RA - in baseline communities) compared to lowland lakes (<10% RA). Principal Response Curves showed overall less sensitivity of the bacterial community to human impact compared to Lake Pounui (Chapter 5), with only three of the seven lakes responding to human settlement (either Māori or European). This may be because the Tasman region is less populated compared to the Wellington region where Lake Pounui is located, and therefore human activities could have been less intense and more progressive. The difference in sequencing depths across samples within the same lake could also play a part.

Chloroflexi and Spirochaetes dominated the baseline community of all lakes and mostly disappeared in recent samples. It is difficult to say whether the increase in Proteobacteria is a response to human impact since this trend was even seen in high alpine lake (Lakes Peel and Lockett). Similarly, the increase in cyanobacteria in Lakes Kaihoka 1 and Killarney could be attributed to human-impact, except the same trends was observed in the small and shallow alpine lake, Lake Peel. It is possible that, with drivers similar to the ones for Lake Pounui playing a part (land-use, non-native species, climate change), even alpine lakes may experience a decrease in water quality due to land clearance and climate change.

The most degraded lake, Lake Killarney, showed a succession of shifts which could be related to human impact, with the succession of Firmicutes, Caldiserica, and Cyanobacteria in recent times. Other lakes with similar catchments and / or trophic status should be analysed in New Zealand to see if these trends are due to general human impact or if they are specific to Lake Killarney. Similar to Lake Pounui, the high relative abundance of Chloroflexi could be an indicator of good water quality, since some strains prefer to live deeper in clear water columns (Okazaki et al., 2018). The high relative abundance of spirochaetes was surprising (compared to Lake Pounui) and may suggest a regional difference.

In summary, bacteria from sedimentary ancient DNA showed promising insights into the history of New Zealand lakes. Cyanobacteria were a negligible part of all communities, showing that lakes currently undergoing cyanobacterial blooms have deviated from their baseline communities. Due to the lack of robustness of this dataset, more thorough analyses could not be done, such as linking human impacts to specific changes in bacteria.

A.4.2 Comparison with lake bacterial communities from overseas

The most striking difference of this dataset compared to published literature was that Actinobacteria were a very minor part of the community (max 5%), while

in European lakes they can make up to 60% of the community (Bertilsson et al., 2021). Lake Kaihoka 1 is the lake which displayed the highest concentration of Actinobacteria, which only increased after European arrival. It is possible that these bacteria were introduced by European activities, though if there were simply related to European impact there should be more of them in the most impacted lake, Lake Killarney.

Appendix B

Historical bacteria levels in sediment cores

B.1 Context

This data was produced using droplet digital PCR (ddPCR) at the same time as historical cyanobacterial levels were established with ddPCR from lake sediment cores from Chapter 2 (Lakes Paringa, Pounui, Rotoehu, Johnson, Hayes, and Wairarapa), but it was not published in the corresponding paper (Picard et al., 2022a).

I originally wanted a DNA proxy that would stay constant over time (i.e. not affected by ecological trends) to see when sedaDNA was affected by DNA degradation. We followed methods from Pal et al. (2015) to explore whether cyanobacterial levels decreasing downcore were not solely an artifact of diagenesis but also an ecological trend showing historical shifts in cyanobacteria gene copy numbers. The initial rationale was that bacterial levels in lakes should be stable over time, as there is always bacterial activity even in a healthy lake ecosystem, therefore we should be observing generally stable bacteria levels while cyanobacterial levels would be increasing in lakes showing blooms in contemporary times.

To target all bacteria, we selected the glutamine synthetase gene, which codes for an essential enzyme for nitrogen metabolism in bacteria. This gene (glnA) has been used as a house-keeping gene in (lake) sediment in previous studies to reconstruct

historical bacterial abundance (Hurt et al., 2001; Stoeva et al., 2014), and it has been used once as a proxy for DNA degradation (Pal et al., 2015). If GlnA was a true proxy of DNA degradation, we expected it to be stable downcore and exponentially increase between Māori and European arrival (800 to 200 years ago), at most during the European phase. Sedimentary ancient DNA was degraded to its maximum 100 years after deposition in a tropical swamp in Uganda (Dommain et al., 2020), therefore we would expect DNA to degrade slower in our colder and darker lakes (discussed in Chapter 2).

B.2 Methods

Methods were identical to Chapter 2, with the addition of a ddPCR assay targeting bacterial glutamine synthetase activity, using the GS1β-F (5'-GATGCCGCCGATG TAGTA-3') and GS2-R (5'-AAGACCGCGACCTTYATGCC-3') primers targeting an approximate 153 to 156 base-pairs (bp) region of the glutamine synthetase gene (Hurt et al., 2001).

All samples were diluted for quantification by ddPCR. Dilutions ranged from 1/10 to 1/1,000. The ddPCR was undertaken using a BioRad QX200 system, following methods from Chapter 2. The GlnA assay included 0.25 µL of primer at 10 µM, 10 µL of 2×BioRad QX200™ ddPCR EvaGreen Supermix, 10.5 µL of DNA/RNA free water (Life Technologies) and 1 µL of diluted DNA for a total reaction volume of 22 µL per well. After droplet creation, samples were amplified using the following conditions: initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 1 min, and extension at 57°C for 1 min, and finally 4°C for 5 min and 90°C for 5 min. At least one negative control (all reagents and RNA/DNA-free water) and one positive control (genomic DNA extracted from a cyanobacteria culture) were included on each plate. Droplets counts were normalised with the same formula than cyanobacteria levels, replacing the template DNA volume to 1 µL (see Equation 2.1 in Chapter 2).

Trends were analysed using the R and RStudio software (R Core Team, 2021;

RStudio Team, 2022). Downcore trends were plotted using ggplot2 and correlations in trends were analysed using the Spearman correlation coefficient.

B.3 Results

Overall, GlnA profiles and cyanobacteria 16S rRNA gene copy numbers were positively and significantly correlated in all six lakes, though the correlation was weaker in Lake Wairarapa (Table B.1). When looking at the correlation per phase, GlnA was only correlated to cyanobacteria profiles in the PH phase for Lake Paringa. GlnA was positively correlated to cyanobacteria levels in all individual phases for Lake Pounui, and only for the PES phase for all remaining lakes. The PH phase of Lake Wairarapa was the only lake/phase combination which showed a negative correlation. This was most obvious in Lake Hayes and Pounui when looking at the different phases independently (Table B.1, Figure B.1).

Table B.1: Spearman correlation (R^2 and p-value) summarising the fit of GlnA concentration to 16S cyanobacteria concentration for all phases and per phase. Significant p-values (α=0.05) and their associated R^2 are in bold.

	All Phases		PES		EMS		PH	
	R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value
Paringa	**0.52**	**<0.01**	-0.37	0.44	0.5	0.07	0.7	**0.04**
Pounui	**0.93**	**<0.01**	**0.75**	**<0.01**	**0.79**	**0.03**	**0.88**	**<0.01**
Rotoehu	**0.69**	**<0.01**	**0.75**	**<0.01**	0.4	0.29	0.32	0.5
Hayes	**0.88**	**<0.01**	**0.87**	**<0.01**	0.62	0.09	NA	NA
Johnson	**0.82**	**<0.01**	**0.91**	**<0.01**	0.68	0.05	1	0.08
Wairarapa	**0.34**	**0.04**	**0.58**	**0.01**	0.38	0.25	**-0.68**	**<0.01**

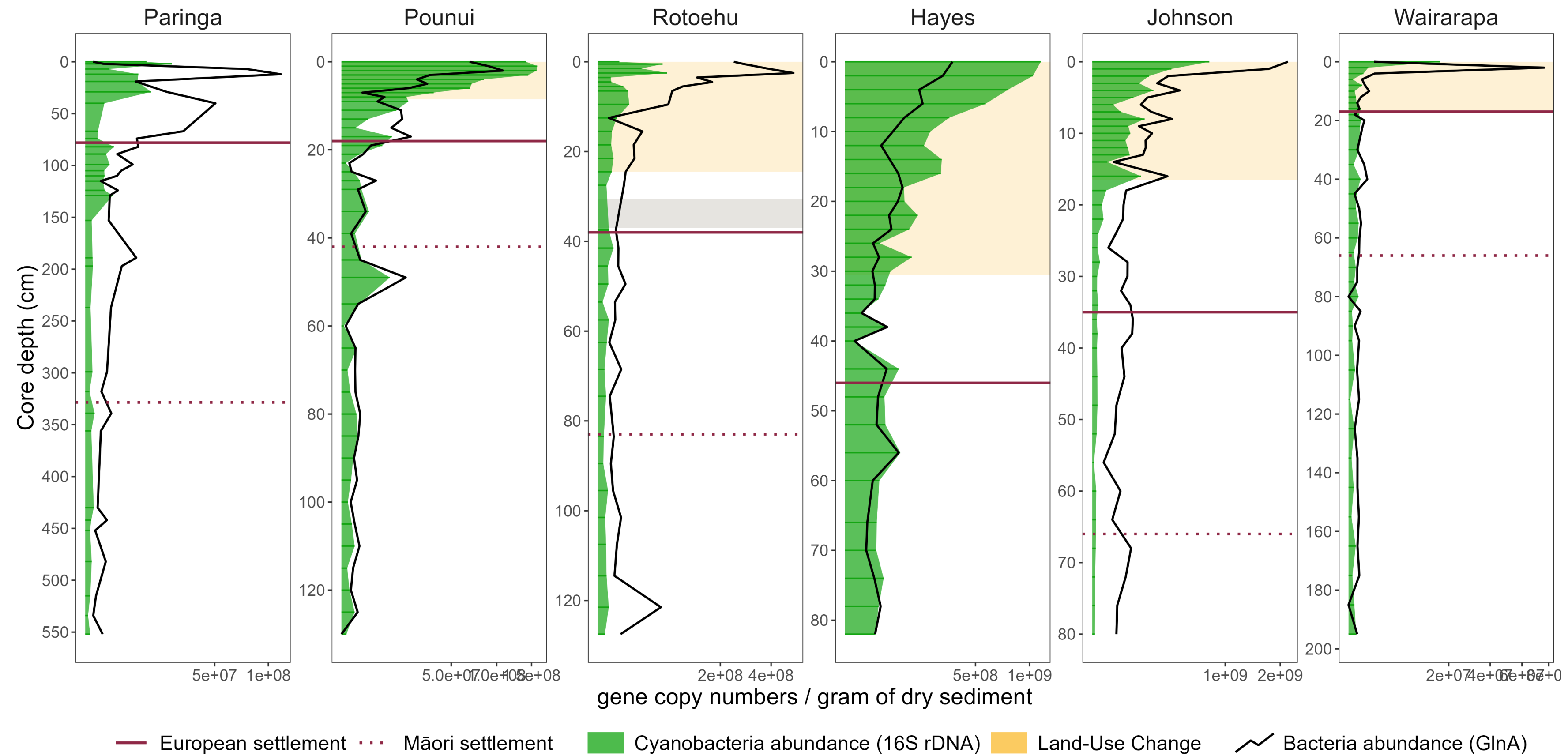


Figure B.1: Historical trends of cyanobacterial abundances and bacterial abundances using droplet digital PCR. The horizontal lines separate the three phases, pre-human (PH; bottom), evidence of Māori settlement (EMS; middle), and post European settlement (PES; top); note: the sediment core from Lake Hayes does not include any PH phase. Yellow shades indicate the depths associated with land-use intensification in relevant lakes and the grey shade indicates the 1886 Tarawera tephra layer in Lake Rotoehu.

B.4 Discussion

The GlnA assay worked well on all samples. A steady increase between Māori and European settlement (800 to 200 years ago) was only observed in Lake Paringa, which could indicate less DNA degradation and fits the expected timeframe. But GlnA levels increased markedly after European arrival in all lakes. This correlation either revealed a DNA degradation rate much higher than expected (<100 years), or most likely, the inadequacy of GlnA as a proxy for DNA degradation in our study lakes.

All study lakes showed different patterns of cyanobacteria and GlnA levels, and contrary to the lakes from Pal et al. (2015) which were in a national park, these study lakes were all directly impacted by human activities (see Chapter 2). Furthermore, because most cyanobacteria also have the GlnA gene (Gibson et al., 2006), the GlnA trends are highly likely just following the patterns of increases in cyanobacteria levels. In summary, a number of factors could impact GlnA trends in lakes undergoing cyanobacterial blooms:

- Co-amplification of cyanobacteria
- Lake eutrophication, leading to general increase in biological biomass
- Faecal contaminants, leading to the specific of increase bacteria levels
- Major sedimentary increase (storm, landslide / earthquake, human-induced terraforming)

Lakes Pounui, Hayes, and Johnson were good examples of lake eutrophication and co-amplification of cyanobacteria affecting GlnA levels. Bacteria levels increased during European presence in concert with cyanobacterial levels, and in the case of Lake Johnson, matching every increase and decrease of cyanobacteria levels (Figure B.1).

GlnA trends in Lake Rotoehu were interesting since they did not match the cyanobacterial trends as much as the three other lakes (Pounui, Hayes, Johnson). It is therefore possible that GlnA trends were less affected by cyanobacteria levels, and perhaps more affected by catchment activities (pasture - faecal contaminants,

pine plantation - repeated erosion) or by in-lake processes (warm geothermal waters from an underground source). The Land Air Water website mentions that pine plantations have increased since 1977 (lawa.org.nz), which could fit with the GlnA increase from 11 cm.

The GlnA trend in Lake Paringa was an interesting example of sedimentary inputs from the catchment, since major peaks tended to correlate with earthquakes (prior to human arrival), and then with road building (Paringa-Haast cattle track c. 50-60 cm, then modern highway c. 20 cm). The difference in bacteria levels before and after European arrival could be due to DNA degradation, or a to a difference in the type of sedimentary impact: large-scale and high magnitude earthquakes but vegetation to buffer the lake, or small-scale road building which removed the vegetation acting as nutrient buffer.

Running the GlnA assay on Lake Wairarapa revealed that DNA content was very low in this core. The GlnA trend does not reflect any of the catchment modifications (input diversion, heavy nutrients and sewage run-offs, extensive loss of wetlands) that have happened since European settlement. The negative correlation during the PH phase may just be due to very low DNA levels. It also may be the only core where DNA degradation is observable, since GlnA levels have a unique peak at 2cm.

Overall, this data was very interesting but raised a lot of new questions which would need to be investigated to yield more robust claims. My analysis indicates that using the GlnA gene to assess DNA degradation in lake sediment cores is not feasible.

Appendix C

Spatial variation in fish community composition from metabarcoding and relationship to droplet digital PCR

C.1 Context

As demonstrated in Chapter 4, it can be very useful to be able to detect fish using eDNA. This data can be crucial to inform conservation or eradication projects, however most studies still use metabarcoding instead of more sensitive methods such as qPCR/ddPCR.

Comparisons between metabarcoding and qPCR/ddPCR have already been published, especially in the marine environment (Wood et al., 2019), but the knowledge gap is greater in lakes. This appendix is based on the same samples as Chapter 4: 14 sites in Lakes Pounui, Tomarata, and Waitawa, with replicates of water and sediment samples. We first intended for this data to be published with the rest of Chapter 4, however the sequencing depth was not enough to yield to a robust analysis. Therefore Chapter 4 only focused on droplet digital PCR (ddPCR), while this appendix presents the difference in detection between metabarcoding (fish community) and ddPCR (species-specific perch and rudd) in the samples used in Chapter 4.

The goal of this study was to compared detection rates of fish eDNA (perch and rudd) between ddPCR and metabarcoding, with the hypothesis that metabarcoding would yield less detections, especially in sediment samples where we would expect more inhibitors.

C.2 Methods

The same DNA extracts were used for ddPCR and metabarcoding, therefore the methods are identical to Chapter 4 from sampling to DNA extraction. Droplet digital PCR was used to target perch (*Perca fluviatilis*) and rudd (*Scardinius erythrophthalmus*) eDNA.

For metabarcoding, DNA extracts were pooled by site and replicate type and sent to Wilderlab Lt (Miramar, Wellington, NZ) for fish community metabarcoding. The primers used were F: 5'-TCTTCGGTTGGGGCGAC-3' and R: 5'-GGATTGCGCTG TTATCCCT-3'. The forward primer was developed in-house by Wilderlab Lt, and the reverse primer was developed by Deagle et al. (2010).

Raw fish reads from metabarcoding were summarised by lake and sample type and plotted as bar graphs. For further analysis, all data were transformed to binary (presence/absence). The ddPCR detections were merged by site to match the metabarcoding data, where fish eDNA was successfully detected if at least one of the two replicates detected the target fish eDNA. The dataset was split per lake, sample type, and species (e.g., Waitawa perch water, Waitawa perch sediment, etc.) and the ddPCR data was compared to metabarcoding data using Receiver Operating Characteristics (ROC).

For the purpose of this analysis, ddPCR was considered the gold standard (control), the "true" method able to detect fish eDNA, and metabarcoding was considered the response (case, classifier) tested by the ROC analysis to see whether it could detect presence and absence of fish eDNA as accurately as ddPCR. The pROC package was used in R (Robin et al., 2011; R Core Team, 2021; RStudio Team, 2022).

The plots resulting from the ROC analysis show a diagonal line (bottom left to

top right) which is the control (ddPCR), and a broken line (case, metabarcoding) which can vary from the bottom right corner to the top left corner. The area between the diagonal and the broken line (area under the curve, AUC) indicates how well the case matches the control. An AUC smaller than or equal to 50% indicates the case is not able to discriminate between true positive and true negatives, 70 to 80% shows an acceptable prediction ability, and 80 to 90% shows an excellent diagnosis ability. Ideally, the AUC would be 100%, which would indicate a perfect match of the case to the control / gold standard.

The AUC is influenced by two factors, sensitivity and specificity. The sensitivity indicates whether the case detects as many presences and absences as the control, and the specificity indicates whether these detections are in the same samples as the control (paired analysis).

C.3 Results

Fish reads could not always be assigned down to species level (Figure C.1), but eight species were identified – shortfin eel, common smelt, European perch, black mudfish, koi carp, rudd, tench, and goldfish. Read numbers were very low even though they were merged from 14 samples, grouped per lake and sample type (Figure C.1). Maximum read numbers were higher in the sediment of Lakes Pounui and Tomarata, with more taxonomical groupings detected, while there were no obvious differences in Lake Waitawa.

The ROC analysis could not be performed on the sediment samples of Lake Pounui (perch) nor on the water samples of Lake Waitawa (perch and rudd) because all ddPCR samples of these subsets only showed detection (presence, Figure C.1). The ROC analysis requires the control to have presence and absence values to analyse specificity, therefore ROC could only be calculated on the water samples of Lake Pounui (perch), sediment samples of Lake Waitawa (perch and rudd) and in all samples of Lake Tomarata (rudd).

A simple comparison of detection rates indicated that metabarcoding was much

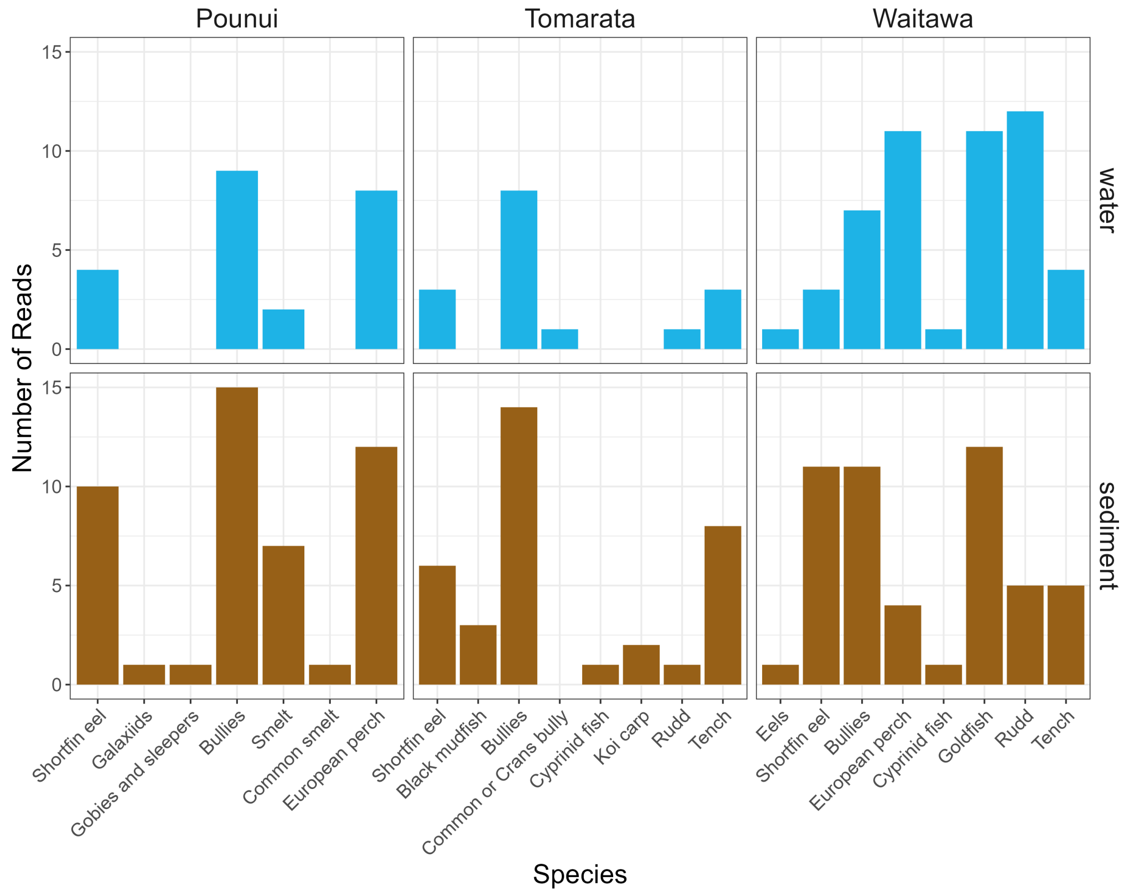


Figure C.1: Fish species and read numbers amplified by 16S mitochondria DNA, per lake and sample type. Read numbers were summed per site of the same lake/sample type subset.

worse than ddPCR in Lake Pounui (sediment) and Lake Waitawa (water samples), since all ddPCR samples detected the target fish eDNA and metabarcoding samples did not. The ROC analysis indicated that for Lake Tomarata and Pounui (water), metabarcoding was unable to accurately discriminate between true presence and absence, with the AUCs under 50% (Figure C.2). The AUC for Lake Pounui (water) was influenced by a low specificity (30%), while for Lake Tomarata it was the sensitivity which was very low (<10%). The AUC was much higher for metabarcoded sediment samples in Lake Waitawa, with 82% for perch sedDNA and 68% for rudd sedDNA. Specificity and sensitivity were both quite high (64 to 93%).

Table C.1: Perch and rudd detection (0 = absence, 1 – presence) from ddPCR and metabarcoding, for each site. Positive detection are highlighted per site (blue for perch, orange for rudd).

Lake	Sample type	Site	Perch ddPCR	Perch metabar-coding	Rudd ddPCR	Rudd metabar-coding
Pounui	sediment	1	1	0	0	0
Pounui	sediment	2	1	0	0	0
Pounui	sediment	3	1	0	0	0
Pounui	sediment	4	1	1	0	0
Pounui	sediment	5	1	1	0	0
Pounui	sediment	6	1	0	0	0
Pounui	sediment	7	1	1	0	0
Pounui	sediment	8	1	1	0	0
Pounui	sediment	9	1	1	0	0
Pounui	sediment	10	1	1	0	0
Pounui	sediment	11	1	1	0	0
Pounui	sediment	12	1	1	0	0
Pounui	sediment	13	1	1	0	0
Pounui	sediment	14	1	1	0	0
Pounui	water	1	0	1	0	0
Pounui	water	2	0	0	0	0
Pounui	water	3	0	0	0	0
Pounui	water	4	0	0	0	0
Pounui	water	5	1	1	0	0
Pounui	water	6	1	1	0	0
Pounui	water	7	1	1	0	0
Pounui	water	8	1	1	0	0
Pounui	water	9	1	0	0	0
Pounui	water	10	1	1	0	0
Pounui	water	11	1	1	0	0
Pounui	water	12	1	0	0	0
Pounui	water	13	1	0	0	0
Pounui	water	14	1	1	0	0
Tomarata	sediment	1	0	0	0	0
Tomarata	sediment	2	0	0	0	0
Tomarata	sediment	3	0	0	1	0
Tomarata	sediment	4	0	0	0	0
Tomarata	sediment	5	0	0	1	0
Tomarata	sediment	6	0	0	1	0
Tomarata	sediment	7	0	0	1	1
Tomarata	sediment	8	0	0	1	0
Tomarata	sediment	9	0	0	1	0
Tomarata	sediment	10	0	0	0	0
Tomarata	sediment	11	0	0	0	0
Tomarata	sediment	12	0	0	0	0
Tomarata	sediment	13	0	0	0	0

Continued on next page

Table C.1: Perch and rudd detection (0 = absence, 1 – presence) from ddPCR and metabarcoding, for each site. Positive detection are highlighted per site (blue for perch, orange for rudd). (Continued)

Tomarata	sediment	14	0	0	0	0
Tomarata	water	1	0	0	0	0
Tomarata	water	2	0	0	0	0
Tomarata	water	3	0	0	0	0
Tomarata	water	4	0	0	0	0
Tomarata	water	5	0	0	0	0
Tomarata	water	6	0	0	1	0
Tomarata	water	7	0	0	0	0
Tomarata	water	8	0	0	0	0
Tomarata	water	9	0	0	0	0
Tomarata	water	10	0	0	0	0
Tomarata	water	11	0	0	1	0
Tomarata	water	12	0	0	0	1
Tomarata	water	13	1	0	0	0
Tomarata	water	14	0	0	0	0
Waitawa	sediment	1	1	0	0	0
Waitawa	sediment	2	1	0	0	0
Waitawa	sediment	3	0	1	1	0
Waitawa	sediment	4	1	1	1	0
Waitawa	sediment	5	1	0	0	0
Waitawa	sediment	6	1	0	1	0
Waitawa	sediment	7	1	0	1	1
Waitawa	sediment	8	1	0	1	0
Waitawa	sediment	9	1	0	1	1
Waitawa	sediment	10	1	0	1	0
Waitawa	sediment	11	1	0	1	0
Waitawa	sediment	12	1	1	0	1
Waitawa	sediment	13	1	0	1	1
Waitawa	sediment	14	1	1	1	1
Waitawa	water	1	1	1	1	1
Waitawa	water	2	1	1	1	1
Waitawa	water	3	1	1	1	0
Waitawa	water	4	1	1	1	1
Waitawa	water	5	1	1	1	1
Waitawa	water	6	1	1	1	1
Waitawa	water	7	1	0	1	1
Waitawa	water	8	1	0	1	1
Waitawa	water	9	1	1	1	1
Waitawa	water	10	1	1	1	1
Waitawa	water	11	1	0	1	0
Waitawa	water	12	1	1	1	1
Waitawa	water	13	1	1	1	1
Waitawa	water	14	1	1	1	1

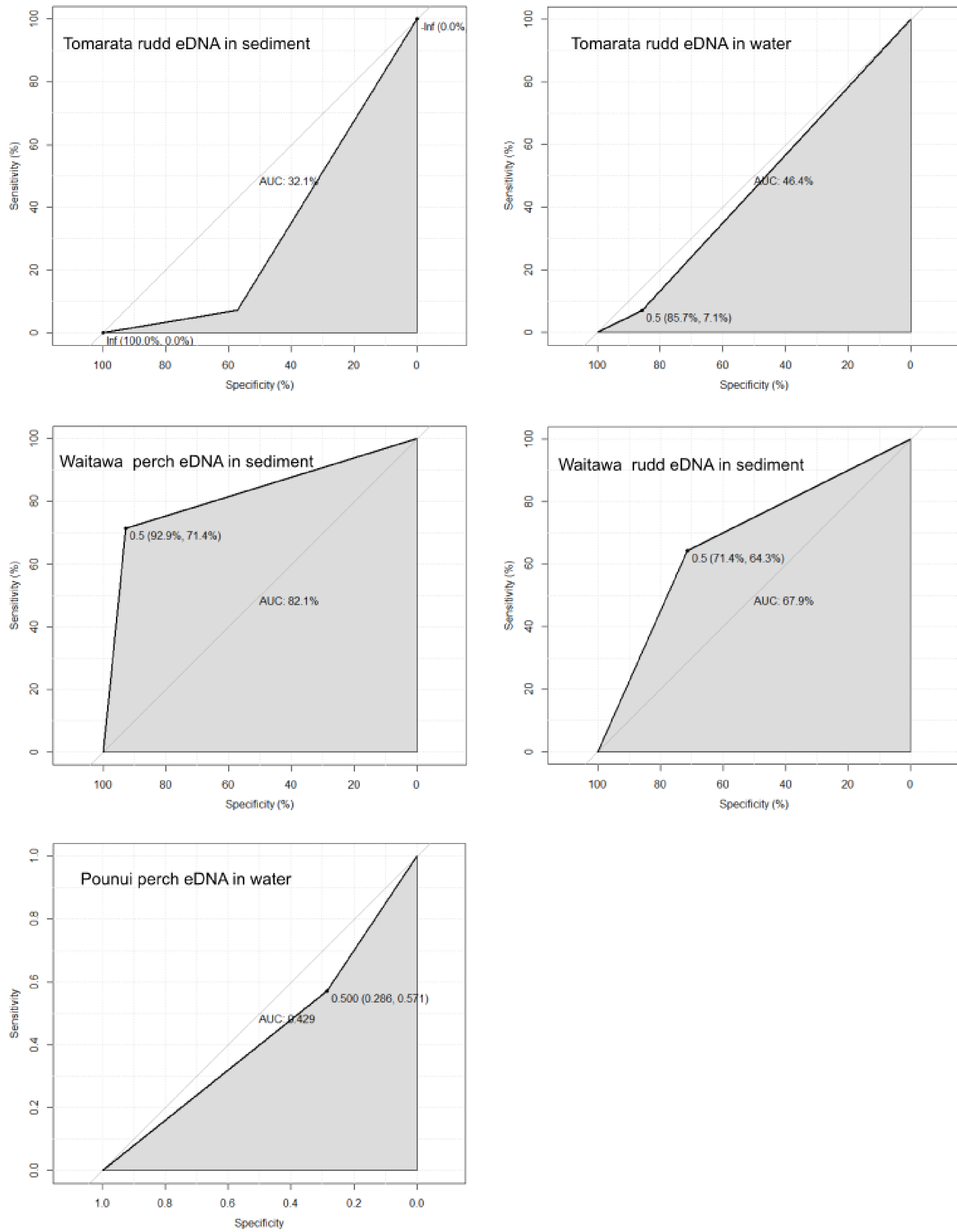


Figure C.2: Receiver Operating Characteristics analysis comparing ddPCR to metabarcoding in Lake Tomarata, Lake Waitawa (sediment samples) and Lake Pounui (water samples).

C.4 Discussion

C.4.1 Fish community composition

Metabarcoding revealed diverse fish population in all lakes. Non-native fish reads were more easily assigned to species level compared to native fish, especially for bullies and smelt. It is probable that that some native fish are very close genetically and the 16S mitochondrial DNA (mtDNA) amplicon used in this study is always not able to identify a specific species.

Apart from perch, only native fish were detected in Lake Pounui. Fish surveys have found brown trout (*Salmo trutta*) in very low abundances, but this species was not identified by metabarcoding. Bullies (*Gobiomorphus* sp.) eDNA showed the highest abundance, but those sequences were not attributed to the common bully (*Gobiomorphus cotidianus*) which have previously been identified in this lake (Jellyman, 1991). A new species of bullies was recently discovered through small morphological and molecular differences from another species (*Gobiomorphus mataraerore*, distinct from *G. breviceps*; Thacker et al., 2021). *G. mataraerore* does occur within the Wairarapa region, therefore the sequences attributed to this genus could belong to this new species. It is also possible that the 16S mtDNA amplicon produced in this study was not precise enough to be attributed to the common bully. Both shortfin and longfin eels also occur in Lake Pounui but only shortfin eels were detected. Shortfin eels seem in higher abundances and are more easily detectable throughout eDNA (Thomson-Laing, unpublished) therefore this could be the reason, or, once again, it could also be that the 16S mtDNA amplicon is not able to differentiate the two species which are very close genetically. Common smelt (*Retropinna retropinna*) have also been identified in Lake Pounui, but most amplicons were not identified down to species level. Similarly, galaxiids sequences were amplified from sedDNA which could correspond to giant kokopu (*Galaxias argenteus*), which are known to occur around the lake (Jellyman, 1979; McEwan, 2010).

In Lake Tomarata, all fish species identified by metabarcoding have been identi-

fied by a 2019 fish survey (Ling et al., 2019) except for koi carp (*Cyprinus rubrofiscus*) which has apparently been sighted by locals, but never captured (Nick Ling, pers. comm.). Most importantly, black mudfish eDNA was identified in the sediment but not in water samples. This species does not live in Lake Tomarata but in its adjacent wetland, which highlights the advantage of sedDNA to record fish presence in the recent past.

Lake Waitawa showed a high proportion on non-native fish (perch, koi, goldfish, rudd, tench) all known to occur in this lake. The high number of reads assigned to goldfish was surprising, as was the high read numbers of native eels and bullies eDNA since Lake Waitawa is known for its degraded water quality and heavy cyanobacterial blooms. Native fish may live in the nearby wetlands and visit the lake occasionally, since the read numbers were higher in the sediment compared to water. Due to the very low sequencing depths, no robust conclusions can be drawn on the most abundant fish species within each lake. Studies are only just starting to link fish eDNA to their population size or biomass, and different species may have different DNA shedding rates. At the very least, the sampling design (14 sites throughout the lake) should provide an integrated survey of fish eDNA throughout the study lakes. Comparisons with fish surveys showed the present design was able to detect the most abundant fish species identified by fish surveys. Species with low densities will need an adapted sampling design (Chapter 4, Furlan et al. (2019)).

C.4.2 Metabarcoding comparison to ddPCR

Many studies have investigated the best way to reliably detect fish using their eDNA (e.g., Doi et al., 2015; Baldigo et al., 2017; Wood et al., 2019; Cantera et al., 2019; Capo et al., 2021c). The present study is another example that metabarcoding generally performs worse than quantitative PCR, more specifically worse than ddPCR. This is not surprising, given than ddPCR is less sensitive to inhibitors (Rački et al., 2014). Moreover, restriction enzymes were used to cleave non-target DNA in the ddPCR reaction (Chapter 4), which theoretically unfolds secondary structures

and so that the polymerase enzyme has a better access to target DNA. Restriction enzymes were not used in the metabarcoding PCR reaction. In the present study, ddPCR was used to target one specific species per assay compared to metabarcoding which targeted the whole Actinopteri Class (most fish), which would lead to rare species possibly not being amplified. Finally, with the very low sequencing depth in all samples it is also not surprising that low levels of perch and rudd eDNA were not detected.

An interesting result from the ROC analysis was that metabarcoding was only an acceptable predictor for rudd sedDNA and perch sedDNA in Lake Waitawa, not in Lakes Pounui nor Tomarata. Occupancy modelling from Chapter 4 showed that Lake Waitawa was the only lake were eDNA detection was better in water samples compared to sediment samples. We speculated that Lake Waitawa's geochemistry was hampering sedDNA detection by ddPCR (Chapter 4). It seems that metabarcoding and ddPCR detection were hampered in the same way, leading to similar results detected by the ROC analysis. Furthermore, specificity was higher than sensitivity in both of these subsets, which may indicate that presence and absence were similar in paired samples (high specificity), and that non-detection by metabarcoding may be due to low levels of target eDNA (slightly lower sensitivity).

The present samples would need to be re-sequenced to increase sequencing depth for more robust claims. The present sampling design (two types of samples, 14 sites for small lakes) can produce an insightful eDNA dataset in one afternoon of sampling. Though species with low abundance may still not be detected with metabarcoding, many assays can be applied to extracted DNA for targeted species analysis.

Appendix D

Supplementary material from Chapter 2-5

D.1 Chapter 2 Supplementary material

Table D.1: Sub-sampling depths per core and corresponding dates. Phases are indicated in shades of blue: Post-European Settlement (PES - light blue), Evidence of Māori Settlement (EMS - blue), Pre-Human (PH - dark blue).

Paringa		**Pounui**		**Rotoehu**	**Hayes**	**Johnson**	**Wairarapa**
Depth (cm)	Date	Depth (cm)	Date	Depth (cm)	Depth (cm)	Depth (cm)	Depth (cm)
0	2011	0-1	2013	0.5	0-1	0-1	0-1
2.5	2007	1-2	1999	1.5	2-3	1-2	2-3
7.5	1999	2-3	1992	2.5	4-5	2-3	4-5
12.5	1989	3-4	1985	3.5	6-7	3-4	6-7
19.5	1971	4-5	1978	4.5	8-9	4-5	8-9
29.5	1964	5-6	1963	5.5	10-11	5-6	10-11
40	1950	6-7	1956	6.5	12-13	6-7	12-13
67.5	1873	7-8	1950	9.5	14-15	7-8	14-15
74.5	1856	8-9	1937	12.5	16-17	8-9	16-17
82	1837	9-10	1930	15.5	18-19	9-10	18-19
89	1811	11-12	1911	18.5	20-21	10-11	20-21
99.5	1782	13-14	1891	21.5	22-23	11-12	22-23
105	1765	15-16	1878	24.5	24-25	12-13	30-31
110	1754	17-18	1859	27.5	26-27	13-14	35-36
115	1745	19-20	1845	37.5	28-29	14-15	40-41
124	1726	21-22	1773	41.5	30-31	16-17	45-46
129	1724	23-24	1746	45.5	32-33	18-19	50-51
153	1716	25-26	1719	49.5	34-35	20-21	55-56
189	1652	27-28	1693	53.5	36-37	22-23	60-61
197	1599	29-30	1675	57.5	38-39	24-25	65-66
237.5	1463	34-35	1613	62.5	40-42	26-27	70-71
299	1392	39-40	1551	68.5	44-46	28-29	75-76
318.5	1344	44-45	1489	74.5	48-50	30-31	80-81
339.5	1303	49-50	1440	83.5	52-54	32-33	85-86
356.5	1241	55-56	1383	89.5	56-58	34-35	90-91
430.5	1151	60-61	1338	95.5	60-62	36-37	95-96
442.5	1077	65-66	1273	101.5	66-68	38-39	105-106
452.5	998	70-71	1200	107.5	70-72	40-42	115-116
482	963	75-76	1127	114.5	74-76	44-46	125-126
515.5	939	80-81	1074	121.5	78-80	48-50	135-136
534	866	85-86	1028	127.5	82-84	52-54	145-146
552.2	790	90-91	982			56-58	155-156
		95-96	929			60-62	165-166
		100-101	883			64-66	175-176
		105-106	834			68-70	185-186
		110-111	784			72-74	195-196
		115-116	727			76-78	
		120-121	670			80-82	
		125-126	612				
		130-131	562				

Table D.2: Description of non-molecular analyses performed on the cores to establish pre-human, evidence of Māori settlement, and post-European settlement phases. Core chronology was only done for Lakes Pounui and Paringa, while pollen and charcoal counts were undertaken on all six lakes. Note that the methods varied slightly depending on the lake, therefore for detailed methods see Howarth et al. (2012) for Lake Paringa, Cochrane (2017) for Lake Pounui, Khan et al. (n.d.) for Lakes Hayes and Johnson, Waters et al. (2018) for Lake Wairarapa.

Type of analysis	**Description and method**
Core chronology (14C)	Carbon-14 activity was measured from terrestrial leaf macrofossils in Lakes Paringa and Pounui. Macrofossils were cleaned and pre-treated then converted to CO2 and measured by accelerator mass spectrometry. Age-depth modelling was conducted using a Bayesian framework with the OXCAL 4.4 program. Dates are presented in Table S4 and Table S5.
Pollen	Extraction was carried out from sediment samples using 10% hot hydrochloric acid and acetolysis (6-micron sieving). Exotic *Lycopodium* tablets were added to each sample for pollen concentration calculations.
Charcoal	Charcoal was counted as number of fragments, grouped by fragment size, and presented as concentration per cm^3.

Table D.3: Primers used in this study, with reagent mix and cycling condition details. ddPCR = droplet digital PCR, and HTS = High-Throughout Sequencing.

	Target	**Amplicon length**	**Primer name**	**Sequence**	**Source**	**Reaction mix (one sample)**	**Cycling conditions**
ddPCR	Total cyanobacteria 16S rRNA	282 bp	CYAN 108F CYAN 377R	Forward: 5'-ACGGGT GAGTAACRCGTRA-3' Reverse: 5'-CCATGGC GGAAAATTCCCC-3'	Urbach et al., 1992; Nübel et al., 1997	❯0.2 µL of primer at 10 µM ❯10 µL of 2×BioRad QX200™ ddPCR EvaGreen Supermix ❯7.6 µL of DNA/RNA free water (Life Technologies) ❯4 µL of diluted DNA	❯95°C for 5 min ❯50 cycles ❯95°C for 30 s ❯56°C for 1 min ❯4°C for 5 min ❯90°C for 5 min
HTS	Total cyanobacteria 16S rRNA	400 bp	CYB359-F CYB784-R	Forward: 5'-GGGGAAT YTTCCGCAATGGG-3' Reverse: 5'-ACTACWGG GGTATCTAATCCC-3'	Nübel et al., 1997	❯25 µL of AmpliTaq Gold® 360 Master Mix (Life Technologies) ❯5 µL of 360 GC enhancer (Life Technologies) ❯5 µl of BSA (Sigma) ❯1 µL of primer at 10 µM ❯10 µL of DNA/RNA free water (Life Technologies) ❯1 µL of extracted sedDNA	❯95°C for 10 min ❯35 cycles ❯95°C for 30 s ❯52°C for 30 s ❯72°C for 1 min ❯72°C for 7 min

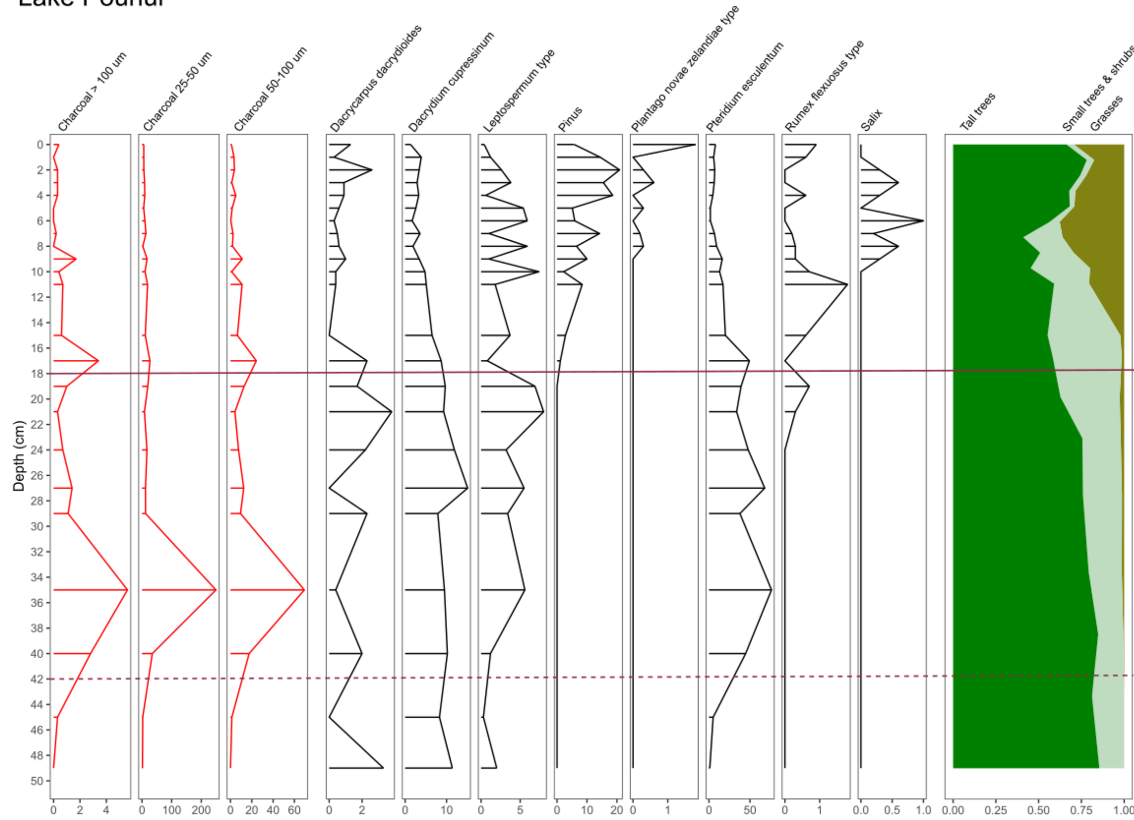


Figure D.1: Selection of pollen and charcoal data from the top of the Lake Pounui core. *Pinus*, *Salix*, and *Rumex* are exotic plants indicators of European presence, charcoal is an indication of vegetation clearance in the catchment (Māori then European presence). Adapted from Cochrane (2017). This data was used to identify the three phases (separated by horizontal purple lines) used in this study: pre-human (PH; bottom), evidence of Māori settlement (EMS; middle), and post European settlement (PES; top).

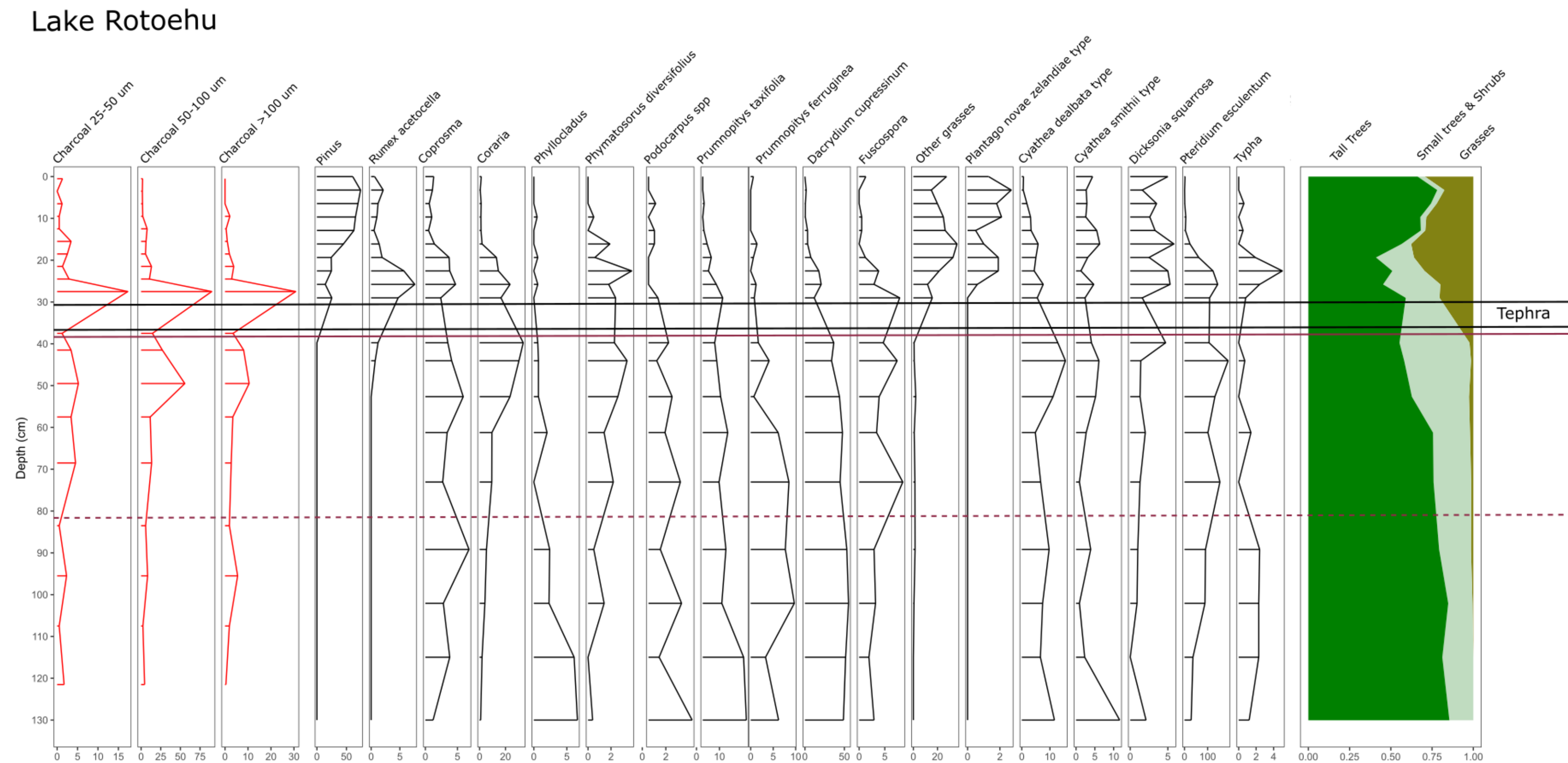


Figure D.2: Pollen and charcoal data from the Lake Hayes core. *Pinus*, *Salix*, and *Rumex* are exotic plants indicators of European presence, charcoal is an indication of vegetation clearance in the catchment. Adapted from Khan et al. (n.d.). This data was used to identify the three phases (separated by a horizontal purple line) used in this study: evidence of Māori settlement (EMS; bottom), and post European settlement (PES; top).

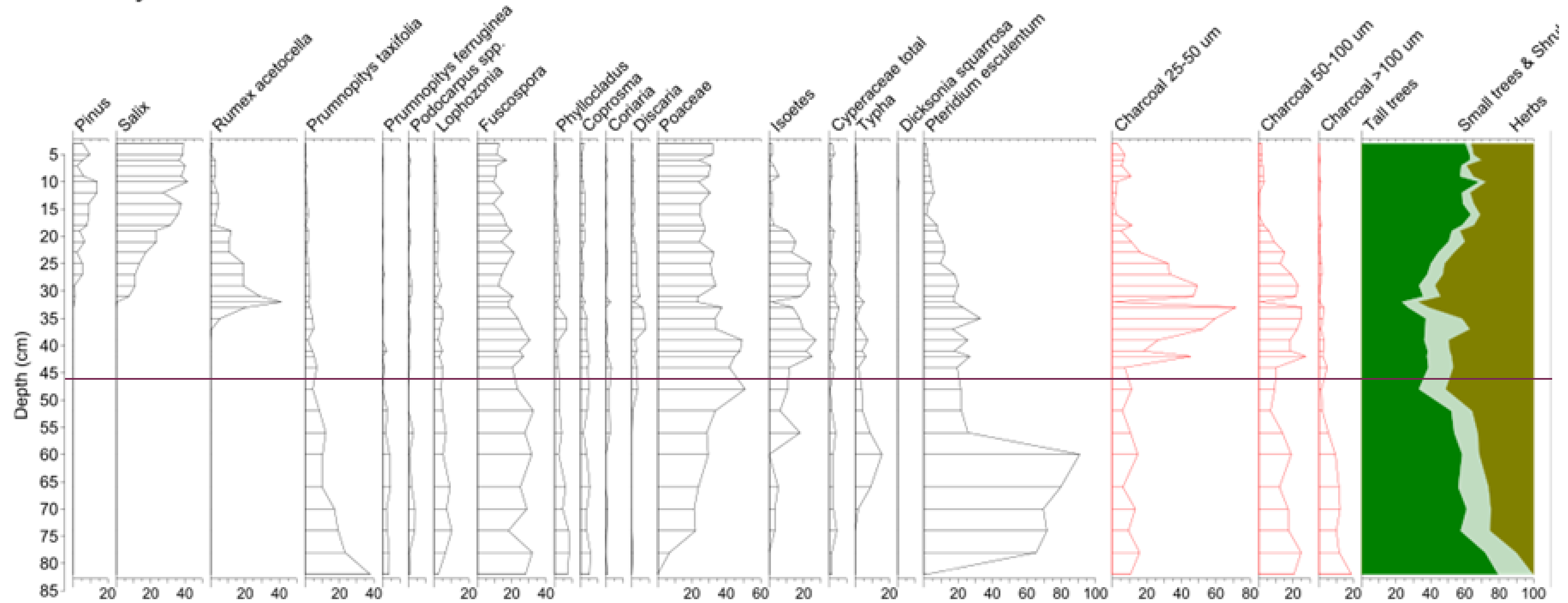


Figure D.3: Pollen and charcoal data from the Lake Hayes core. *Pinus*, *Salix*, and *Rumex* are exotic plants indicators of European presence, charcoal is an indication of vegetation clearance in the catchment. Adapted from Khan et al. (in review). This data was used to identify the three phases (separated by a horizontal purple line) used in this study: evidence of Māori settlement (EMS; bottom), and post European settlement (PES; top).

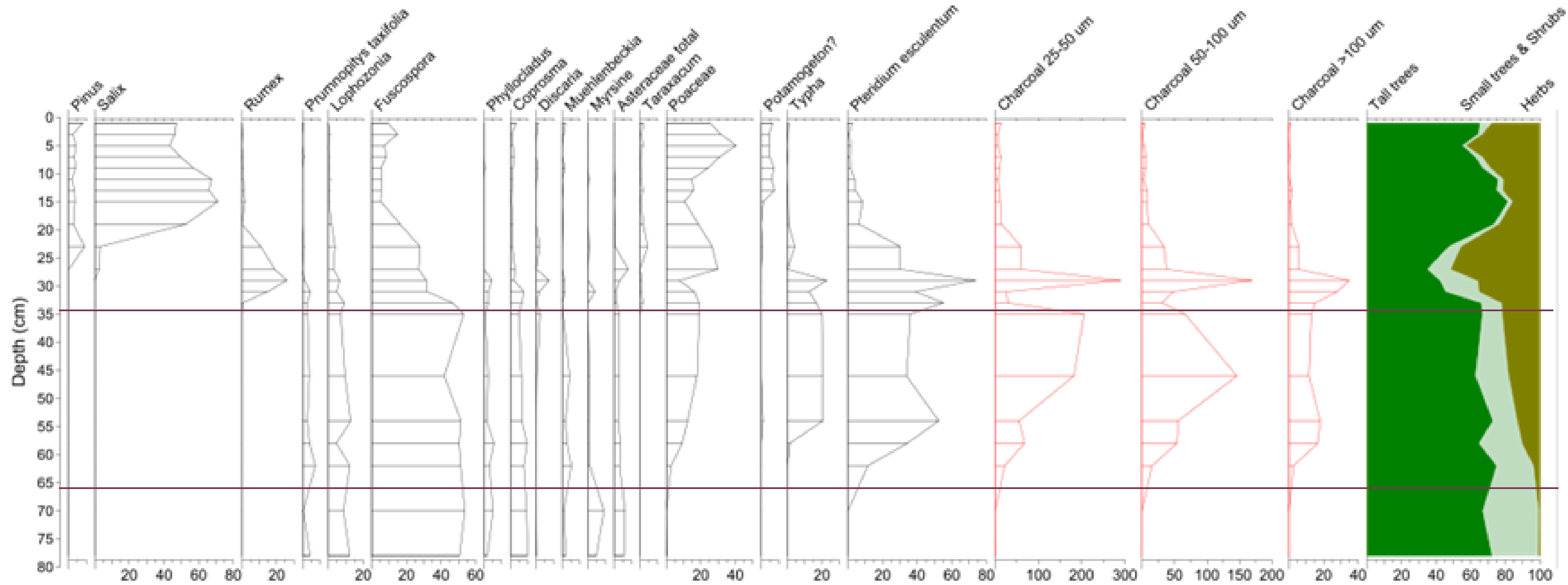


Figure D.4: Selection of pollen and charcoal data from the Lake Johnson core, used to determine occupation phases. *Pinus*, *Salix*, and *Rumex* are exotic plants indicators of European presence, charcoal is an indication of vegetation clearance in the catchment (Māori then European presence). Adapted from Khan et al. (in review). This data was used to identify the three phases (separated by horizontal purple lines) used in this study: pre-human (PH; bottom), evidence of Māori settlement (EMS; middle), and post European settlement (PES; top).

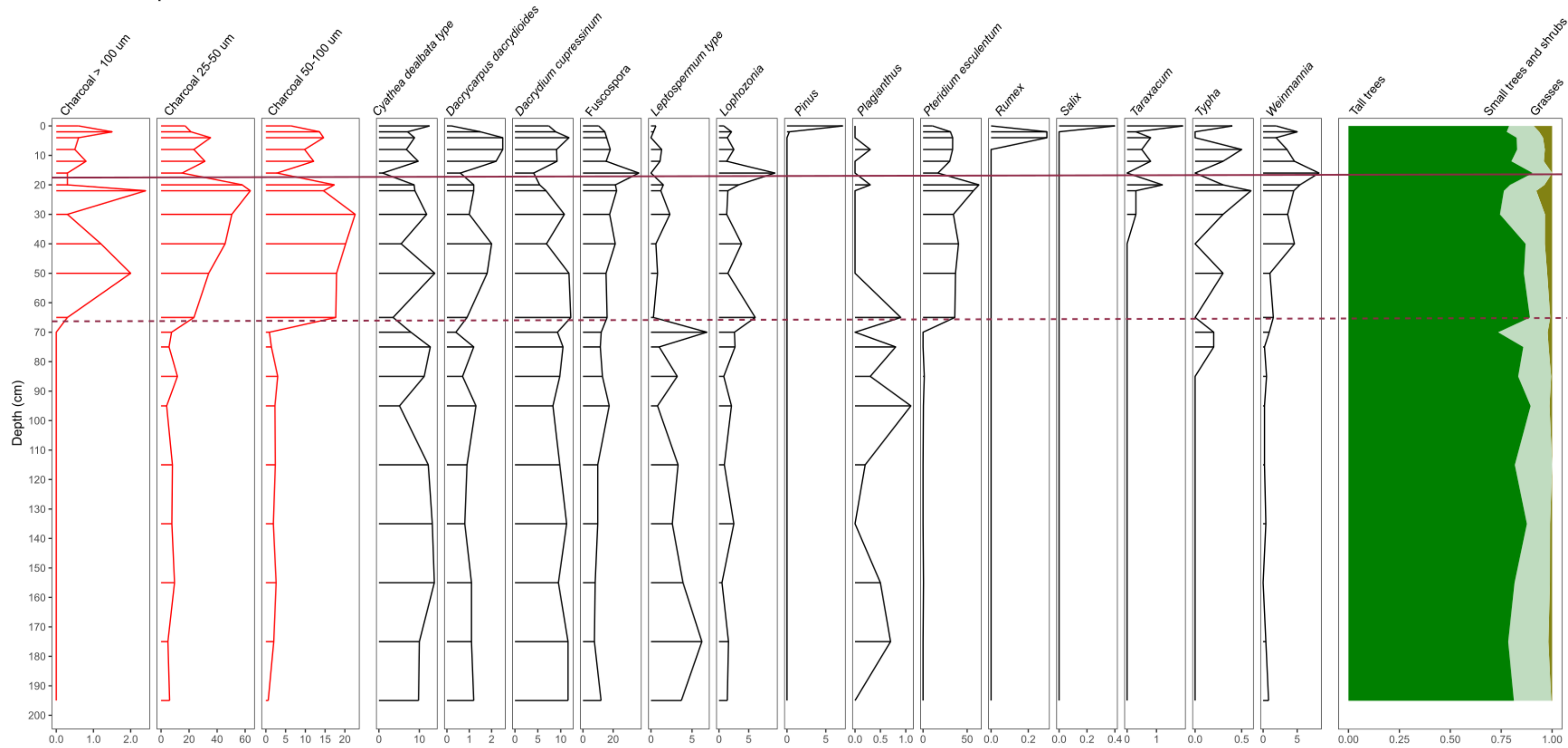


Figure D.5: Selection of pollen and charcoal data from the Lake Wairarapa core, used to determine occupation phases. *Pinus*, *Salix*, and *Rumex* are exotic plants indicators of European presence, charcoal is an indication of vegetation clearance in the catchment (Māori then European presence). Adapted from Waters et al. (2018). This data was used to identify the three phases (separated by horizontal purple lines) used in this study: pre-human (PH; bottom), evidence of Māori settlement (EMS; middle), and post European settlement (PES; top).

Table D.4: Chronology for Lake Paringa – adapted from Howarth et al. (2012).

Sample	**Sub-bottom depth (cm)**	**RDL depth (cm)**	**Mean age (AD)**	**1 sigma uncer-tainty**	**Lower 2 sigma (AD)**	**Upper 2 sigma (AD)**
PA1 3.5 cm D	0	0	2011	0	2010	2011
PA1 5.5 cm D	1.5	1.5	2007	4	1995	2011
PA1 10.5 cm D	6.5	6.5	1999	8.3	1981	2011
PA1 14.5 cm D	11.5	11.5	1989	9.4	1969	2008
PA1 25.0 cm D	21	21	1971	7	1958	1986
PA1 32.5 cm D	29	27.5	1964	4.3	1952	1970
PA1 43.5 D	39	37	1950	6.3	1934	1960
PA6 36 cm D	68	63.5	1873	25.4	1821	1922
PA6 43 cm D	74	69.5	1856	26.3	1802	1908
PA6 52 cm D	80.5	76	1837	26.6	1783	1890
PA6 59.5 cm D	89.5	85	1811	25.7	1760	1863
PA6 69.5 cm D	99.5	95	1782	22.8	1738	1827
PA6 76 cm D	105.5	101	1765	19.8	1729	1804
PA6 81.5 cm D	110.5	105	1754	18.4	1720	1791
PA6 87 cm D	115	108	1745	17.2	1714	1780
PA6 96.5 cm D	125	115	1726	12.5	1702	1753
PA6 101 cm D	129.5	115.5	1724	12	1702	1751
PA6 126 cm D	153.5	118.5	1716	8.1	1700	1733
PA6 S2 1.0 cm D	189.5	132.5	1652	10.3	1632	1671
PA6 S2 13.0 cm D	201.5	144.5	1599	17.4	1558	1626
PA6 S2 55 cm D	238.5	167	1463	15.4	1436	1497
PA6 S2 121.5 cm D	300	187.5	1392	7.1	1374	1406
PA6 S2 142.5 cm D	319.5	200.5	1344	12.5	1319	1369
PA6 S3 1.5 cm D	336	213	1303	17.6	1266	1343
PA6 S3 20.5 cm D	357.5	233	1241	16.6	1212	1276
PA6 S3 100 cm D	431	253.5	1151	17.6	1109	1180
PA6 S3 116 cm D	442.5	264	1077	29.3	1024	1133
PA6 S3 132cm D	453.5	275	998	17.8	964	1030
PA6 S4 15.5 cm D	484	286	963	10.3	941	984
PA6 S4 52 cm D	517	291	939	27.7	865	974
PA6 S4 74 cm D	536.5	310.5	866	47.3	766	942
PA6 S4 98 cm D	558	330.5	790	63.1	659	901

Table D.5: Chronology for Lake Pounui. All ages are indicated in radiocarbon years (BP) except when specified (calendar years – AD). Adapted from Cochrane, 2017.

Depth (cm)	**Mean age**	**Mean AD age**	**sigma**	**Median age**	**Lower CI (95)**	**Upper CI (95)**	**Lower CI (99)**	**Upper CI (99)**
0	-63	2013	1	-63	-61	-66	-61	-66
1.625	-49	1999	13	-52	-21	-66	-7	-66
2.4375	-42	1992	14	-44	-14	-66	-5	-66
3.25	-35	1985	15	-35	-7	-61	-2	-66
4.0625	-28	1978	15	-26	-4	-59	0	-66
5.6875	-13	1963	10	-10	1	-39	1	-61
6.5	-6	1956	3	-5	0	-12	9	-31
7.23125	0	1950	11	-3	31	-14	68	-31
8.69375	13	1937	17	9	52	-11	92	-19
9.425	20	1930	20	16	62	-9	97	-15
11.6187	39	1911	23	38	84	-4	106	-9
13.8125	59	1891	23	60	103	12	116	-6
15.275	72	1878	22	74	111	26	129	-3
17.4688	91	1859	17	94	121	52	147	18
19	105	1845	11	105	126	81	161	71
21.2219	177	1773	27	173	241	106	303	89
23.3875	204	1746	36	199	293	147	359	101
25.5531	231	1719	42	226	323	157	393	112
27.7188	257	1693	46	253	353	171	424	133
29.1625	275	1675	48	272	375	183	439	150
34.2156	337	1613	49	338	435	237	470	189
39.2687	399	1551	43	406	475	307	491	245
44.575	461	1489	27	466	500	393	504	339
49.2	510	1440	8	510	528	495	544	481
55.6125	567	1383	25	568	616	518	635	502
60.6	612	1338	20	616	646	552	659	543
65.2125	677	1273	44	674	777	595	842	554
70.3375	750	1200	49	750	852	648	916	601
75.4625	823	1127	40	820	924	753	948	684
80.25	876	1074	40	868	961	811	1036	773
85.125	922	1028	45	918	1017	837	1084	809
90	968	982	49	965	1068	873	1125	836
95.6875	1021	929	49	1020	1126	926	1160	879
100.562	1067	883	47	1064	1161	981	1178	935
105.344	1116	834	50	1111	1207	1008	1266	985
110.031	1166	784	56	1166	1282	1056	1343	1003
115.305	1223	727	59	1224	1343	1103	1409	1037
120.578	1280	670	60	1282	1401	1156	1460	1084
125.852	1338	612	58	1341	1451	1217	1500	1131
130.539	1388	562	54	1393	1490	1276	1530	1178

Table D.6: Description of the variables considered in Generalised Linear Models against cyanobacteria abundances and richness.

Variable name	Description	Type
Depth	individual sample depths in cm (e.g., 0, 2, 4, 6...)	Continuous auto-correlated variable
Lake	individual lakes (Paringa, Pounui, Rotoehu, Hayes, Johnson, Wairarapa)	Categorical fixed variable
Phase	Time frame corresponding to the presence of absence of specific activities in/around the lake (PES, EMS, PH)	Categorical explanatory variable
Phase2	pre-European settlement vs post-European settlement	Categorical (binary) explanatory variable
Exotic Fish	presence/absence of exotic fish introduced in each lake. Whenever possible it was aligned with known dates of introduction (H, J, Pa, Po), otherwise it was aligned with European settlement (R, W)	Categorical (binary) explanatory variable
Native Vegetation Change	before/after start of high charcoal peaks (land burning)	Categorical (binary) explanatory variable
Land-Use intensification	presence/absence of European-related land-use in lake catchment. Paringa was considered pristine	Categorical (binary) explanatory variable

Table D.7: Categories attributed to each taxonomic entity at Genus level and plotted as Figure 2.6 (bubble plot).

Genus	Type	Toxic
Aliterella	> 3 µm	No
Anabaena_XPORK15F	> 3 µm	Potentially
Annamia_HOs24	> 3 µm	Potentially
Aphanizomenon_NIES81	> 3 µm	Potentially
Calothrix_KVSF5	> 3 µm	Potentially
Calothrix_PCC-6303	> 3 µm	Potentially
CENA359	> 3 µm	No
Chroococcopsis	> 3 µm	No
Cuspidothrix_LMECYA_163	> 3 µm	Potentially
Cyanobium_PCC-6307	Picocyanobacteria	No
Cyanothece_PCC_7425	> 3 µm	No
Cylindrospermum_PCC-7417	> 3 µm	Potentially
Dolichospermum_NIES41	> 3 µm	Potentially
Geitlerinema_PCC-7105	> 3 µm	Potentially

Continued on next page

Table D.7: Categories attributed to each taxonomic entity at Genus level and plotted as Figure 2.6 (bubble plot). (Continued)

Geminocystis_PCC-6308	> 3 µm	No
Gleocapsa	> 3 µm	No
HAVOmat113	> 3 µm	No
JSC-12	> 3 µm	No
Leptolyngbya_ANT.L52.2	> 3 µm	Potentially
Limnolyngbya_CHAB4449	> 3 µm	No
Microcoleus_SAG_1449-1a	> 3 µm	Potentially
Microcystis_PCC-7914	> 3 µm	Potentially
Nodosilinea_PCC-7104	> 3 µm	No
Nostoc_PCC-7524	> 3 µm	Potentially
Nostoc_PCC-8976	> 3 µm	Potentially
Oscillatoria_PCC-6304	> 3 µm	Potentially
Oscillatoria_SAG_1459-8	> 3 µm	Potentially
Phormidesmis_ANT.LACV5.1	> 3 µm	No
Phormidium_MBIC10003	> 3 µm	Potentially
Planktothrix_NIVA-CYA_15	> 3 µm	Potentially
Pseudanabaena_PCC-6802	> 3 µm	No
Pseudanabaena_PCC-7429	> 3 µm	No
Richelia_HH01	> 3 µm	No
Scytolyngbya_XSP1	> 3 µm	No
Snowella_0TU37S04	> 3 µm	No
Sphaerospermopsis_BCCUSP55	> 3 µm	No
SU2_symbiont_group	> 3 µm	No
Synechococcus_MBIC10613	Picocyanobacteria	Potentially
Synechocystis_BDHKU-20401	Picocyanobacteria	Potentially
Tychonema_CCAP_1459-11B	> 3 µm	Potentially
Unknown Cyanobacteriaceae	> 3 µm	No
Unknown Cyanobacteriales	> 3 µm	No
Unknown Cyanobiaceae	Picocyanobacteria	No
Unknown Eurycoccales	> 3 µm	No
Unknown Leptolyngbyaceae	> 3 µm	No
Unknown Microcystaceae	> 3 µm	Potentially
Unknown Nostocaceae	> 3 µm	Potentially
Unknown Oscillatoriaceae	> 3 µm	No
Unknown Synechococcaceae	Picocyanobacteria	No
Unknown Synechococcales	Picocyanobacteria	No
Unknown Xenococcaceae	> 3 µm	No
Xenococcus_CRM	> 3 µm	No

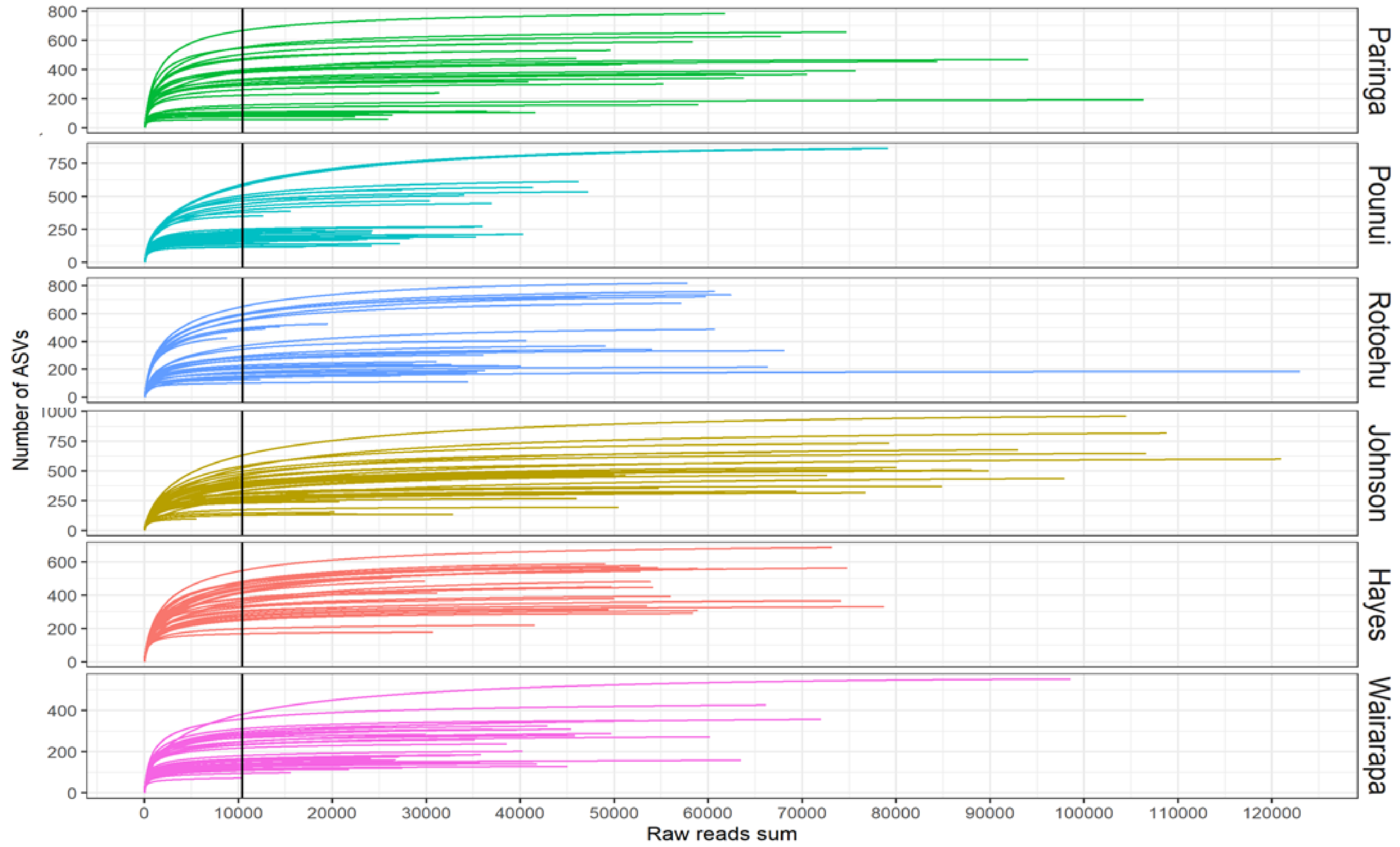
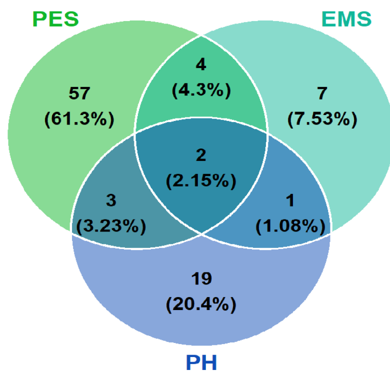
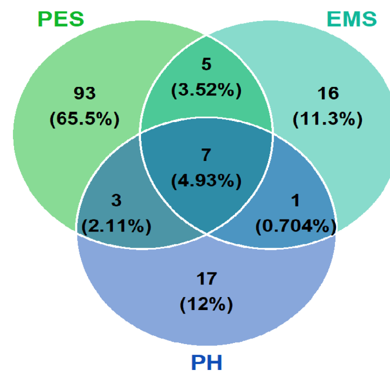


Figure D.6: Individual rarefaction curves, calculated on all bacterial reads. The vertical lines show the rarefaction threshold at 10,400 reads for univariate data (richness analyses).

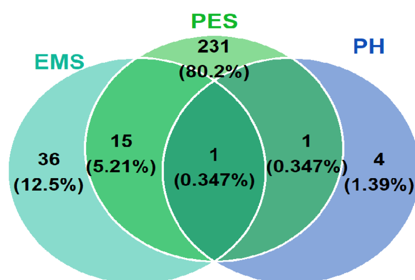
A) Lake Paringa



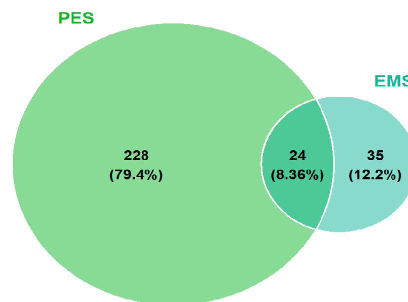
B) Lake Pounui



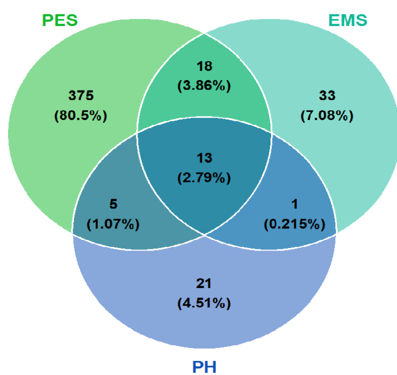
C) Lake Rotoehu



D) Lake Hayes



E) Lake Johnson



F) Lake Wairarapa

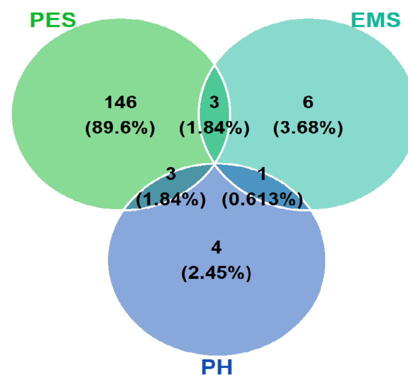


Figure D.7: Venn diagrams per lake showing the number and percentages of shared and unique Amplicon Sequence Variants across phases. Phases anagrams refer to occupation phases: PH = Pre-Human, EMS = Evidence of Māori Settlement, PES = Post-European Settlement. Drawn with the VennDiagram R package (Chen et al., 2011)

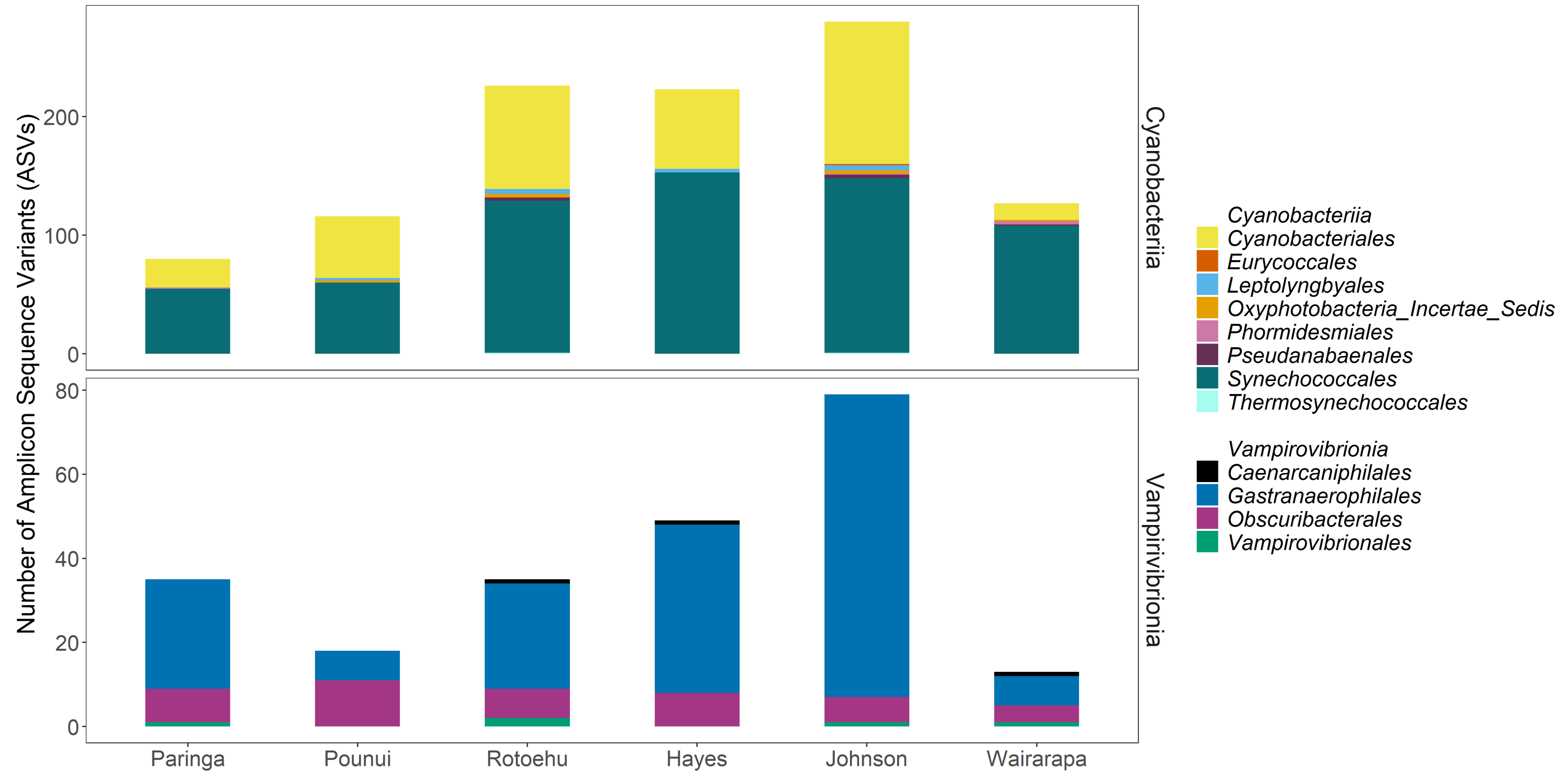


Figure D.8: Taxonomic composition and richness (number of Amplicon Sequence Variants) per lake at Class level and corresponding detail of Order level per Class. Since Cyanobacteriia is twice as abundant as Vampirovibrionia, different scales are used to improve visualisation.

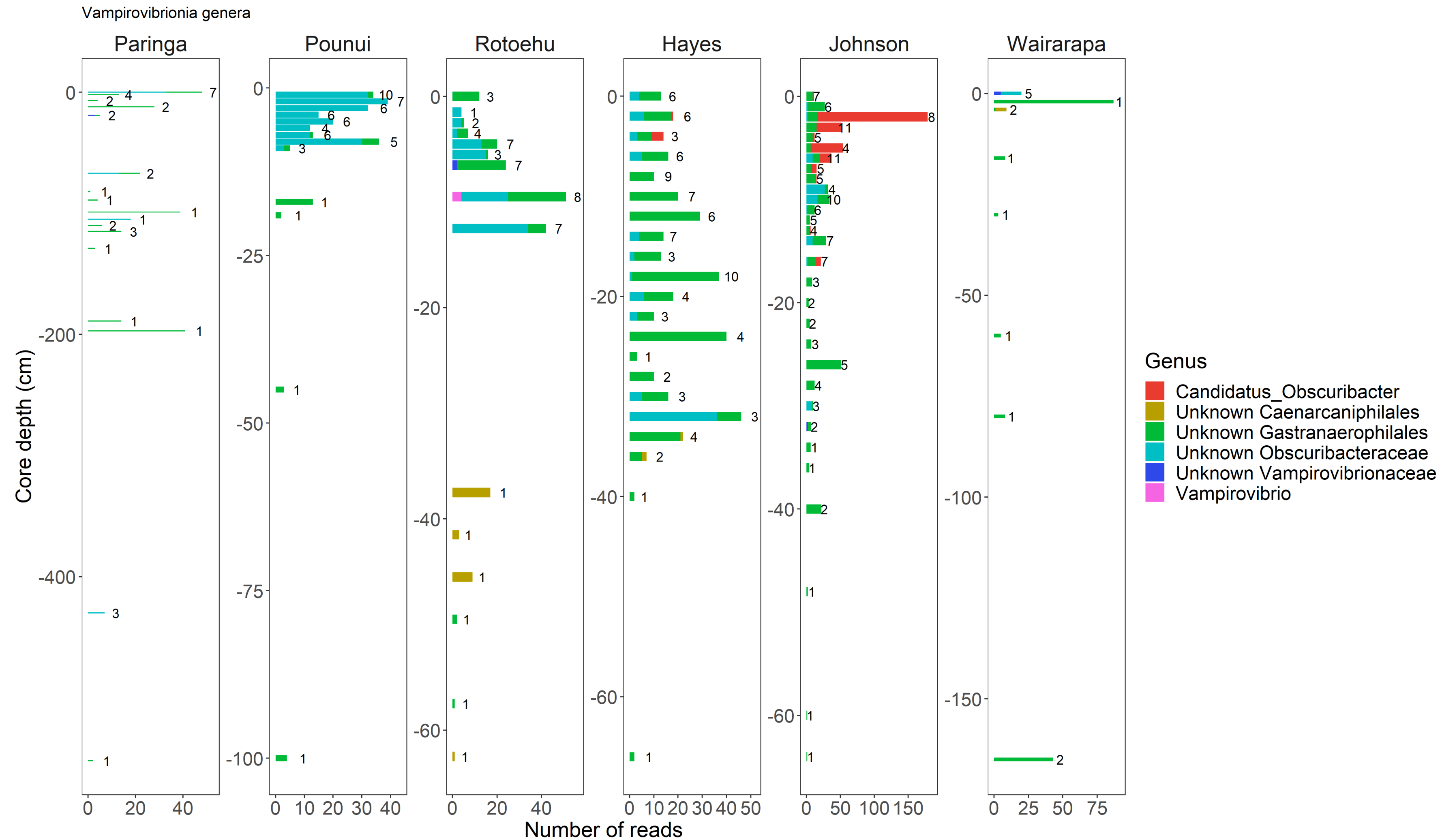


Figure D.9: Abundance of non-photosynthetic cyanobacteria (Class Vampirovibrionia) for each sample. Composition at Genus level (or closest resolved taxonomical level) is color-coded, and the number of Amplicon Sequence Variants (ASV) per sample (richness) is indicated on the right of each bar.

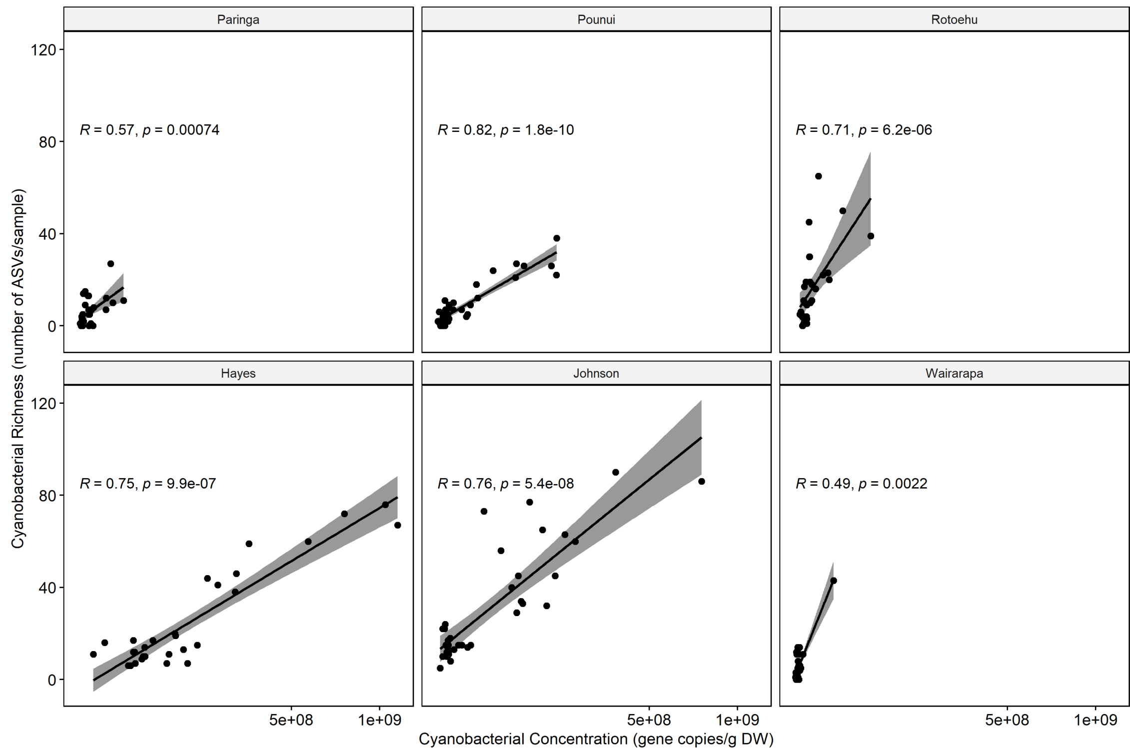


Figure D.10: Relationship between metabarcoding richness (number of amplicon sequence variants (ASVs)) and cyanobacterial abundance (16S rRNA cyanobacterial gene copy numbers per gram of dry sediment), lakes plotted individually, x-axis was transformed with square-root.

Table D.8: Lake pairwise comparisons of cyanobacterial abundance (16S rRNA gene, droplet digital PCR). Results were averaged by Phase and given on the log scale, degrees-of-freedom method was Satterthwaite and p-value adjustment method was tukey.

contrast	**estimate**	**std.error**	**df**	**statistic**	**adj.p.value**
Paringa - Hayes	-4.28442	0.487311	56.51464	-8.79196	< **0.001**
Paringa - Johnson	-1.62373	0.48031	70.05239	-3.38058	**0.01**
Paringa - Pounui	-1.43915	0.428028	42.00901	-3.36228	**0.02**
Paringa - Rotoehu	-1.12957	0.427571	42.81542	-2.64184	0.11
Paringa - Wairarapa	1.076898	0.396292	55.62571	2.717437	0.09
Hayes - Johnson	2.660692	0.568402	85.74592	4.681005	< **0.001**
Hayes - Pounui	2.845272	0.551843	63.00539	5.155947	< **0.001**
Hayes - Rotoehu	3.154846	0.533699	62.75399	5.911286	< **0.001**
Hayes - Wairarapa	5.361317	0.527012	87.28901	10.17305	< **0.001**
Johnson - Pounui	0.18458	0.535947	81.58546	0.344399	0.99
Johnson - Rotoehu	0.494154	0.528002	84.91633	0.935894	0.94
Johnson - Wairarapa	2.700625	0.511749	76.64022	5.277243	< **0.001**
Pounui - Rotoehu	0.309574	0.483094	67.36705	0.640816	0.99
Pounui - Wairarapa	2.516045	0.440256	48.17593	5.714955	< **0.001**
Rotoehu - Wairarapa	2.206471	0.456369	50.91589	4.834834	< **0.001**

Table D.9: Lake pairwise comparisons of cyanobacterial richness (16S rRNA gene, metabarcoding). Results were averaged by Phase and given on the sqrt scale, degrees-of-freedom method was Satterthwaite and p-value adjustment method was tukey.

contrast	**estimate**	**std.error**	**df**	**statistic**	**adj.p.value**
Paringa - Hayes	-1.76359	0.65456	26.54497	-2.69432	0.11
Paringa - Johnson	-2.56889	0.658925	56.12537	-3.89861	**<0.01**
Paringa - Pounui	-0.61988	0.564298	9.29285	-1.0985	0.87
Paringa - Rotoehu	-0.90071	0.560841	9.949678	-1.606	0.61
Paringa - Wairarapa	-0.2071	0.50584	8.759694	-0.40942	0.99
Hayes - Johnson	-0.8053	0.806557	33.68649	-0.99845	0.92
Hayes - Pounui	1.143706	0.763921	25.31611	1.497152	0.67
Hayes - Rotoehu	0.862882	0.740507	23.94373	1.165258	0.85
Hayes - Wairarapa	1.556489	0.721803	28.33236	2.15639	0.29
Johnson - Pounui	1.949011	0.758587	22.10189	2.569266	0.15
Johnson - Rotoehu	1.668187	0.745508	20.6873	2.23765	0.26
Johnson - Wairarapa	2.361794	0.717055	24.39192	3.293743	**0.03**
Pounui - Rotoehu	-0.28082	0.66811	13.05836	-0.42033	0.99
Pounui - Wairarapa	0.412782	0.606933	12.66998	0.680111	0.98
Rotoehu - Wairarapa	0.693607	0.620543	13.40042	1.117742	0.87

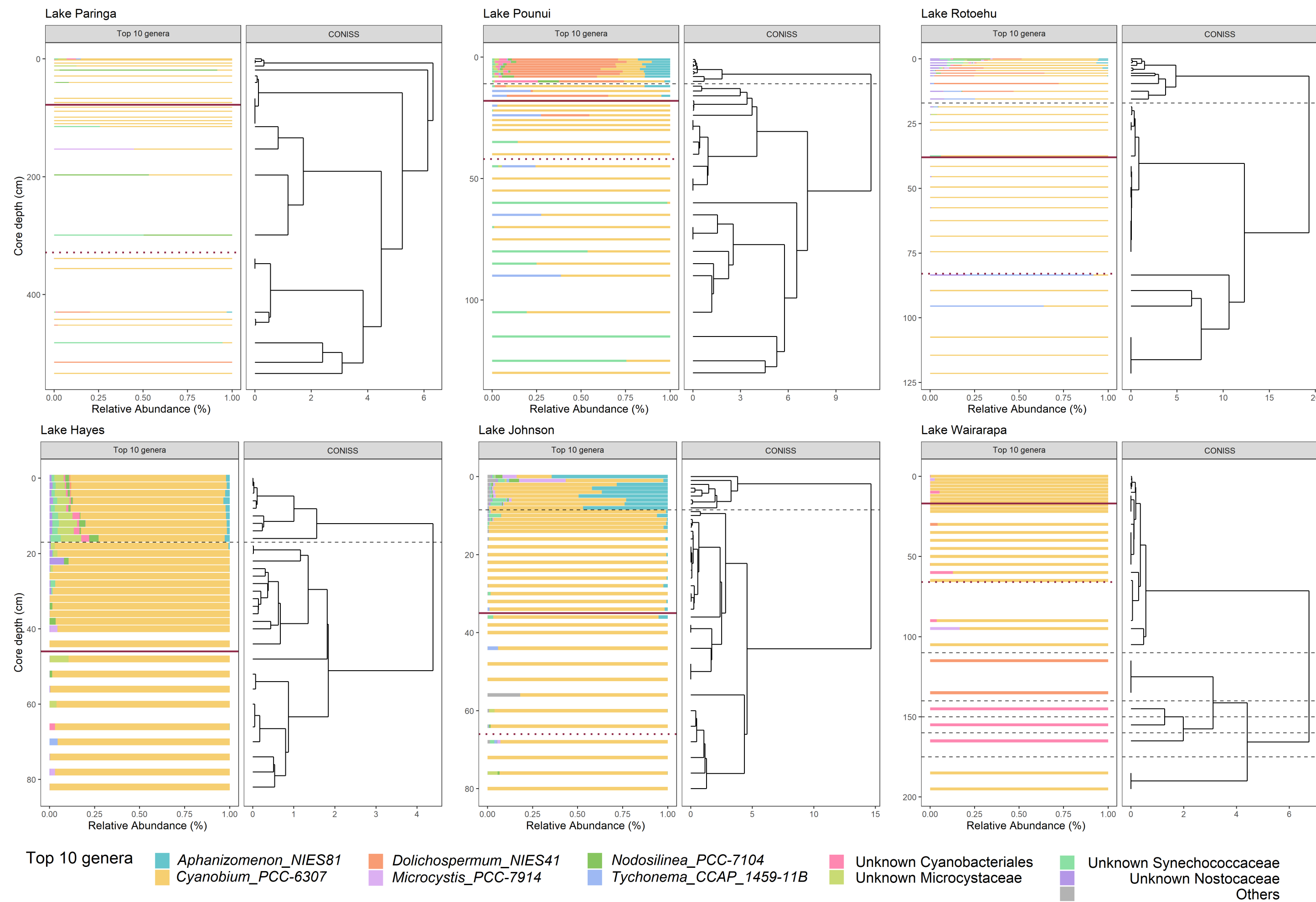


Figure D.11: Cyanobacteria composition at Genus level and CONISS analysis per lake. Only the top 10 genera are shown, the rest were grouped as “Others”. Occupation phases defined by the pollen data are indicated for each lake on the barplots (purple full line for European settlement, purple dotted line for Māori settlement). The CONISS analysis also identified main eras for all lakes except Lake Paringa, these are indicated by the grey dotted line going across the barplot and the dendrogram.

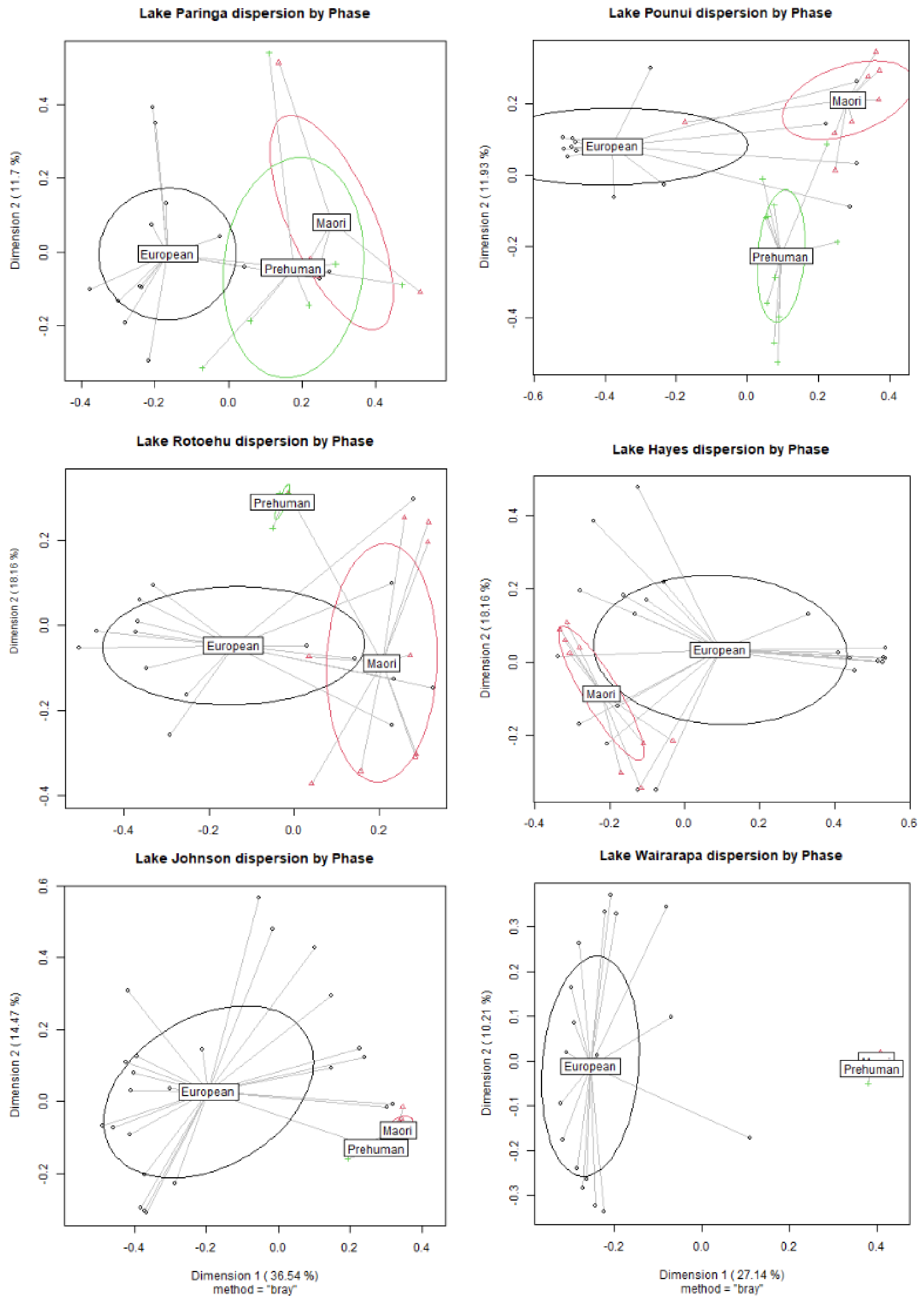


Figure D.12: Dispersion plots from the betadisper analysis of the R vegan package for phases within individual lakes.

Table D.10: PERMANOVA and pairwise test on cyanobacterial communities across phases within individual lakes. Since the core from Lake Hayes only retraced PES and EMS, no pairwise comparisons were needed (grey shading).

	PERMANOVA			**Pairwise test**		
	F value	R2	p-value		PES	EMS
Paringa	1.9099	0.14	< **0.001**	EMS	**0.003**	-
				PH	**0.002**	0.81
Pounui	4.4398	0.22	< **0.001**	EMS	< **0.004**	-
				PH	< **0.001**	< **0.001**
Rotoehu	2.8506	0.17	< **0.001**	EMS	< **0.004**	-
				PH	< **0.001**	< **0.006**
Hayes	4.2593	0.13	< **0.005**			
Johnson	5.3147	0.23	< **0.001**	EMS	< **0.001**	-
				PH	< **0.003**	**0.027**
Wairarapa	5.9314	0.31	< **0.001**	EMS	< **0.001**	-
				PH	< **0.001**	0.18

Table D.11: ANOVA and Tukey multiple comparisons of means, testing the homogeneity of multivariate dispersions in cyanobacterial communities across phases within individual lakes. Since the core from Lake Hayes only retraced PES and EMS, no pairwise comparisons were needed (grey shading).

	ANOVA		**Tukey**		
	F value	p-value		PES	EMS
Paringa	6.7255	< **0.01**	EMS	**0.04**	-
			PH	**0.02**	0.99
Pounui	4.3828	**0.02**	EMS	0.48	-
			PH	**0.02**	0.19
Rotoehu	5.4046	**0.01**	EMS	0.77	-
			PH	< **0.01**	0.06
Hayes	9.421	< **0.01**			
Johnson	22.421	< **0.001**	EMS	< **0.001**	-
			PH	< **0.001**	0.99
Wairarapa	23.738	< **0.001**	EMS	< **0.001**	-
			PH	< **0.001**	0.99

Table D.12: Summary of known events / lake states from the literature. European settlement started in 1840 after the signature of the Treaty of Waitangi.

	Lake Paringa	**Lake Pounui**	**Lake Rotoehu**
Post-European Settlement	Present day: lake open to the public, used for trout fishing. Pristine native catchment, dark waters (tannin). Oligotrophic. 1962-1965: Main road built next to lake. 1930: Brown trout (*Salmo trutta*) introduction. 1875: Haast-Paringa cattle track (rough track used to move stock) provides some access to lake.	Present day: land around lake privately owned, cyanobacterial blooms mostly summer. Tens of thousands of perch (*Perca fluviatilis*) estimated to live in the lake. One of the last lowland lakes with a mostly native catchment and high native macrophyte presence/diversity. Eutrophic. 1970s: flood gates downstream found to prevent fish passage back to Lake Pounui. 1960s: perch (*Perca fluviatilis*) introduction. 1941: south-western catchment cut down (aerial image).	Present day: lake open to the public, recreational use. Cyanobacterial blooms every summer since 1993, especially in the multiple shallow arms. Eutrophic. 1993: marked decrease in lake level (4.2 m). 1960s: mesotrophic state, first land-use changes (forest and scrubland converted to pasture), first cyanobacterial blooms observed. 1900s: introduction of rainbow trout (*Oncorhynchus mykiss*).

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Table D.12: Summary of known events / lake states from the literature. European settlement started in 1840 after the signature of the Treaty of Waitangi. (Continued)

Post-European Settlement		1938 to 1938: yearly stocking of fingerlings rainbow trout. 1855 – M 8.2: earthquake on nearby fault.	1886 – eruption of Mount Tarawera covered the region with ash (c. 5cm thick tephra in core).
Evidence of Māori Settlement	No known Māori settlement – no evidence of land clearance. Lake is situated near the Paringa fault: repeated earthquakes every c. 300 years. Last earthquakes > 7.6 magnitude: c. AD 925, c. AD 1150, c. AD 1400 and AD 1717.	Māori settlement in the region estimated to c. 1500s-1600s (some land clearance) but no major settlement in the vicinity of the lake.	Māori settlement in the Rotorua region estimated to c. 1250s but no major settlement in the vicinity of the lake.
Pre-human		Lake existed in high nutrient state (before 0 AD) and then lower nutrient state (after 0 AD) until European arrival (diatom reconstruction, Cochrane, 2017).	Lake situated near active volcanoes. Long history of so impact from eruptions expected (tephra layers observed).

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Table D.12: Summary of known events / lake states from the literature. European settlement started in 1840 after the signature of the Treaty of Waitangi. (Continued)

	Lake Johnson	**Lake Hayes**	**Lake Wairarapa**
Post-European Settlement	Present day: lake open to the public, popular for trout fishing. Surrounded by pasture. Eutrophic. Contains rainbow trout. 1962: known beginning of cyanobacterial blooms. Also start of yearly stocking of fingerlings rainbow trout (*Oncorhynchus mykiss*). 1955: beginning of superphosphate fertiliser application in the catchment. (unknown date) original outlet dammed to ensure water supply for irrigation. 1870s: Brown trout (*Salmo trutta*) and perch (*Perca fluviatilis*) introduced.	Present day: lake open to the public, popular for recreational (swimming, boating, fishing). Surrounded by pasture and a few settlements. Eutrophic. 2006: Blooms of *Ceratium hirundinella*. 1970: Bottom waters became anoxic, releasing phosphate. Now eutrophic. 1969: First cyanobacteria bloom recorded (Dolichospermum flos-aquae). 1961: Significant catchment drainage work began. 1959: Lake approaching eutrophic state.	Present day: lake open to the public, very degraded state (super-eutrophic). Surrounded by pastures and a few towns. Supertrophic. 1963-1983 – Construction to prevent regular flooding of the valley (Lower Wairarapa Valley Development Scheme). Inflow (Ruamahanga River) diverted permanently from Lake Wairarapa. 1855 – M 8.2 earthquake on nearby fault. Increase in burning, deforestation, agricultural land-use. 1844 - European settlement.

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Table D.12: Summary of known events / lake states from the literature. European settlement started in 1840 after the signature of the Treaty of Waitangi. (Continued)

Post-European Settlement		1910-50: Increasing land-use around the lake (cheese factory releasing effluents, improved farming practices spreading superphosphate fertiliser). 1870s – Brown trout (*Salmo trutta*) and perch (*Perca fluviatilis*) introduced.	
Evidence of Māori Settlement	No major Māori settlement.	No major Māori settlement.	Māori settlement in the region estimated to c. 1500s.
Pre-human			

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Table D.13: Lake Rototoa age model. Some rapidly deposited layers were observed between 24.7-28.1 cm and 44.3-53.9 cm, therefore theses depths were removed from the age model.

Depth (cm)	Year (AD)	sigma	median	sed	from_95_4	to_95_4
0	2018	0	2018	0.59	2017	2018
1	2016	0	2016	0.352	2016	2016
2	2012	0	2012	0.25	2012	2012
3	2008	0	2008	0.223	2007	2009
4	2003	0	2003	0.221	2002	2004
5	1999	1	1999	0.222	1998	2000
6	1994	1	1994	0.203	1993	1995
7	1989	1	1989	0.216	1988	1991
8	1985	1	1985	0.2	1983	1986
9	1979	1	1979	0.186	1977	1981
10	1974	1	1974	0.193	1972	1976
11	1969	1	1969	0.18	1967	1971
12	1963	1	1963	0.165	1960	1965
13	1957	1	1957	0.15	1954	1960
14	1950	2	1950	0.151	1947	1953
15	1944	2	1943	0.152	1940	1947
16	1936	2	1936	0.131	1933	1940
17	1928	2	1928	0.122	1924	1932
18	1920	2	1920	0.14	1916	1924
19	1914	2	1914	0.157	1910	1918
20	1907	3	1908	0.152	1899	1916
21	1901	4	1901	0.152	1893	1910
22	1894	4	1894	0.152	1885	1903
23	1888	3	1888	0.171	1878	1893
24	1882	4	1883	0.196	1872	1892
29	1874	5	1874	0.195	1863	1885
30	1869	5	1869	0.195	1857	1881
31	1864	6	1864	0.195	1851	1877
32	1859	6	1859	0.195	1846	1871
33	1854	6	1854	0.195	1840	1867
34	1849	7	1849	0.195	1834	1863
35	1844	7	1844	0.196	1829	1859
36	1838	7	1838	0.195	1823	1854
37	1833	8	1833	0.195	1817	1850
38	1828	8	1828	0.195	1812	1844
39	1823	8	1823	0.195	1806	1840
40	1818	8	1818	0.195	1801	1836
41	1813	9	1813	0.196	1795	1831
42	1808	9	1808	0.196	1789	1827
43	1803	9	1802	0.196	1784	1822
44	1797	9	1797	0.196	1779	1818
54	1795	9	1794	0.195	1776	1816
55	1790	9	1790	0.195	1772	1810
56	1785	10	1784	0.196	1765	1806
57	1780	10	1779	0.196	1760	1801
58	1774	10	1774	0.196	1754	1797
59	1769	10	1769	0.196	1749	1791
60	1764	10	1763	0.196	1744	1787

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Table D.13: Lake Rototoa age model. Some rapidly deposited layers were observed between 24.7-28.1 cm and 44.3-53.9 cm, therefore theses depths were removed from the age model. (Continued)

61	1759	11	1758	0.196	1739	1782
62	1754	11	1753	0.196	1733	1777
63	1749	11	1748	0.196	1728	1773
64	1744	11	1743	0.196	1723	1768
65	1739	11	1737	0.196	1718	1763
66	1734	11	1732	0.196	1713	1758
67	1729	11	1727	0.196	1707	1754
68	1723	12	1722	0.196	1702	1748
69	1718	12	1716	0.196	1697	1745
70	1713	12	1712	0.196	1693	1738
71	1708	12	1706	0.196	1687	1735
72	1703	12	1701	0.196	1683	1729
73	1698	12	1696	0.196	1676	1724
74	1693	12	1690	0.196	1673	1721
75	1688	12	1685	0.196	1667	1714

Table D.14: Lake details. All lakes were cored at their deepest point (depocenter).

	Lake Nganoke	Lake Okataina	Lake Pounui	Lake Rototoa
Latitude	41° 21' 19" S	38° 6' 36" S	41° 20' 39" S	36° 30' 43" S
Longitude	175° 11' 13" E	176° 25' 17" E	175° 6' 48" E	174° 14' 19" E
Max depth (m)	2	78	9	26.1
Current water quality	Eutrophic	Oligotrophic	Eutrophic	Oligotrophic
Recent cyanobacterial blooms	Yes	No	Yes	No
Main land type in catchment	Pasture	Native / Pine forestry	Native	Pine forestry / Native

Table D.15: Mastermix and cycling conditions for the 107F and 377R_mod primer set.

Primer set	Master Mix (for one sample)	Cycling conditions
107F and 377R_mod	10 µL Evagreen (2x) 7.6 µL DNA-free water 0.2 µL Forward primer (10 µM) 0.2 µL Reverse primer (10 µM) 4 µL template DNA	Initial denaturation: 95°C - 5 minutes 50 cycles: Denaturation: 95°C - 30 seconds Annealing: 55°C - 1 minute 4°C - 5 minutes 90°C - 5 minutes

16S cyanobacteria.

	108F	5'- ACGGGTGAGTAACRCGTRA -3'
	107F	5'- GACGGGTGAGTAACRCGTRRG -3'
Forward	*Aphanizomenon flos-aquae*	5'- GACGGGTGAGTAACGCGTAAG -3'
	Calothrix sp.	5'- GACGGGTGAGTAACGCGTGAG -3'
	Cyanobium gracile	5'- GACGGGTGAGTAACGCGTGGG -3'
	Dolichospermum lemmermannii	5'- GACGGGTGAGTAACGCGTAAG -3'
	Microcoleus vaginatus	5'- GACGGGTGAGTAACGCGTGAG -3'
	Microcystis aeruginosa	5'- GACGGGTGAGTAACGCGTGAG -3'
	Nostoc punctiformes	5'- GACGGGTGAGTAACGCGTGAG -3'
	Oscillatoria sp.	5'- GACGGGTGAGTAACGCGTGAG -3'
	Phormidium automnale	5'- GACGGGTGAGTAACGCGTGAG -3'
	Synechococcus elongatus	5'- GACGGGTGAGTAACGCGTGAG -3'
	Tychenoma bourrelyi	5'- GACGGGTGAGTAACGCGTGAG -3'
	377R	5'- GGGGAATTTTCCGCCATGG -3'
	377R_mod	5'- GGGGAATTTTCCGCAATGG -3'
Reverse	*Aphanizomenon flos-aquae*	5'- GGGGAATTTTCCGCAATGG -3'
	Calothrix sp.	5'- GGGGAATTTTCCGCAATGG -3'
	Cyanobium gracile	5'- GGGGAATTTTCCGCAATGG -3'
	Dolichospermum lemmermannii	5'- GGGGAATTTTCCGCAATGG -3'
	Microcoleus vaginatus	5'- GGGGAATTTTCCGCAATGG -3'
	Microcystis aeruginosa	5'- GGGGAATTTTCCGCAATGG -3'
	Nostoc punctiformes	5'- GGGGAATTTTCCGCAATGG -3'
	Oscillatoria sp.	5'- GGGGAATTTTCCGCAATGG -3'
	Phormidium automnale	5'- GGGGAATTTTCCGCAATGG -3'
	Synechococcus elongatus	5'- GGGGAATTTTCCGCAATGG -3'
	Tychenoma bourrelyi	5'- GGGGAATTTTCCGCAATGG -3'

Figure D.13: Selection of a few sequences from the nucleotide alignment of the cyanobacterial 16S rRNA gene with the two primer sets and a few cyanobacteria sequences. The primer CYAN107F was improved from CYAN108F and CYAN377R_mod was improved from CYAN377R, both from Rinta-Kanto et al. (2005). Nucleotides highlighted in yellow show which nucleotides from the CYAN108F/CYAN377R primer set were modified in the 107R/CYAN377R_mod set. Nucleotides in red show the nucleotides added to the new forward primer. Strain and GenBank accession numbers are the following: *Aphanizomenon flos-aquae* (strain NIES81) AJ293131, *Calothrix* sp. (strain PCC 7714) AJ133164, *Cyanobium gracile* (strain PCC-6307) NR_102447, *Dolichospermum lemmermannii* (strain NIES-833) LC228978, *Microcoleus vaginatus* (no strain) EF654064, *Microcystis aeruginosa* (strain AB2017/10) LR794173, *Nostoc punctiforme* (strain PCC73102) NR_114430, *Oscillatoria nigro-viridis* (strain PCC-7112) AB074509, *Phormidium automnale* (strain SAG 35.90) EF654081, *Synechococcus elongatus* (strain PCC 6301) NR_074309), *Tychonema bourrelyi* (no strain) LM651417.

Table D.16: List and details of the new chloroplasts (plastids) detected by the new primer set compared to the original set. Run in-silico on the SILVA database, with 1 bp mismatch.

Scientific Name	Common name	Found in
Nephroselmis olivacea	green algae	freshwater
Zygnema circumcarinatum	charophycean alga	freshwater (not NZ)
Pelargonium x hortorum	zonal geranium	global
Austrotaxus spicata	New Caledonia yew / southern yew	New Caledonia
uncultured bacterium ARCTIC13_E_12		
uncultured bacterium ARCTIC24_F_09		
Micromonas commoda	green algae	marine environment
Bathycoccus prasinos	marine green alga	marine environment
Cephalotaxus hainanensis	Hainan plum-yew	Hainan peninsula (China)
Ecdeiocolea monostachya	tufted perennial	Western Australia
Passiflora ciliata	passionflower	natural range Mexico to North Colombia, South Florida to Caribbean
Wisteria floribunda	Japanese wisteria	Japan
Aquilaria sinensis	Aquilaria tree	China
Hafniomonas laevis	green algae	freshwater
Cylindrocystis brebissonii	green algae	freshwater
Thorea hispida	red algae	British Isles
Otohimella japonica	red algae	Japan (freshwater)
Oryza rufipogon	wild rice / red rice	East, South, Southeast Asia
Dermonema virens	red algae	marine environment
Izziella formosana	red algae	marine environment
Liagoropsis maxima	red algae	marine environment
Trichogloeopsis pedicellata	red algae	marine environment
Dichanthelium oligosanthes	Heller's rosette grass	North America
Polysiphonia schneideri	red algae	marine environment
Hemitomes congestum	Gnome plant	Western United States
Mantoniella squamata	green algae	marine environment

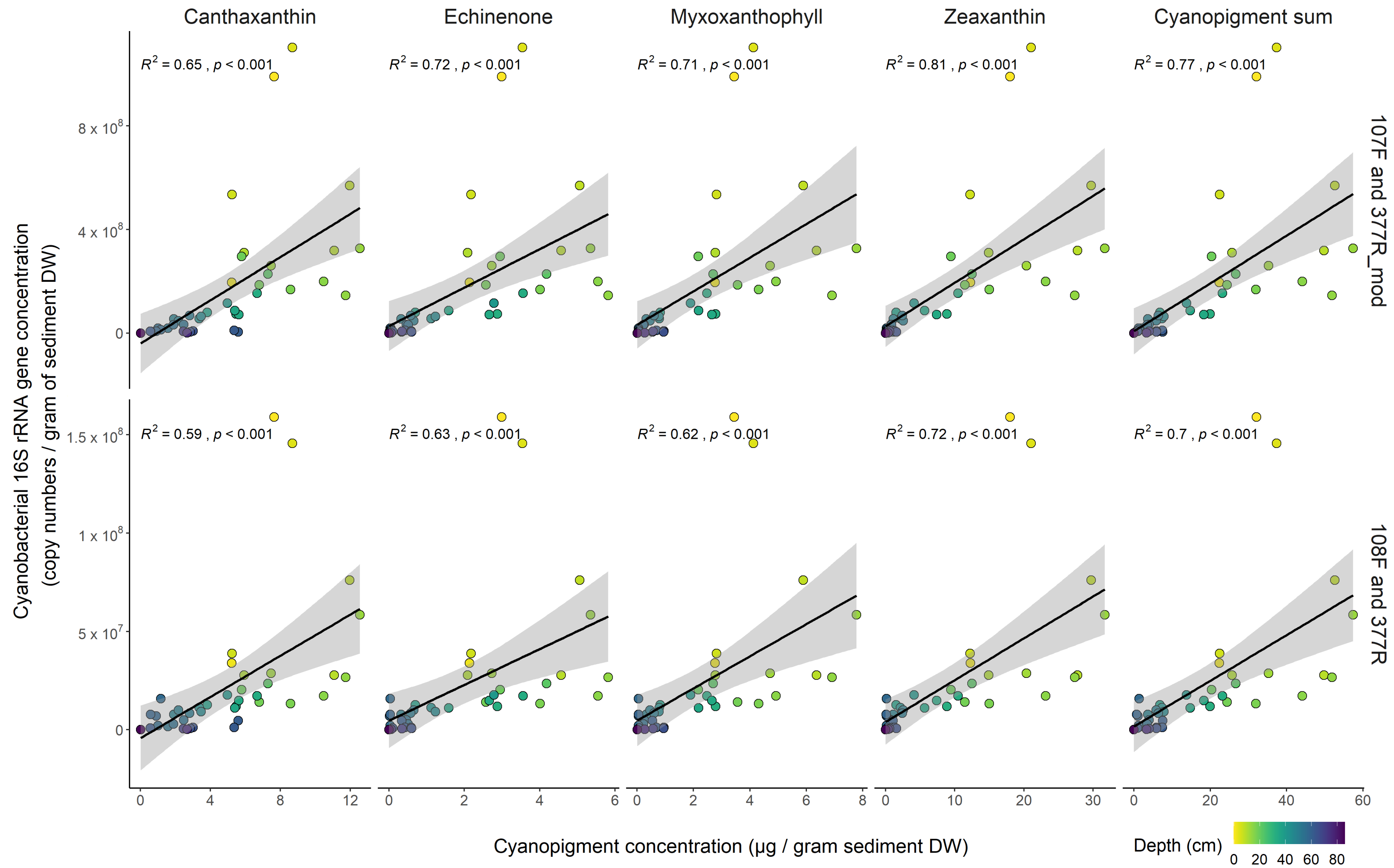

Figure D.14: Correlations between the new and original primer sets (107F / 377R_mod and 108F / 377R, respectively) with individual and summed cyanopigments in Lake Nganoke. Spearman's correlations are displayed for each plot, DW stands for dry weight of sediment.

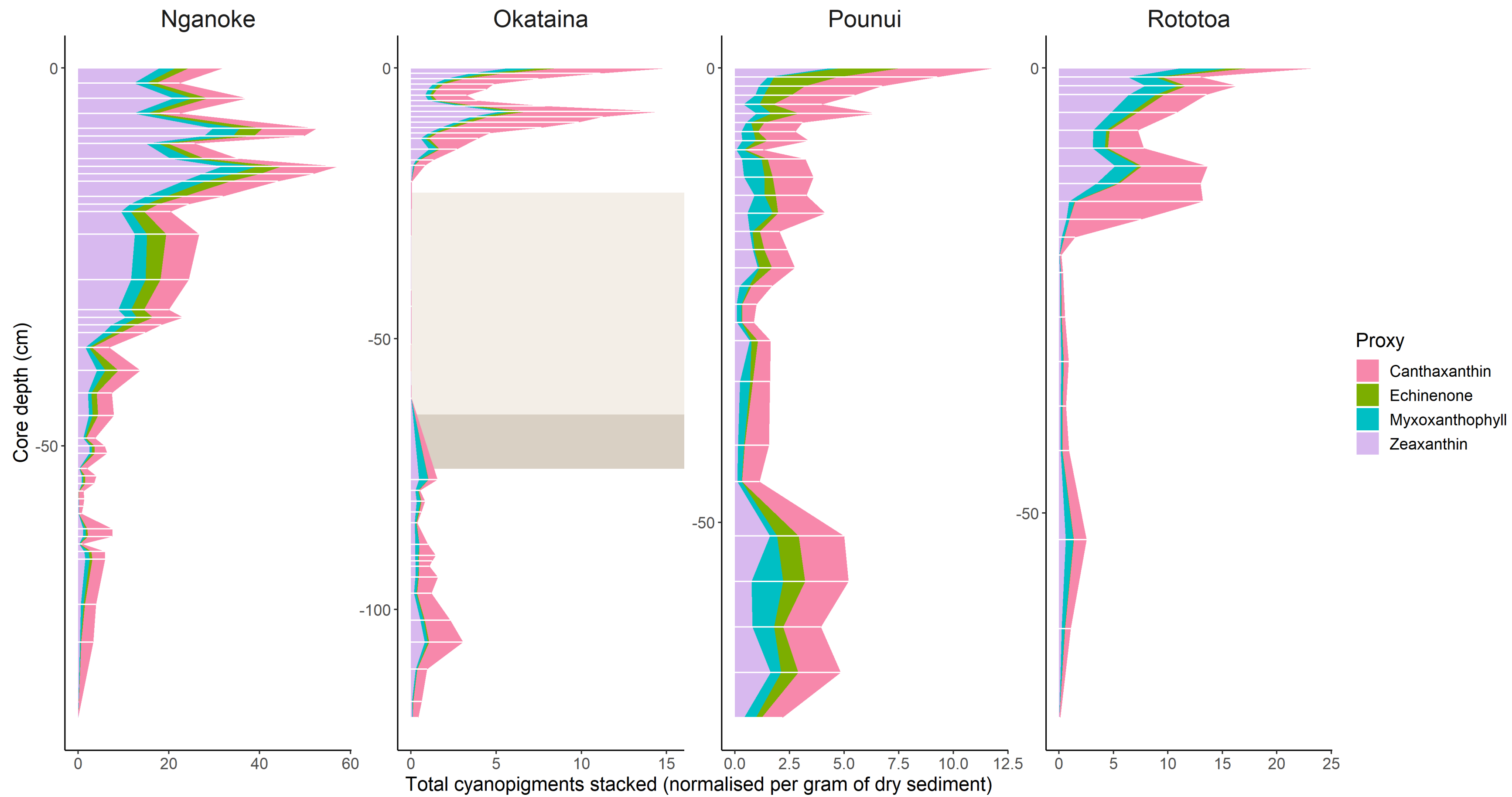

Figure D.15: Stacked view of the individual cyanopigments to visualise the contribution of each cyanopigment to total cyanopigment. White horizontal lines indicate sub-samples depths for each lake sediment core.

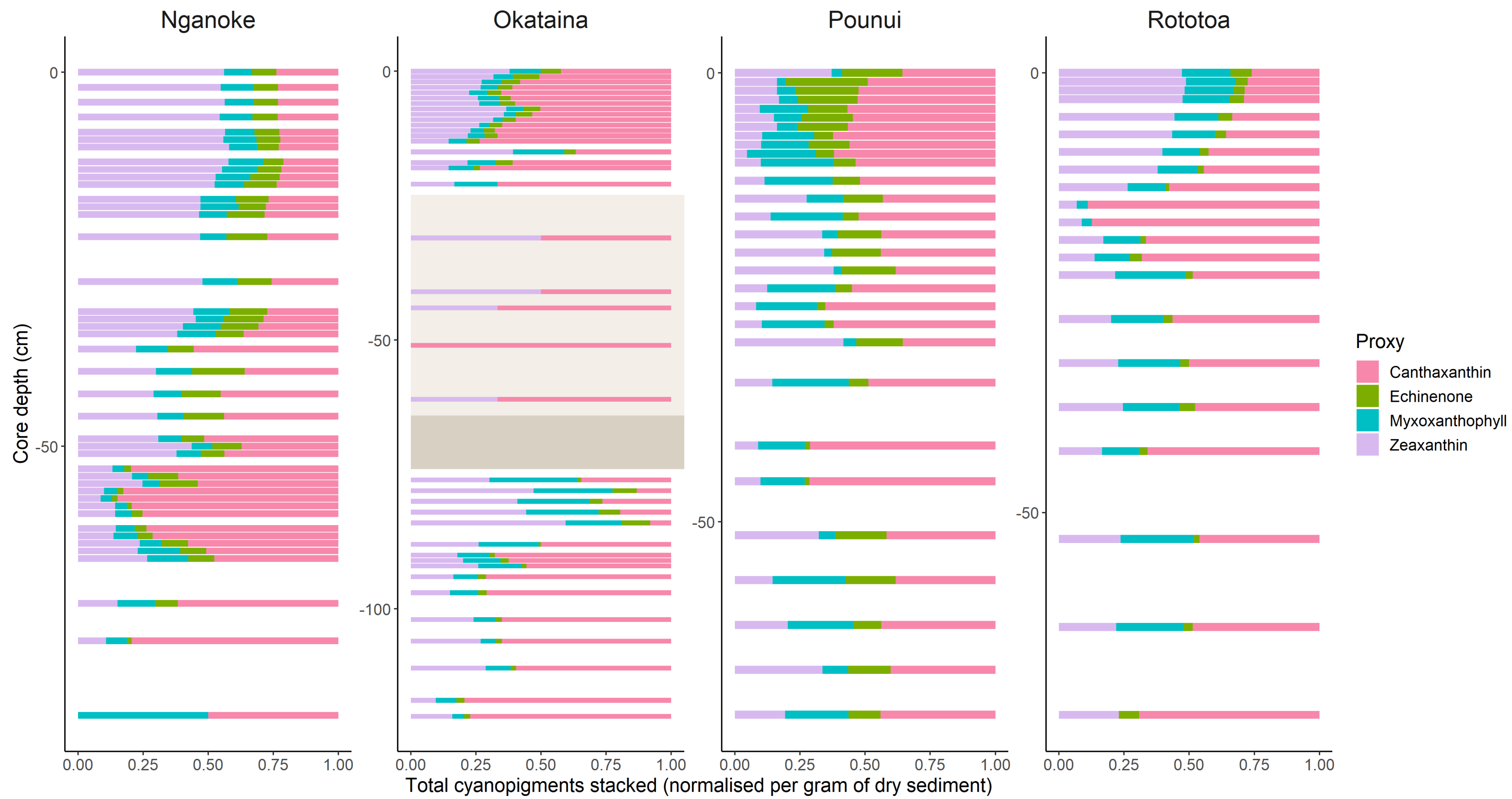

Figure D.16: Percent stacked barplots showing the relative contribution of individual pigments to total cyanopigments.

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Figure D.17: Selection of the rudd alignment and position of the primers and probe. The alignment was realised on 87 sequences, and identical sequences from the same species were removed for this figure. Rudd is set as the reference sequence compared to all other sequences. Dots indicate identical nucleotides at the same position as the reference sequence, while nucleotides are indicated and color-coded for all disagreements.

Table D.17: Details of the sampling sites.

Lake	Sampling date	Location	Site	Depth (m)	Easting	Northing
Pounui	4/12/2018	Mid-lake	1	6	175.1148	-41.3445
			2	9.1	175.1124	-41.3445
			3	9.8	175.1086	-41.3437
			4	7.3	175.1172	-41.3452
			5	7.5	175.1133	-41.3465
			6	8.4	175.1154	-41.3462
			7	5.3	175.1118	-41.3471
		Near-shore	8	2.1	175.1181	-41.3431
			9	2.5	175.1127	-41.3423
			10	1.4	175.1097	-41.3431
			11	1.8	175.1068	-41.3431
			12	2	175.1094	-41.3474
			13	2.1	175.1137	-41.3472
			14	1.5	175.1184	-41.3456
Tomarata	18/11/2018	Near-shore	1	2.8	174.6474	-36.19243
			2	2.7	174.6482	-36.1939
			3	3.7	174.6504	-36.19547
			4	2.9	174.6531	-36.19456
			5	3.1	174.6515	-36.1927
			6	5.1	174.6503	-36.19234
			7	4	174.6482	-36.19171
		Mid-lake	8	5.4	174.648	-36.19256
			9	5.1	174.6487	-36.19349
			10	4.7	174.6503	-36.19442
			11	4.7	174.6514	-36.19446
			12	4.8	174.6505	-36.19347
			13	5.6	174.6488	-36.19244
			14	5.2	174.6494	-36.19338
Waitawa	13/10/2019	Mid-lake	1	6.5	178.3612	-54.89617
			2	6.5	178.3557	-54.89575
			3	6.8	178.346	-54.89537
			4	6.9	178.335	-54.89545
			5	7	178.3261	-54.89558
			6	4.5	178.318	-54.89443
			7	6.5	178.332	-54.8938
		Near-shore	8	2.5	178.3476	-54.89295
			9	1.8	178.3125	-54.89401
			10	2.9	178.3227	-54.89625
			11	2.7	178.3633	-54.89786
			12	2.6	178.3671	-54.89863
			13	2.2	178.3772	-54.89609
			14	2.7	178.3874	-54.89647

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Table D.18: List of fish species against which the rudd assay has been tested. The method column indicates how the specificity was checked: qPCR on tissue = fish tissue extracts were directly tested on a qPCR machine; metabarcoding and qPCR = samples of known species composition (metabarcoding) were also tested with the rudd assay and returned a negative signal. The full list of species tested in the metabarcoding samples is in Table D.19

Method	**Fish common name**	**Fish latin name**
qPCR on tissue	Brown bullhead catfish	*Ameiurus nebulosus*
	Goldfish	*Carassius auratus*
	Koi carp	*Cyprinus rubrofiscus*
	Gambusia	*Gambusia affinis*
	European perch	*Perca fluviatilis*
	Tench	*Tinca tinca*
Metabarcoding & qPCR	Brown bullhead catfish	*Ameiurus nebulosus*
	Short-finned eel	*Anguilla australis*
	Goldfish	*Carassius auratus*
	Gambusia	*Gambusia affinis*
	Skipjack tuna	*Katsuwonus pelamis*
	Rainbow trout	*Oncorhynchus mykiss*
	Smelt	*Retropinna retropinna*
	Brown trout	*Salmo trutta*
	Tench	*Tinca tinca*

Table D.19: Details of the metabarcoding samples on which the rudd assay was tested. Fish amplicon sequence variants were summed by species.

Collection date	**Latitude**	**Longitude**	**Volume filtered (mL)**	**Rudd qPCR**	**Fish detected using metabarcoding (number of reads)**
27/11/2019	-38.0317	176.3514	300	Negative	*Ameiurus nebulosus* (58); *Carassius auratus* (841); *Retropinna retropinna* (30); *Gobiomorphus* (2309); *Gambusia affinis* (14)
27/11/2019	-38.0317	176.3514	240	Negative	*Ameiurus nebulosus* (99); *Carassius auratus* (1748); *Retropinna retropinna* (93); *Gobiomorphus* (932); *Gambusia affinis* (36)
28/08/2019	-38.1286	176.2501	130	Negative	*Ameiurus nebulosus* (220); *Katsuwonus pelamis* (31); *Oncorhynchus mykiss* (322); *Oncorhynchus mykiss* (547); *Salmo trutta* (53); *Retropinna retropinna* (119); *Retropinna retropinna* (6116); *Gobiomorphus* (69); *Gobiomorphus* (20463); *Gambusia affinis* (126)
16/12/2019	-39.0113	174.2434	300	Positive	*Scardinius erythrophthalmus* (82); *Anguilla australis* (1151); *Anguilla dieffenbachii* (383); *Anguilla australis* (786); *Perca fluviatilis* (374); *Gobiomorphus* (6947)
16/12/2019	-39.0113	174.2434	480	Positive	*Scardinius erythrophthalmus* (52); *Anguilla australis* (450); *Anguilla dieffenbachii* (21); *Perca fluviatilis* (80); *Gobiomorphus* (1183)

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Table D.19: Details of the metabarcoding samples on which the rudd assay was tested. Fish amplicon sequence variants were summed by species. (Continued)

16/12/2019	-39.0113	174.2434	480	Positive	*Carassius auratus* (52); *Scardinius erythrophthalmus* (94); *Anguilla australis* (193); *Perca fluviatilis* (108); *Gobiomorphus* (22)
16/12/2019	-39.0113	174.2434	600	Positive	*Scardinius erythrophthalmus* (26); *Anguilla australis* (215); *Perca fluviatilis* (421); *Gobiomorphus* (517)
16/12/2019	-39.0113	174.2434	480	Positive	*Carassius auratus* (40); *Scardinius erythrophthalmus* (13); *Anguilla australis* (72); *Anguilla australis* (296); *Perca fluviatilis* (28); *Gobiomorphus* (56)
22/11/2019	-37.9448	175.2821	60	Positive	*Anguilla australis* (45); *Scardinius erythrophthalmus* (56); *Cyprinus rubrofuscus* (908); *Carassius auratus* (729); *Carassius auratus* (19841); *Ameiurus nebulosus* (13); *Cyprinus rubrofuscus* (4008); *Carassius auratus* (39244); *Scardinius erythrophthalmus* (55); *Ameiurus nebulosus* (16); *Anguilla australis* (46); *Gobiomorphus* (26)
22/11/2019	-37.9279	175.2883	110	Positive	*Gambusia affinis* (100); *Gambusia affinis* (100); *Anguilla australis* (39); *Scardinius erythrophthalmus* (548); *Carassius auratus* (4352); *Ameiurus nebulosus* (380); *Carassius auratus* (25701); *Scardinius erythrophthalmus* (2827); *Ameiurus nebulosus* (157); *Anguilla australis* (59); *Gambusia affinis* (235)

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Table D.19: Details of the metabarcoding samples on which the rudd assay was tested. Fish amplicon sequence variants were summed by species. (Continued)

22/11/2019	-37.9606	175.2949	60	Positive	*Anguilla australis* (4); *Carassius auratus* (304); *Carassius auratus* (1904); *Ameiurus nebulosus* (151); *Cyprinus rubrofuscus* (41807); *Carassius auratus* (36974); *Scardinius erythrophthalmus* (202); *Retropinna retropinna* (2); *Ameiurus nebulosus* (20); *Gobiomorphus* (88); *Gambusia affinis* (9)
22/11/2019	-37.9258	175.2917	75	Positive	*Scardinius erythrophthalmus* (18); *Carassius auratus* (440); *Ameiurus nebulosus* (101); *Carassius auratus* (2960); *Scardinius erythrophthalmus* (130); *Ameiurus nebulosus* (11); *Anguilla australis* (4); *Gambusia affinis* (5)
22/11/2019	-37.9548	175.2938	70	Positive	*Gambusia affinis* (9); *Gambusia affinis* (9); *Anguilla australis* (40); *Scardinius erythrophthalmus* (54); *Cyprinus rubrofuscus* (89); *Carassius auratus* (15332); *Ameiurus nebulosus* (37); *Carassius auratus* (331); *Cyprinus rubrofuscus* (1502); *Carassius auratus* (107434); *Scardinius erythrophthalmus* (289); *Anguilla australis* (297); *Gambusia affinis* (83)

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Table D.19: Details of the metabarcoding samples on which the rudd assay was tested. Fish amplicon sequence variants were summed by species. (Continued)

22/11/2019	-37.9497	175.3203	360	Negative	*Gambusia affinis* (14144); *Gobiomorphus* (194); *Gobiomorphus* (22142); *Gobiomorphus* (578); *Anguilla australis* (2727); *Carassius auratus* (4618); *Ameiurus nebulosus* (19); *Carassius auratus* (393); *Retropinnidae* (424); *Ameiurus nebulosus* (830); *Anguilla australis* (78); *Gobiomorphus* (857); *Gobiomorphus* (293); *Gambusia affinis* (801)
22/11/2019	-37.9274	175.2932	88	Positive	*Cypriniformes* (90); *Cyprinoidei* (128); *Carassius auratus* (233); *Carassius auratus* (44171); *Cyprinoidei* (19); *Cypriniformes* (267); *Carassius auratus* (1260); *Cyprinidae* (46); *Carassius auratus* (1596); *Scardinius erythrophthalmus* (125); *Ameiurus nebulosus* (2963)
22/11/2019	-37.9511	175.3206	390	Negative	*Gambusia affinis* (977); *Gobiomorphus* (11028); *Gobiomorphus* (297); *Anguilla australis* (545); *Carassius auratus* (5618); *Ameiurus nebulosus* (531); *Carassius auratus* (4762); *Retropinnidae* (348); *Retropinnidae* (416); *Ameiurus nebulosus* (476); *Anguilla australis* (334); *Anguilla australis* (110); *Retropinna retropinna* (799); *Gobiomorphus* (5969); *Gobiomorphus* (3558); *Gambusia affinis* (866)

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Table D.19: Details of the metabarcoding samples on which the rudd assay was tested. Fish amplicon sequence variants were summed by species. (Continued)

22/11/2019	-37.9496	175.3202	480	Negative	*Gambusia affinis* (236); *Gambusia affinis* (236); *Gobiomorphus* (2394); *Anguilla australis* (110); *Scardinius erythrophthalmus* (7); *Carassius auratus* (830); *Ameiurus nebulosus* (302); *Carassius auratus* (3425); *Retropinnidae* (73); *Ameiurus nebulosus* (98); *Anguilla australis* (190); *Retropinna retropinna* (1175); *Gobiomorphus* (4746); *Gobiomorphus* (5492); *Gambusia affinis* (1053

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Table D.19: Details of the metabarcoding samples on which the rudd assay was tested. Fish amplicon sequence variants were summed by species. (Continued)

20/02/2020	-37.7927	175.2913	500	Positive	*Gambusia affinis* (13); *Galaxias maculatus* (28); *Mugil cephalus* (31); *Mugil cephalus* (68); *Gobiomorphus* (482); *Cheimarrichthys fosteri* (50); *Salmo trutta* (16); *Anguilla australis* (217); *Scardinius erythrophthalmus* (65); *Cyprinus rubrofuscus* (324); *Carassius auratus* (40); *Cheimarrichthys fosteri* (195); *Cyprinus rubrofuscus* (1326); *Carassius auratus* (184); *Scardinius erythrophthalmus* (407); *Retropinnidae* (229); *Retropinna retropinna* (214); *Ameiurus nebulosus* (17); *Anguilla reinhardtii* (43); *Anguilla australis* (624); *Anguilla dieffenbachii* (168); *Salmo trutta* (35); *Retropinna retropinna* (745); *Retropinna retropinna* (1002); *Gobiomorphus* (949); *Gobiomorphus* (264); *Gambusia affinis* (100); *Mugil cephalus* (113); *Galaxias maculatus* (104)

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Table D.19: Details of the metabarcoding samples on which the rudd assay was tested. Fish amplicon sequence variants were summed by species. (Continued)

19/02/2020	-37.7927	175.2913	500	Positive	*Mugil cephalus* (42); *Gobiomorphus* (408); *Cheimarrichthys fosteri* (110); *Anguilla australis* (94); *Scardinius erythrophthalmus* (23); *Cyprinus rubrofuscus* (330); *Carassius auratus* (33); *Cheimarrichthys fosteri* (262); *Cyprinus rubrofuscus* (1619); *Carassius auratus* (304); *Scardinius erythrophthalmus* (119); *Retropinnidae* (120); *Retropinna retropinna* (154); *Anguilla australis* (463); *Anguilla dieffenbachii* (70); *Salmo trutta* (74); *Retropinna retropinna* (1500); *Retropinna retropinna* (1524); *Retropinna retropinna* (205); *Gobiomorphus* (2205); *Gobiomorphus* (82); *Mugil cephalus* (291); *Galaxias maculatus* (70); *Galaxias maculatus* (67); *Galaxias maculatus* (55); *Galaxias maculatus* (163); *Galaxias maculatus* (64)

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Table D.19: Details of the metabarcoding samples on which the rudd assay was tested. Fish amplicon sequence variants were summed by species. (Continued)

20/02/2020	-37.7927	175.2913	500	Positive	*Mugil cephalus* (181); *Mugil cephalus* (251); *Gobiomorphus* (2167); *Gobiomorphus cotidianus* (81); *Cheimarrichthys fosteri* (493); *Salmo trutta* (148); *Anguilla australis* (551); *Anguilla* (58); *Cyprinus rubrofuscus* (1525); *Carassius auratus* (281); *Cheimarrichthys fosteri* (23); *Cyprinus rubrofuscus* (570); *Carassius auratus* (48); *Scardinius erythrophthalmus* (30); *Retropinnidae* (549); *Retropinna retropinna* (1035); *Anguilla australis* (195); *Salmo trutta* (72); *Retropinna retropinna* (542); *Retropinna retropinna* (457); *Gobiomorphus* (650); *Gobiomorphus* (131); *Mugil cephalus* (107); *Mugil cephalus* (25); *Galaxias maculatus* (16)
11/02/2020	-41.1596	172.9975	600	Negative	*Carassius auratus* (5397); *Tinca tinca* (4278); *Anguilla australis* (533); *Anguilla australis* (213)

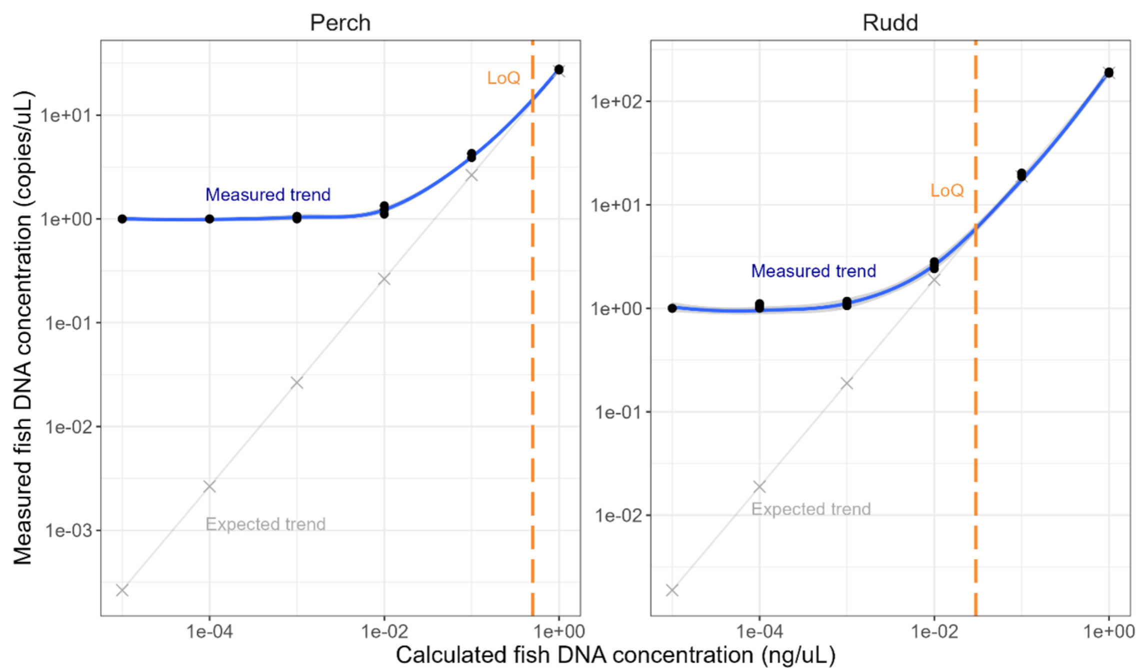

Figure D.18: Limit of quantification (LoQ) obtained by measuring diluted perch and rudd DNA extracts with the perch-rudd duplex assay on a droplet digital PCR system. Raw extracts were diluted to obtain a starting concentration of 1 ng/µL, and further diluted (0.1 ng/µL, 0.01 ng/µL, 0.001 ng/µL, 0.0001 ng/µL, 0.00001 ng/µL). Dilutions were run in triplicate. Calculated concentrations were plotted against measured concentrations (log scale for both axis), with the grey line representing the expected trend, and the blue line representing the measured trend using a loess function. The LoQ was set as the point where the measured trend diverged from the expected trend: 13 copies/µL for perch and 5 copies/µL for rudd. Measured concentrations are in copies/µL per ddPCR sample analysed.

Table D.20: Surface sediment geochemistry (single spot sampling from the deepest part of each lake).

	Lake Pounui	**Lake Tomarata**	**Lake Waitawa**
Date Surveyed	25/09/2018	14/11/2018	24/09/2018
Dry Weight (kg.m^{-3})	128.9	205.4	21.4
Total Produced Water (%)	86.8	86.7	97.9
Ash (g/100g dry wt)	85.1	74.5	66.1
Organic Matter (g/100g dry wt)	14.9	25.5	33.9
Organic Matter / Ash	0.18	0.34	0.51
Carbonates (g/100g dry wt)	1.1	2	8.8
Total Organic Carbon (g/100g dry wt)	0.41	9.1	17.9
Total Nitrogen (g/100g dry wt)	$<$0.13	0.85	1.95
Total Phosphorus (g/kg dry wt)	2260	888	2010
Iron (g/kg dry wt)	51700	74100	19600
Manganese (g/kg dry wt)	2750	991	497
Aluminum (g/kg dry wt)	19000	24600	19300
Calcium (g/kg dry wt)	3990	3030	4410
Lead (g/kg dry wt)	32.4	33.1	14.1
Copper (g/kg dry wt)	23	21.3	23.3
Cadmium (g/kg dry wt)	0.11	0.15	0.15
Zinc (g/kg dry wt)	107	46.5	63.3
Sulfur (g/kg dry wt)	No results	4950	9170
Grain Size $\geq$ 63 µm (%)		57.74	27.93
Grain size 2 to 63 µm (%)		42.26	72.07
Grain Size $\leq$ 2 µm (%)		0	0

Methods for eDNA normalisation to gene copies per litre and gene copies per gram (for Figure D.19):

Water content determination

Subsamples of sediment replicates were pooled for each site (0.5 g per site), transferred into pre-weighed glass vials, lyophilized (Gamma 1–16 LSC freeze-dryer; Martin Christ Gefriertrocknungsanlagen, Osterode am Harz, Germany), and re-weighed. The water content was determined using the following formula:

$$Water\ content\ of\ sediment = \frac{(wet\ weight\ (g) - dry\ weight\ (g))}{(wet\ weight\ (g))} \tag{D.1}$$

Droplet digital PCR normalisation

Fish gene copy concentrations were then standardized to fish gene copy numbers per gram of dry sediment or per liter of water using the following formulas:

$$Gene\ copies\ (sediment) = \frac{ddPCR\frac{22.45\,\mu L}{6\,\mu L} \times 100\,\mu L}{sediment\ sample\ weight \times (1 - water\ content)} \tag{D.2}$$

$$Gene\ copies\ (water) = \frac{ddPCR\frac{22.45\,\mu L}{6\,\mu L} \times 100\,\mu L}{volume\ filtered} \tag{D.3}$$

where gene copies = fish gene concentrations (12S rRNA gene for perch and 16S rRNA gene for rudd), ddPCR = concentration of gene copies per µL, 22.45 µL = total reaction volume, 6 µL = volume of DNA template added to the PCR reaction, 100 µL = volume of DNA eluted after extraction, sed. weight = exact weight of each sample extracted for DNA (3 g), water content = water content of the core subsample in % from Equation (1), and volume filtered = 500 mL.

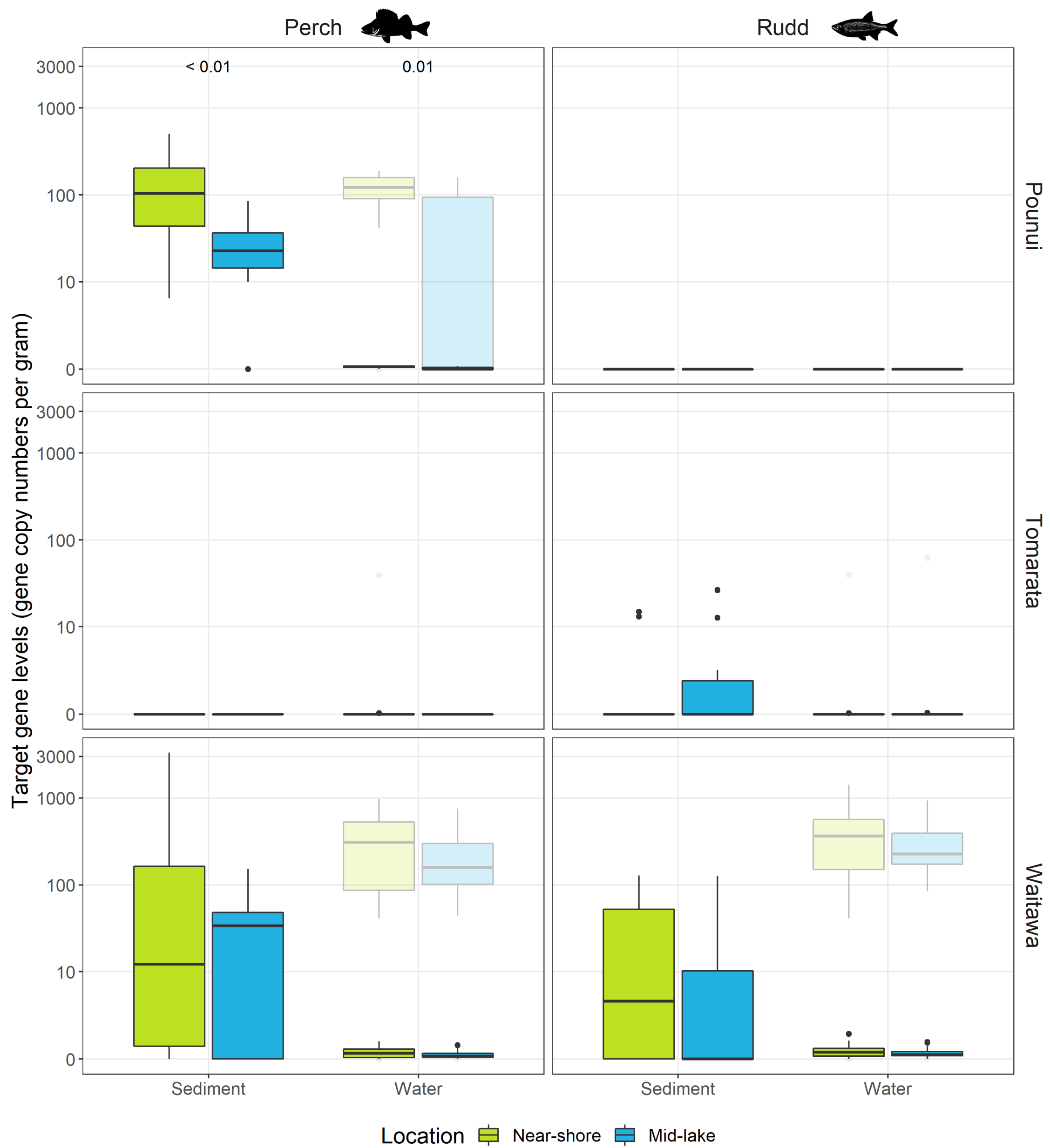

Figure D.19: Gene copy levels for each fish species per lake (12S rDNA for perch and 16S rDNA for rudd), separated by sampling method (sediment and water) and sampling location (near-shore and mid-lake). Gene levels in water were plotted based on an equivalence of 1 g of water and 1 mL of water. Shaded boxplots indicate eDNA levels in water multiplied by 1,000 (concentrations per L) to visualise difference across locations. Target gene levels are plotted on a log10 scale with null values transformed to one.

Table D.21: Ranking of the 16 model variants tested for perch eDNA in Lake Pounui made by the PRESENCE software. The best model has the lower Akaike Information Criterion (AIC) and is displayed on the first row (highlighted in blue). Parameters are constant unless indicated otherwise. Abbreviations as follow: psi = Ψ (large-scale occupancy), theta = ϑ (small-scale occupancy), p = detection probability, m = sampling method (water or sediment), Littoral = location (near-shore or mid-lake). M*Littoral indicates a method and location interaction.

Model	AIC	deltaAIC	AIC wgt	Model Likelihood	no.Par.	-2*LogLike
psi,theta(.),p(m*Littoral)	47.12	0.00	0.9480	1.0000	5	37.12
psi,theta(.),p(Littoral)	53.44	6.32	0.0402	0.0424	3	47.44
psi(Littoral),theta(.),p(m*Littoral)	56.82	9.70	0.0074	0.0078	5	46.82
psi,theta(.),p(m)	58.72	11.60	0.0029	0.0030	4	50.72
psi,theta(Littoral),p(m*Littoral)	61.00	13.88	0.0009	0.0010	5	51.00
psi,(Littoral),theta(.),p(Littoral)	63.15	16.03	0.0003	0.0003	3	57.15
psi,theta(.),p(.)	64.19	17.07	0.0002	0.0002	3	58.19
psi(Littoral),theta(.),p(m)	68.43	21.31	0.0000	0.0000	4	60.43
psi,theta(Littoral),p(Littoral)	69.63	22.51	0.0000	0.0000	3	63.63
psi,theta(Littoral),p(m)	70.31	23.19	0.0000	0.0000	4	62.31
psi(Littoral),theta(Littoral),p(m*Littoral)	70.71	23.59	0.0000	0.0000	5	60.71
psi(Littoral),theta(.),p(.)	73.90	26.78	0.0000	0.0000	3	67.90
psi,theta(Littoral),p(.)	77.09	29.97	0.0000	0.0000	3	71.09
psi(Littoral),theta(Littoral),p(Littoral)	79.34	32.22	0.0000	0.0000	3	73.34
psi(Littoral),theta(Littoral),p(m)	80.01	32.89	0.0000	0.0000	4	72.01
psi(Littoral),theta(Littoral),p(.)	86.79	39.67	0.0000	0.0000	3	80.79

Table D.22: Ranking of the 16 model variants tested for rudd eDNA in Lake Tomarata made by the PRESENCE software. The best model has the lower Akaike Information Criterion (AIC) and is displayed on the first row (highlighted in blue). Parameters are constant unless indicated otherwise. Abbreviations as follow: psi = Ψ (large-scale occupancy), theta = ϑ (small-scale occupancy), p = detection probability, m = sampling method (water or sediment), Littoral = location (near-shore or mid-lake). M*Littoral indicates a method and location interaction.

Model	AIC	deltaAIC	AIC wgt	Model Likelihood	no.Par.	-2*LogLike
psi,theta(.),p(m)	51.51	0.00	0.1993	1.0000	4	43.51
psi,theta(.),p(.)	51.93	0.42	0.1616	0.8106	3	45.93
psi,theta(Littoral),p(m)	52.78	1.27	0.1056	0.5299	4	44.78
psi,theta(.),p(m*Littoral)	52.89	1.38	0.1000	0.5016	5	42.89
psi,theta(Littoral),p(.)	53.68	2.17	0.0674	0.3379	3	47.68
psi(Littoral),theta(.),p(m)	54.11	2.60	0.0543	0.2725	4	46.11
psi,theta(Littoral),p(m*Littoral)	54.14	2.63	0.0535	0.2685	5	44.14
psi,theta(Littoral),p(Littoral)	54.32	2.81	0.0489	0.2454	3	48.32
psi(Littoral),theta(.),p(m*Littoral)	54.66	3.15	0.0413	0.2070	5	44.66
psi(Littoral),theta(.),p(.)	54.72	3.21	0.0400	0.2009	3	48.72
psi,theta(.),p(Littoral)	54.77	3.26	0.0391	0.1959	3	48.77
psi(Littoral),theta(.),p(Littoral)	55.56	4.05	0.0263	0.1320	3	49.56
psi(Littoral),theta(Littoral),p(m)	55.99	4.48	0.0212	0.1065	4	47.99
psi(Littoral),theta(Littoral),p(Littoral)	56.41	4.90	0.0172	0.0863	3	50.41
psi(Littoral),theta(Littoral),p(m*Littoral)	57.06	5.55	0.0124	0.0623	5	47.06
psi(Littoral),theta(Littoral),p(.)	57.16	5.65	0.0118	0.0593	3	51.16

Table D.23: Ranking of the 16 model variants tested for perch eDNA in Lake Waitawa made by the PRESENCE software. The best model has the lower Akaike Information Criterion (AIC) and is displayed on the first row (highlighted in blue). Parameters are constant unless indicated otherwise. Abbreviations as follow: psi = Ψ (large-scale occupancy), theta = ϑ (small-scale occupancy), p = detection probability, m = sampling method (water or sediment), Littoral = location (near-shore or mid-lake). M*Littoral indicates a method and location interaction.

Model	AIC	deltaAIC	AIC wgt	Model Likelihood	no.Par.	-2*LogLike
psi,theta(.),p(m)	63.57	0.00	0.6151	1.0000	4	55.57
psi,theta(.),p(m*Littoral)	65.46	1.89	0.2391	0.3887	5	55.46
psi,theta(.),p(.)	66.69	3.12	0.1293	0.2101	3	60.69
psi(Littoral),theta(.),p(m)	73.27	9.70	0.0048	0.0078	4	65.27
psi,theta(.),p(Littoral)	73.91	10.34	0.0035	0.0057	3	67.91
psi(Littoral),theta(.),p(Littoral)	73.91	10.34	0.0035	0.0057	3	67.91
psi(Littoral),theta(.),p(m*Littoral)	75.16	11.59	0.0019	0.0030	5	65.16
psi,theta(Littoral),p(m)	75.99	12.42	0.0012	0.0020	4	67.99
psi(Littoral),theta(.),p(.)	76.39	12.82	0.0010	0.0016	3	70.39
psi,theta(Littoral),p(m*Littoral)	77.95	14.38	0.0005	0.0008	5	67.95
psi,theta(Littoral),p(.)	79.92	16.35	0.0002	0.0003	3	73.92
psi(Littoral),theta(Littoral),p(m)	85.69	22.12	0.0000	0.0000	4	77.69
psi(Littoral),theta(Littoral),p(m*Littoral)	87.65	24.08	0.0000	0.0000	5	77.65
psi(Littoral),theta(Littoral),p(.)	89.62	26.05	0.0000	0.0000	3	83.62
psi,theta(Littoral),p(Littoral)	90.10	26.53	0.0000	0.0000	3	84.10
psi(Littoral),theta(Littoral),p(Littoral)	99.81	36.24	0.0000	0.0000	3	93.81

Table D.24: Ranking of the 16 model variants tested for rudd eDNA in Lake Waitawa made by the PRESENCE software. The best model has the lower Akaike Information Criterion (AIC) and is displayed on the first row (highlighted in blue). Parameters are constant unless indicated otherwise. Abbreviations as follow: psi = Ψ (large-scale occupancy), theta = ϑ (small-scale occupancy), p = detection probability, m = sampling method (water or sediment), Littoral = location (near-shore or mid-lake). M*Littoral indicates a method and location interaction.

Model	AIC	deltaAIC	AIC wgt	Model Likelihood	no.Par.	-2*LogLike
psi,theta(.),p(m)	60.65	0.00	0.5124	1.0000	4	52.65
psi,theta(.),p(m*Littoral)	60.79	0.14	0.4778	0.9324	5	50.79
psi(Littoral),theta(.),p(m)	70.36	9.71	0.0040	0.0078	4	62.36
psi(Littoral),theta(.),p(m*Littoral)	70.50	9.85	0.0037	0.0073	5	60.50
psi,theta(Littoral),p(m)	73.02	12.37	0.0011	0.0021	4	65.02
psi,theta(Littoral),p(m*Littoral)	74.11	13.46	0.0006	0.0012	5	64.11
psi,theta(.),p(Littoral)	76.31	15.66	0.0002	0.0004	3	70.31
psi,theta(.),p(.)	76.33	15.68	0.0002	0.0004	3	70.33
psi(Littoral),theta(Littoral),p(m)	82.72	22.07	0.0000	0.0000	4	74.72
psi(Littoral),theta(Littoral),p(m*Littoral)	83.81	23.16	0.0000	0.0000	5	73.81
psi(Littoral),theta(.),p(Littoral)	86.01	25.36	0.0000	0.0000	3	80.01
psi(Littoral),theta(.),p(.)	86.03	25.38	0.0000	0.0000	3	80.03
psi,theta(Littoral),p(.)	90.87	30.22	0.0000	0.0000	3	84.87
psi,theta(Littoral),p(Littoral)	92.50	31.85	0.0000	0.0000	3	86.50
psi(Littoral),theta(Littoral),p(.)	100.57	39.92	0.0000	0.0000	3	94.57
psi(Littoral),theta(Littoral),p(Littoral)	102.20	41.55	0.0000	0.0000	3	96.20

Table D.25: Simulation for minimum sampling effort for sediment samples – worst-case scenario (p=0.21). The number of sites (S) and replicates (K) were increased until the percentage of empty histories was 0% (no false negative detection) and until the error rates of the estimator were less than 5% (see values in bold).

Evaluation of design K = 5 S = 6 (TS = 30)
estimator performance (excl empty histories)
psi: bias = -0.0968 var = +0.0276 MSE = **+0.0369**
p: bias = +0.0319 var = +0.0084 MSE = **+0.0094**
covar = -0.0072 critA = +0.0464 critD = +2.964e-04
estimator performance (excl also histories leading to boundary estimates)
psi: bias = -0.2228 var = +0.0354 MSE = +0.0850
p: bias = +0.0646 var = +0.0092 MSE = +0.0133
covar = -0.0093 critA = +0.0984 critD = +1.048e-03
empty histories = **0.0%**
boundary estimates = 56.5%

Table D.26: Simulation for minimum sampling effort for water samples – worst-case scenario (p=0.07). The number of sites (S) and replicates (K) were increased until the percentage of empty histories was 0% (no false negative detection) and until the error rates of the estimator were less than 5% (see values in bold).

Evaluation of design K = 8 S = 20 (TS = 160)
estimator performance (excl empty histories)
psi: bias = -0.1218 var = +0.0335 MSE = **+0.0483**
p: bias = +0.0139 var = +0.0010 MSE = **+0.0011**
covar = -0.0037 critA = +0.0495 critD = +4.207e-05
estimator performance (excl also histories leading to boundary estimates)
psi: bias = -0.2734 var = +0.0338 MSE = +0.1085
p: bias = +0.0297 var = +0.0012 MSE = +0.0020
covar = -0.0039 critA = +0.1106 critD = +2.066e-04v empty histories = **0.0%**
boundary estimates = 55.5%

Table D.27: Simulation for minimum sampling effort for near-shore sediment samples in Lake Pounui (p=0.99). The simulation was run at p = 0.97 due to the function not being able to handle higher detection probabilities. The number of sites (S) and replicates (K) were increased until the percentage of empty histories was 0% (no false negative detection) and until the error rates of the estimator were less than 5% (see values in bold).

Evaluation of design K = 2 S = 2 (TS = 4)
estimator performance (excl empty histories)
psi: bias = -0.0010 var = +0.0005 MSE = **+0.0005**
p: bias = +0.0022 var = +0.0067 MSE = **+0.0067**
covar = -0.0000 critA = +0.0071 critD = +3.170e-06
estimator performance (excl also histories leading to boundary estimates)
psi: bias = -0.0011 var = +0.0005 MSE = +0.0005
p: bias = +0.0300 var = +0.0000 MSE = +0.0009
covar = +0.0000 critA = +0.0014 critD = +4.790e-07
empty histories = **0.0%**
boundary estimates = 10.8%

Table D.28: Simulation for minimum sampling effort for perch eDNA using water samples in Lake Waitawa (p=0.89). The number of sites (S) and replicates (K) were increased until the percentage of empty histories was 0% (no false negative detection) and until the error rates of the estimator were less than 5% (see values in bold).

Evaluation of design K = 2 S = 2 (TS = 4)
estimator performance (excl empty histories)
psi: bias = -0.0102 var = +0.0050 MSE = **+0.0051**
p: bias = +0.0113 var = +0.0209 MSE = **+0.0210**
covar = -0.0010 critA = +0.0261 critD = +1.057e-04
estimator performance (excl also histories leading to boundary estimates)
psi: bias = -0.0156 var = +0.0076 MSE = +0.0078
p: bias = +0.1100 var = +0.0000 MSE = +0.0121
covar = +0.0000 critA = +0.0199 critD = +9.465e-05
empty histories = **0.0%**
boundary estimates = 35.1%

Table D.29: Simulation for minimum sampling effort of rudd eDNA using water samples in Lake Waitawa (p=0.93). The number of sites (S) and replicates (K) were increased until the percentage of empty histories was 0% (no false negative detection) and until the error rates of the estimator were less than 5% (see values in bold).

Evaluation of design K = 2 S = 2 (TS = 4)
estimator performance (excl empty histories)
psi: bias = -0.0037 var = +0.0018 MSE = +0.0018
p: bias = +0.0038 var = +0.0150 MSE = +0.0150
covar = -0.0002 critA = +0.0168 critD = +2.728e-05
estimator performance (excl also histories leading to boundary estimates)
psi: bias = -0.0048 var = +0.0024 MSE = +0.0024
p: bias = +0.0700 var = +0.0000 MSE = +0.0049
covar = +0.0000 critA = +0.0073 critD = +1.183e-05
empty histories = 0.0%
boundary estimates = 24.4%

Table D.30: Comparison of detection of perch DNA using droplet digital PCR (ddPCR) in the sediment samples of Lake Pounui with the PowerSoil kit (0.25g of sediment, columns Binary (0.25g) and RawConc (0.25g)) and with the improved DNA extraction method from Thomson-Laing et al. (2022) (c. 3g of sediment, Binary (3g) and RawConc (3g)). Binary refers to presence/absence of detection and RawConc refers to the raw ddPCR concentration (gene copy numbers per microliter). The replicates where both DNA extraction methods detected perch sedDNA are highlighted in blue.

Lake	**Sample Type**	**Site**	**Replicate**	**Binary (0.25g)**	**RawConc (0.25g)**	**Binary (3g)**	**RawConc (3g)**
Pounui	Sediment	1	A	0	0	1	0.17537
Pounui	Sediment	1	B	0	0	1	0.130996
Pounui	Sediment	2	A	0	0	1	0.453754
Pounui	Sediment	2	B	0	0	1	0.110571
Pounui	Sediment	3	A	0	0	1	0.114716
Pounui	Sediment	3	B	0	0	1	0.118007
Pounui	Sediment	4	A	0	0	0	0
Pounui	Sediment	4	B	0	0	1	0.055195
Pounui	Sediment	5	A	0	0	0	0
Pounui	Sediment	5	B	1	0.0936	1	0.17008
Pounui	Sediment	6	A	0	0	1	0.055142
Pounui	Sediment	6	B	0	0	1	0.08136
Pounui	Sediment	7	A	0	0	1	0.052818
Pounui	Sediment	7	B	0	0	1	0.245246
Pounui	Sediment	8	A	0	0	1	0.204612
Pounui	Sediment	8	B	0	0	1	0.208114
Pounui	Sediment	9	A	1	0.0795	1	0.467441
Pounui	Sediment	9	B	0	0	1	1.883944
Pounui	Sediment	10	A	0	0	1	0.262412
Pounui	Sediment	10	B	0	0	1	0.534346
Pounui	Sediment	11	A	0	0	1	0.104214
Pounui	Sediment	11	B	0	0	1	0.154328
Pounui	Sediment	12	A	0	0	1	0.154791
Pounui	Sediment	12	B	0	0	1	0.10108
Pounui	Sediment	13	A	1	0.0796	1	0.673854
Pounui	Sediment	13	B	1	0.0814	1	1.198951
Pounui	Sediment	14	A	0	0	1	0.055186
Pounui	Sediment	14	B	0	0	1	0.192223

D.4 Chapter 5 Supplementary material

Table D.31: Main events recorded in the history of Lake Pounui.

Event	Proxy in this study	Historical records
Recent climate change – temperature increase	None	*Our atmosphere and climate* (2020)
1460 earthquake / tsunami	None	Schrader (2017) and Hancox (2005)
Māori arrival	Charcoal	Pa sites (Leach, 1981) and charcoal from other cores (Cochrane, 2017)
1844 – biggest land clearance event	Biggest charcoal signal	McQueen (1991)
1850s – land bought by British Crown	None	Most of the Wairarapa land bought by 1965 (Patterson, 1998)
1855 Earthquake	None	Exact date and magnitude, newspaper, articles, etc
Perch introduction / presence	None	Thompson-Laing unpublished, Jellyman (1976)
Brown trout presence	None	Jellyman (1990)
Rainbow trout introduction	None	Wellington Acclimatisation Society: 1938 – 20,000 rainbow fry 1955 – 60,000 rainbow fry 1956 – 10,000 rainbow fingerlings 1957 – 120,000 rainbow fingerlings 1958 – 100,000 rainbow fry
Exotic flora introduction	sedaDNA of *Elodea, Ludwigia*, sedaDNA and pollen of exotic land plants	Jellyman (1990), Lawless (1983), and Winton et al. (2011)
Repeated small-scale European catchment clearance	Charcoal, Pollen	*Retrolens - Historical Imagery Resource* (2021)

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Table D.31: Main events recorded in the history of Lake Pounui. (Continued)

European aerial topdressing (superphosphate fertiliser)	None	Early 1950s - Masterton District Library 2021
Flood gates blocking fish passage downstream of Lake Pounui	None	Mitchell (1996) and Jellyman et al. (1983)
Fish community composition	None	Jellyman (1980), Jellyman (1989a), Jellyman (1990), and Jellyman (1979), Thomson-Laing unpublished
Increase in trophic level	Bacteria, macrophytes, inc/coh, Ti/inc, K/inc	Lawless (1983), Jellyman (1990), and Winton et al. (2011)
Nematodes	18S data (Tobrilidae family)	Hedruridae family, in fish gut (final host) due to amphipod consumption (intermediary host) (Jellyman, 1989b)

Table D.32: Depth and age model of the pollen subsamples (edge core).

Depth (cm)	**Mean (AD)**	**Sigma**	**Median (AD)**	**95% HPDF (lower bound)**	**95% HPDF (higher bound)**
14	1972	2	1972	1968	1976
17	1948	6	1948	1937	1963
20	1924	11	1925	1901	1945
24	1907	13	1907	1879	1934
29	1886	13	1886	1860	1914
39	1850	4	1849	1843	1857
50	1763	36	1767	1692	1828
54	1731	40	1734	1650	1808
59	1684	46	1687	1592	1773
69	1586	52	1586	1483	1690
79	1489	49	1487	1394	1588
87	1411	39	1410	1339	1484
99	1247	50	1251	1138	1345
109	1091	59	1097	982	1194
119	895	59	892	767	1025
129	740	47	759	654	812
139	632	68	635	503	746

Table D.33: Depth and age model of the DNA subsamples (depocenter core).

Depth (cm)	**Mean (AD)**	**Sigma**	**Median (AD)**	**95% HPDF (lower bound)**	**95% HPDF (higher bound)**
0	2021	0	2021	2020	2021
0.5	2014	11	2018	1984	2022
1.5	2003	23	2012	1946	2022
2	1997	26	2007	1937	2022
2.5	1992	28	2000	1929	2022
3	1986	30	1994	1922	2022
3.5	1980	32	1987	1916	2022
4	1974	33	1980	1910	2022
4.5	1969	34	1973	1905	2022
5	1963	34	1966	1901	2022
6.5	1946	34	1944	1887	2014
7.5	1934	33	1930	1877	2004
8.5	1923	31	1917	1870	1991
9.5	1911	27	1907	1865	1971
10.5	1900	20	1897	1862	1942
11.5	1889	14	1889	1860	1917
13.5	1872	15	1870	1847	1902
15	1860	10	1857	1844	1887
17.5	1846	8	1848	1828	1855
19.5	1786	51	1795	1685	1855
22	1716	43	1719	1627	1798
26	1649	57	1653	1531	1758
31	1567	47	1572	1465	1643
36	1477	58	1476	1362	1589
41	1390	34	1392	1330	1453
46	1357	28	1348	1315	1404
51	1333	26	1323	1299	1388
56	1312	21	1306	1284	1379
61	1282	23	1282	1233	1330
66	1252	20	1248	1220	1286

Table D.34: PCR mix and cycling conditions for each primer set. The *rbc*L and *trn*L amplicons were amplified by Wilderlab Ld and exact PCR conditions are not known

Target	Amplicon length	Primer name	Sequence	Source	Reaction mix (one sample)	Cycling conditions
Bacteria 16S rRNA gene	464 bp	341F 805R	F: 5'-CCTACGGG NGGCWGCAG-3' R: 5'-GACTACHVGGG TATCTAATCC-3'	Klindworth et al. (2013)	❯1 µL of each primer at 10 µM ❯10 µL of MyFi ❯4.5 µL of DNA/RNA free water ❯2 µL of BSA ❯1 µL of DNA	❯94°C for 1.30min ❯30 cycles ❯94°C for 30 s ❯52°C for 30 s ❯72°C or 45 s ❯72°C for 5 min
Micro-eukaryotes 18S rRNA gene	260 bp	960f NSR1438	F:5'-GGCTTAATTT GACTCAACRCG-3' R: 5'-GGGCATCAC AGACCTGTTAT-3'	Gast et al. (2004) Van de Peer et al. (2000)	❯2 µL of each primer at 10 µM ❯10 µL of MyFi ❯4 µL of DNA/RNA free water ❯2 µL of DNA	❯95°C 5 min ❯30 cycles ❯95°C for 30s ❯54°C for 30s ❯72°C for 30s ❯72°C for 7min

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Table D.34: PCR mix and cycling conditions for each primer set. The *rbc*L and *trn*L amplicons were amplified by Wilderlab Ld and exact PCR conditions are not known (Continued)

Eukaryotes CO1 gene	313 bp	mlCOIintF jgHCO2198	F: 5'-GGWACWGGWTGAAC WGTWTAYCCYCC-3' R: 5'-TAIACYTCIGGRT GICCRAARAAYCA-3'	Leray et al. (2013) and Folmer et al. (1994)	➤1 µL of primer at 10 µM ➤10 µL of MyFi ➤5 µL of DNA/RNA free water ➤1 µL of DNA	➤95°C 5min ➤30 cycles ➤95°C for 30s ➤62°C for 30s ➤72°C for 30s ➤72°C for 7min
Plant - *rbc*L gene	106 bp	rbcL19b rbcLZ1	F: 5'-CTTCTTCAGG TGGAACTCCAG-3' R: 5'-GTCACCACAAACAG AGACTAAAGCAAGT-3'	Bradley et al., 2007		
Plant - p6 loop of *trn*L gene	variable length	'g''h'	F: 5'-GGGCAATC CTGAGCCAA-3' R: 5'-CCATTGAGTCT CTGCACCTATC-3'	Taberlet et al., 2007		

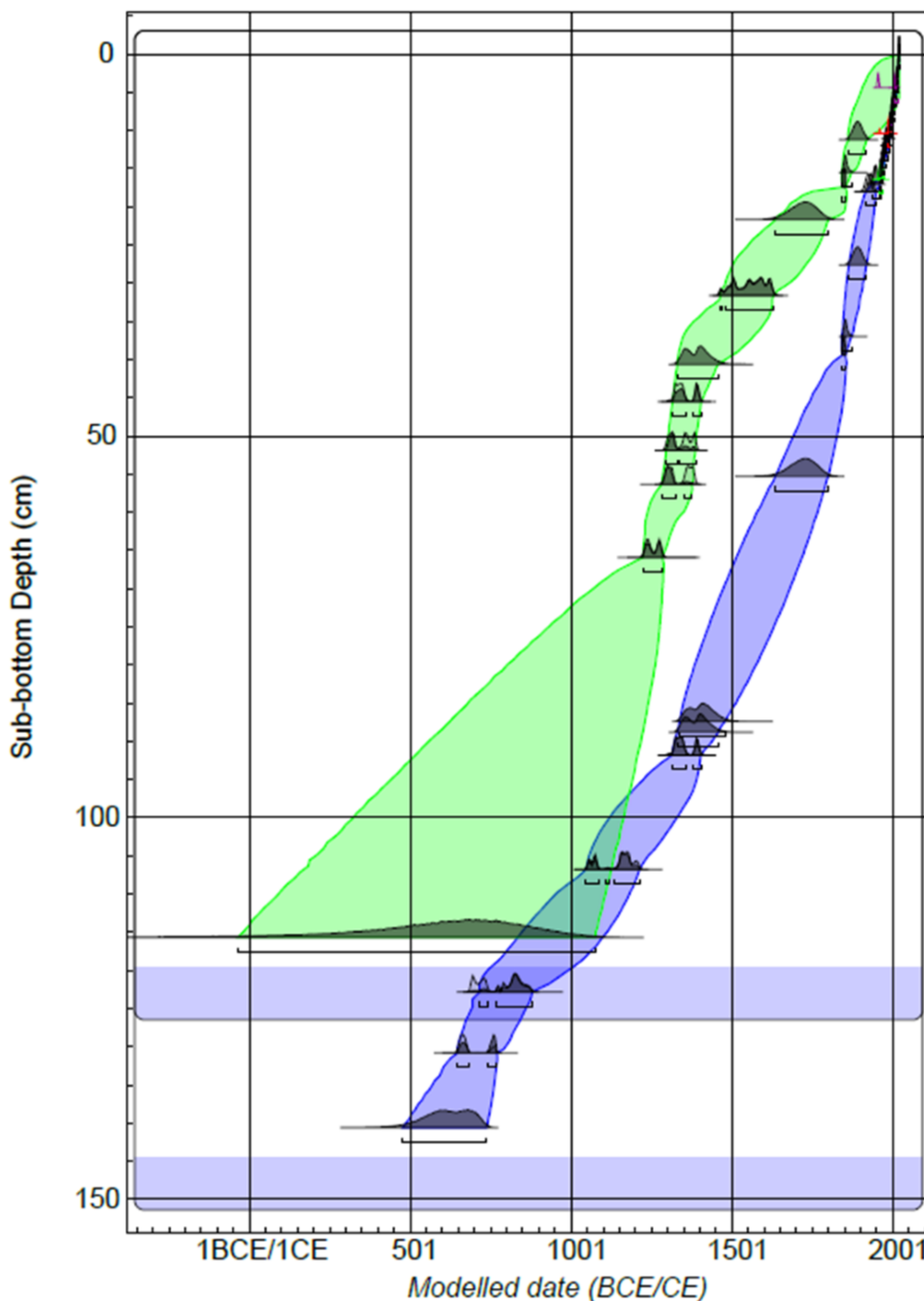

Figure D.20: Bayesian models using the 14C dates from the depocenter core (green) and edge core (blue). The calendar age likelihood is shown in light grey with the posterior probability density functions (dark grey) for the integrated chronologies. The age model uncertainties are derived from the blue and green curves at the 95% level of confidence, and indicated in Table D.32 and D.33 for specific samples. Prepared by Jamie D. Howarth.

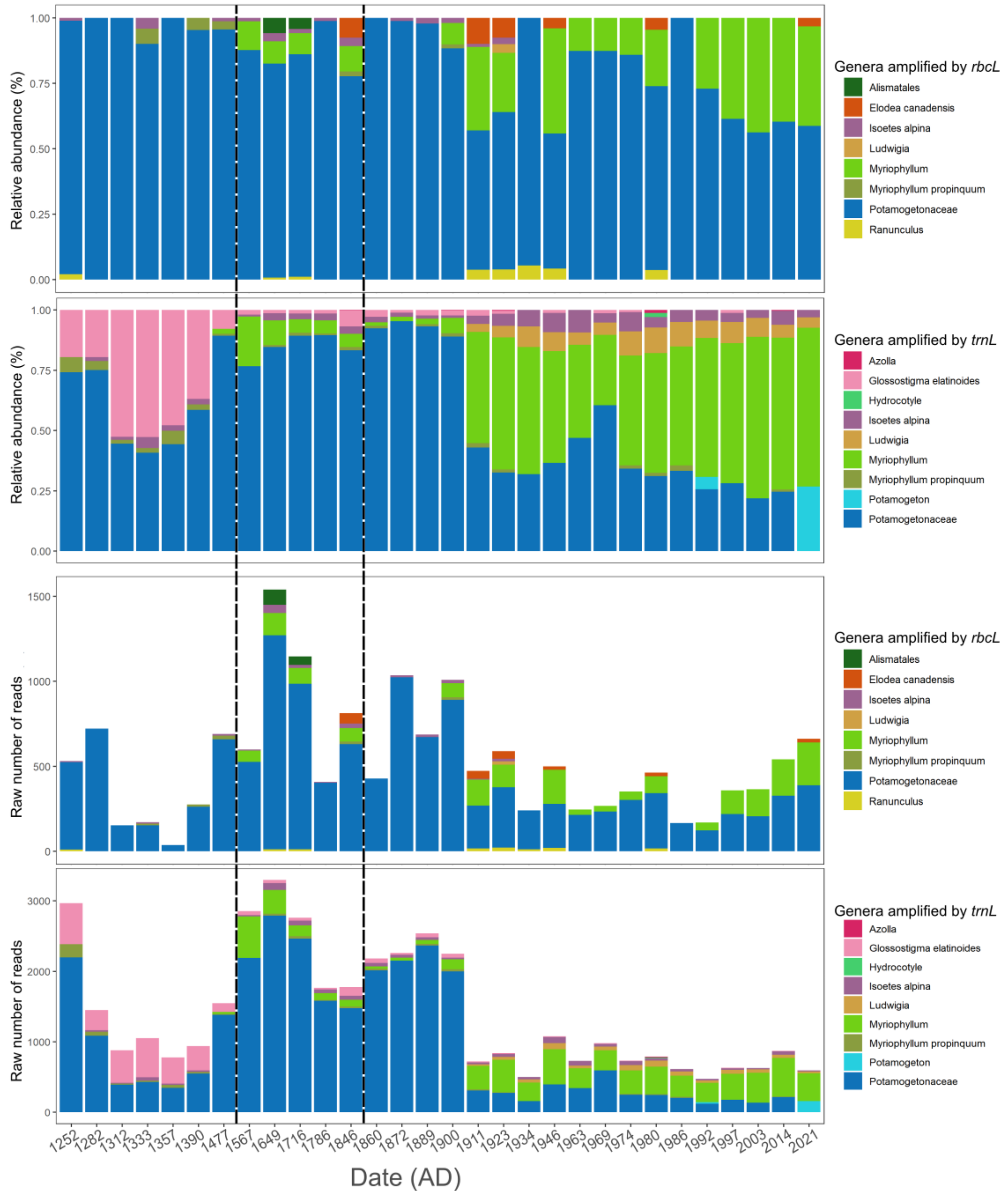

Figure D.21: Macrophyte composition from the rbcL and trnL primer sets. The black dashed lines indicate Māori and European settlements.

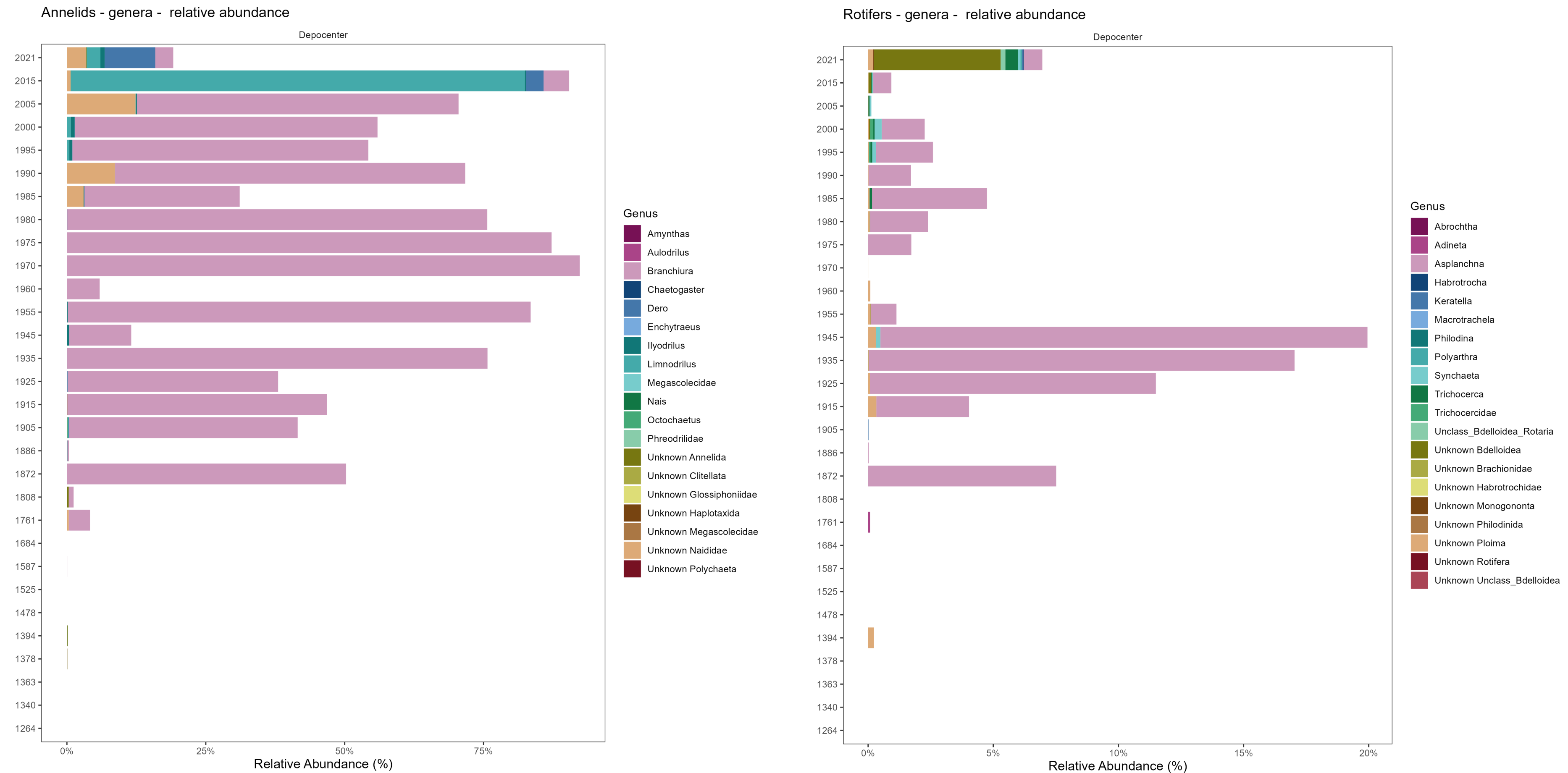

Figure D.22: Community composition of worms (Annelida) and rotifers (Rotifera) at genus level, amplified from their cytochrome oxidase c 1 (CO1) gene.