

eDNA survey of fish communities in Lakes Puketi and Rotoiti



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Executive Summary

This study presents the first combined traditional and environmental DNA (eDNA) fish survey of Lakes Puketi and Rotoiti, two culturally significant dune lakes in the Waikato region of New Zealand. These lakes have populations of invasive rudd (*Scardinius erythrophthalmus*) which pose a threat to native biodiversity and ecosystem health.

In March 2025, University of Waikato researchers conducted a baseline ecological survey of Lakes Puketi and Rotoiti near Port Waikato. This sampling, intended as a "before" stage for a BACI study on rudd control impacts, involved eDNA analysis, traditional fish surveys using nets and traps, and food web sampling (phytoplankton, zooplankton, invertebrates, and fish) for stable isotope analysis. The eDNA sampling involved collecting multiple water samples from near-shore and mid-lake habitats in both lakes, which were then filtered and preserved for subsequent metabarcoding analysis by Wilderlab. Traditional fish sampling utilized standardized fyke nets and minnow traps deployed overnight in both lakes.

The eDNA results hinted at a greater fish diversity than previously understood in these lakes, with Lake Puketi showing a notable increase in its known species richness. While eDNA generally corroborated traditional fyke netting and minnow trapping results, it also suggested the presence of additional non-native species (catfish, goldfish, perch) in the lakes. However, these results require further validation due to the inherent risks of contamination in eDNA workflows. Notably, longfin eels, recorded for the first time in traditional surveys of Lake Puketi, were not detected by eDNA, highlighting the potential for false negatives due to factors like low shedding rates or environmental DNA degradation. Furthermore, the disproportionately high eDNA read counts for abundant, small-bodied common bullies compared to the biomass-dominant eels in Lake Puketi suggests a complex, likely allometric, relationship between eDNA concentrations and fish abundances or biomass.

The stable isotope analyses (SIA) were able to describe key differences in the two lake food webs. SIA highlighted the importance of eels in Lake Puketi as top predators occupying maximum trophic positions, in addition to the flexible trophic roles of common bullies and rudd which occupied higher trophic positions in Lake Rotoiti, where large eels were absent. Overall, $\delta^{13}\text{C}$ values were depleted in Lake Rotoiti, which was potential evidence of a dystrophic state with high inputs of dissolved organic carbon from marginal wetlands.

This research underscores the complementary value of eDNA for comprehensive biodiversity assessments in these vulnerable dune lake ecosystems. However, it also emphasizes the importance of rigorous quality control and further investigation into the factors influencing eDNA detection and interpretation, particularly in the context of invasive species monitoring and native fish conservation. The baseline data generated will be crucial for evaluating the effectiveness of future rudd control efforts and tracking the ecological recovery of these lakes.

Introduction

Rudd (*Scardinius erythrophthalmus*), illegally introduced to New Zealand in 1967, are Eurasian cyprinids that can cause lake regime shifts to phytoplankton dominance (Hicks 2003). Herbivorous adults (>200 mm) act as nutrient pumps, converting macrophytes to excreted nutrients, while benthic feeding stirs bottom sediments (Vanni et al. 2013). The high fecundity of rudd makes them an invasive threat, reducing habitat quality, endangering native plants, and competing with native consumers. First established in Waikato lakes, they have since spread across the North Island and to Nelson and Canterbury (Hicks 2003).

Control efforts in Auckland and Waikato have had mixed success. Pod traps in Lake Kuwakatai removed 71% of rudd, more than fyke netting or electrofishing (Hicks et al. 2015). Since 2000, a pest fish control program in the Lake Rotopiko complex near Ōhaupō has shown gill netting is also effective (Neilson et al. 2004). Targeted gill netting may eradicate small adult rudd populations, allowing macrophyte restoration. However, eradication often requires piscicides like rotenone, successfully used in Lake Kokahuake and in Nelson (Rowe and Champion 1994, Chadderton et al. 2003). Rotenone is expensive and raises concerns about non-target deaths and resistance (Rowe 2003). Therefore, control measures and habitat manipulation remain important tools for rudd control. Understanding the efficacy and ecological implications of targeted removal methods is crucial for lake restoration in affected regions.

Lake Puketī is a small dune lake north of Port Waikato significant to Ngāti Tamaoho, Ngāti te Ata, and Waikato. A 2016 survey confirmed the presence of rudd, shortfin eels (*Anguilla australis*), and common bullies (*Gobiomorphus cotidianus*), but not the rainbow trout (*Oncorhynchus mykiss*) recorded in 1980 and likely extinct in the lake (Özkundakci et al. 2017). There is concern that rudd are contributing to declining water quality in Lake Puketī, thus hampering the restoration of this dune lake. Its small size makes it ideal for a long-term study on the impacts of rudd control on food webs and ecosystem processes.

To robustly assess fish communities in Lake Puketī, we combined traditional methods (fyke netting, Gee's minnow trapping) with environmental DNA (eDNA) sampling—a molecular approach that is revolutionizing biodiversity assessment (Altermatt et al. 2025). Our combined sampling helps to establish the current fish community and ensures methodological consistency for future monitoring after putative control measures are implemented.

Methods

Site location and study design

Lake Puketi is a small dune lake located to the north of Port Waikato. It is part of the “Twin Dune Lakes” complex of Lakes Puketi and Rotoiti, with Lake Puketi being the larger of the two. The lakes have significance to mana whenua and an old pa site is located between the two waterbodies. Lake Puketi has a maximum depth of 6.5 metres and covers an area of approximately 6.4 hectares (Özkundakci et al. 2017). It is a landlocked coastal dune lake situated at an elevation of 95 metres and is surrounded by improved pasture grazed by drystock. Lake Rotoiti is smaller, covering approximately 1 hectare. Lakes Puketi and Rotoiti have been the subject of an intensive ecological investigation in 2016 which assessed plankton, benthic invertebrates, fish, riparian spiders, terrestrial insects, and vegetation communities (Özkundakci et al. 2017).

To understand the impacts of rudd control, "before" sampling is needed for a robust BACI (Before-After, Control-Impact) study to track changes in fish communities and food webs. On 11/12th of March 2025, University of Waikato staff sampled Lakes Puketi and Rotoiti for fish, invertebrates, and basal resources alongside an eDNA survey.

eDNA survey

University of Waikato (UniWaikato) staff collected water samples in each lake before setting the fish nets and traps. In Lake Puketi, 15 1 L water samples were collected, with 10 targeting near-shore habitat, and 5 targeting mid-lake habitat (Fig.1). Due to Lake Rotoiti's smaller area and 'hour-glass' shape, only 6 1 L water samples were collected, with 5 targeting near-shore habitat, and 1 targeting mid-lake habitat (Fig.1). An additional 3 L composite sample was collected from Little Lake Puketi, a small wetland with open water habitat located to the west of Lake Puketi (Fig.A1; Table A1).

UniWaikato staff collected water samples from Lake Puketi using a decontaminated boat ("Hiko Hi Ika"). Because of its smaller size and difficult access, samples were collected from Lake Rotoiti by two UniWaikato staff in a decontaminated inflatable boat.

The water samples were collected in sterile, food-grade 1 L High Density Polyethylene (HDPE) plastic ('jar') containers (Stowers, Auckland, NZ). To collect water samples, a UniWaikato staff member at the boat's front used a Mighty Gripper Pole to submerge a 1 L container approximately 0.6 m below the surface while the boat moved slowly forward. When at the correct depth, the container was flipped right side up, releasing trapped air and allowing it to fill with a water sample. Care was taken to prevent the boat drifting into the sampling area.



Figure 1. Approximate location of eDNA samples collected from Lakes Puketi and Rotoiti on the 11th March 2025.

Water samples were filtered using a Wilderlab eDNA ‘mini-kit’. Each mini-kit contains an encapsulated 30 mm diameter, 5 µm cellulose acetate syringe filter. Water was filtered until the maximum 1 L was reached or the filter had clogged. For the more turbid Lake Rotoiti samples, a caulking gun was used to push the syringe plunger to filter more water. The total filtered volume was recorded and each filter preserved with 350 µL DNA/RNA Shield (Zymo Research). Filters were placed in sealed foil bags for storage.

Six negative control samples were taken from the field and the lab by filtering sterile, ultrapure water with the Wilderlab eDNA mini-kits.

eDNA samples were sent to Wilderlab (Wellington, NZ) to be analyzed using a multispecies metabarcoding approach. The comprehensive suite of assays used target a wide range of biota including fish, invertebrates (e.g., aquatic insects and crustaceans), microbes, and vascular plants. For more information about the DNA extraction, PCR amplification, sequencing and bioinformatics pipeline used by Wilderlab, see Wilkinson et al. (2024).

Fish survey

UniWaikato staff sampled fish in each lake using standardized fyke nets and Gee's minnow traps (dimensions detailed in Joy et al. 2013). In Lake Puketi, 5 fyke nets (with exclusion chambers) and 10 fine mesh minnow traps were deployed, while Lake Rotoiti had 4 fyke nets and 8 minnow traps.

In each lake, two minnow traps were placed within 5 meters of each fyke net, which were set perpendicularly from the shore. A wooden stake secured the fyke leader to the shore, and a weight kept the cod end submerged. Nets were typically deployed by boat (or occasionally from shore in shallow areas), left overnight, and retrieved the next day.

A shore-based team processed all captured fish, which were identified, measured for total length, and released. For each species (excluding eels), the first 50 individuals were measured and weighed, followed by an additional 10 per net. All eels were anesthetized using Aquis® for accurate identification and measurement. Across the two lakes, a total of 9 fyke nets and 18 minnow traps were deployed and retrieved. Specific net locations per lake are shown in Figure 2.

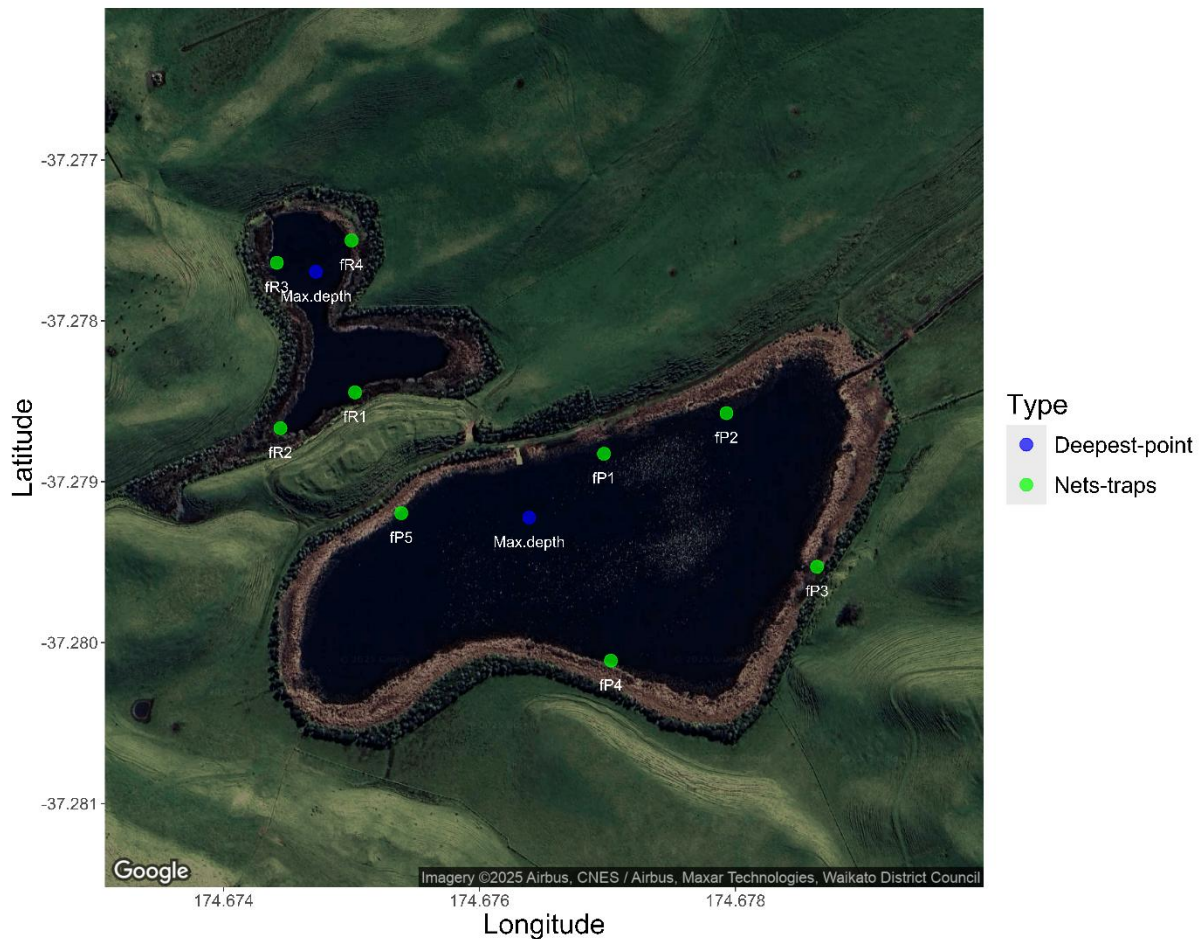


Figure 2. Approximate location of standardized fyke nets and Gee’s minnow traps set at Lakes Puketi and Rotoiti on the 11/12th March 2025.

Food web survey

UniWaikato staff collected samples for stable isotope analysis (SIA) following methods established in previous food web studies of Waikato lakes (Collier et al. 2018, Collier et al. 2019).

Eight 250-mL sub-surface unfiltered water samples were collected for phytoplankton from each lake. Four samples were preserved immediately with Lugol's Iodine and stored in the dark for identification and enumeration. Four samples were each filtered through a 25 mm G/F filter. Filters were stored on ice in the dark and frozen at -20 °C upon return to the laboratory.

Zooplankton samples were collected from each lake by UniWaikato staff. Eight vertical hauls (haul speed 1 ms⁻¹) were taken from the deepest location within each lake. Four samples were

immediately fixed in 90% ethanol for identification and enumeration. The remaining four samples were stored on ice and frozen at -20 °C upon return to the laboratory.

UniWaikato staff collected littoral invertebrates from each lake. Using a 500 µm mesh sweep net, marginal aquatic plants and submerged macrophytes were sampled for 30 seconds twice at four locations (North, South, East, West) per lake, totalling two minutes of effort. Eight samples were collected at each lake. Four of the samples were preserved in 90% ethanol for identification and enumeration. The remaining four samples were stored on ice and frozen at -20 °C upon return to the laboratory.

Representative fish were also sampled for SIA. All rudd and ten common bullies from each lake were euthanised and placed in individual plastic bags. To avoid invasive sampling of *Anguilla* spp., we collected mucus samples from individual eels following Boardman et al. (2022). Fish and mucus samples were stored on ice and frozen at -20 °C upon return to the laboratory. Fish capture, handling and euthanising were conducted in accordance with the University of Waikato Standard Operating Procedures numbers 6 and 7.

Macroinvertebrate and fish samples were prepared for stable isotope analysis using standard methods (Burdon et al. 2020). Stable isotope analyses were carried out on a DELTA V Plus continuous flow stable isotope ratio mass spectrometer linked to a Flash 2000 elemental analyser using a MAS 200 R autosampler (Thermo-Fisher Scientific, Bremen, Germany) at the Earth Sciences New Zealand Environmental and Ecological Stable Isotope Facility in Wellington, New Zealand. Low N-containing samples were analysed on a DELTA Q continuous flow stable isotope ratio mass spectrometer linked to an EA-Isolink elemental analyser using a MAS PLUS autosampler (Thermo-Fisher Scientific, Bremen, Germany). Some samples were reweighed because they were outside the optimal range for analysis.

Data analysis

For this report, only fish data from eDNA and traditional sampling was analysed. eDNA sequence count data was sorted to isolate fish. Marine fish detections were removed as likely contaminants using the World Register of Marine Species (WORMS) database (www.marinespecies.org). Species detections were also compared with historical records in the New Zealand Freshwater Fish Database (NZFFD) to assess their reliability based on biogeography. Fish species detected in only one sample at each lake were removed to reduce false positives (McCull-Gausden et al. 2021); these species are listed in Table B1. Data sorting was done using the tidy package in R (R Core Team 2022). Biomass of *Anguilla* spp. was estimated using length-mass regressions reported in Jellyman et al. (2013).

I assessed differences in trophic niche space (hereafter “isospace”) using stable isotope analysis (SIA). Trophic diversity of invertebrate and fish communities within each lake was quantified using the R package SIBER (Jackson and Parnell 2023). For each fish species, trophic niche width was estimated as the Standard Ellipse Area (SEA) using Bayesian inference. Models were run with a Markov Chain Monte Carlo (MCMC) chain length of 300,000 iterations, a burn-in of 200,000 iterations, thinning every 100th iteration, and three parallel chains. In addition to Bayesian SEA estimates, I calculated the sample size–corrected standard ellipse area (SEA-c) using maximum-likelihood estimation. This metric is less sensitive to small sample sizes and extreme values (Jackson et al. 2011). Differences in ellipse size among species were assessed by estimating the probability that the posterior distribution of one species’ SEA was larger or smaller than another. This was achieved by pairwise comparison of posterior draws between species, with the

proportion of draws indicating a smaller (or larger) ellipse interpreted as the probability of a difference in trophic niche width (Jackson and Parnell 2023). To further explore food web complexity using the stable isotope data, I calculated the Layman metrics (Layman et al. 2007) for each lake with the `laymanMetrics` function in the SIBER package.

I estimated the trophic position (TP) of predators (fish) in each lake using the “OneBaseline” model in the Bayesian mixing-model R package “`tRophicPosition`” (Quezada-Romegialli et al. 2018). This approach includes isotopic variation in the baseline indicator, the consumer, and the TEFs to provide robust estimates of consumer TP. For the baseline indicator, I used mean stable isotope values for *Lymnaeoidea* snails (Mollusca) in each lake. I used mean (± 1 standard deviation) TEF values of $1.3 \pm 0.3\text{‰}$ ($\delta^{13}\text{C}$) and $2.9 \pm 0.32\text{‰}$ ($\delta^{15}\text{N}$) based on McCutchan et al. (2003). Models were run with a MCMC chain length of 40,000 iterations, a burn-in of 10,000 iterations, thinning every 10th iteration, and four parallel chains.

All analyses and data visualisations were performed in R (R Core Team 2022).

Results

eDNA

Five fish species were detected using eDNA in Lake Puketi (Fig.3). These included two native fish species previously recorded from the lake, common bullies and shortfin eels. The non-native species recorded by eDNA in Lake Puketi were rudd, brown bullhead catfish (*Ameiurus nebulosus*), and goldfish (*Carassius auratus*).

Common bullies and rudd were detected in all samples (Fig.3a). Shortfin eels were detected in 12 samples, and catfish detected in 5. Goldfish were only detected in 2 samples.

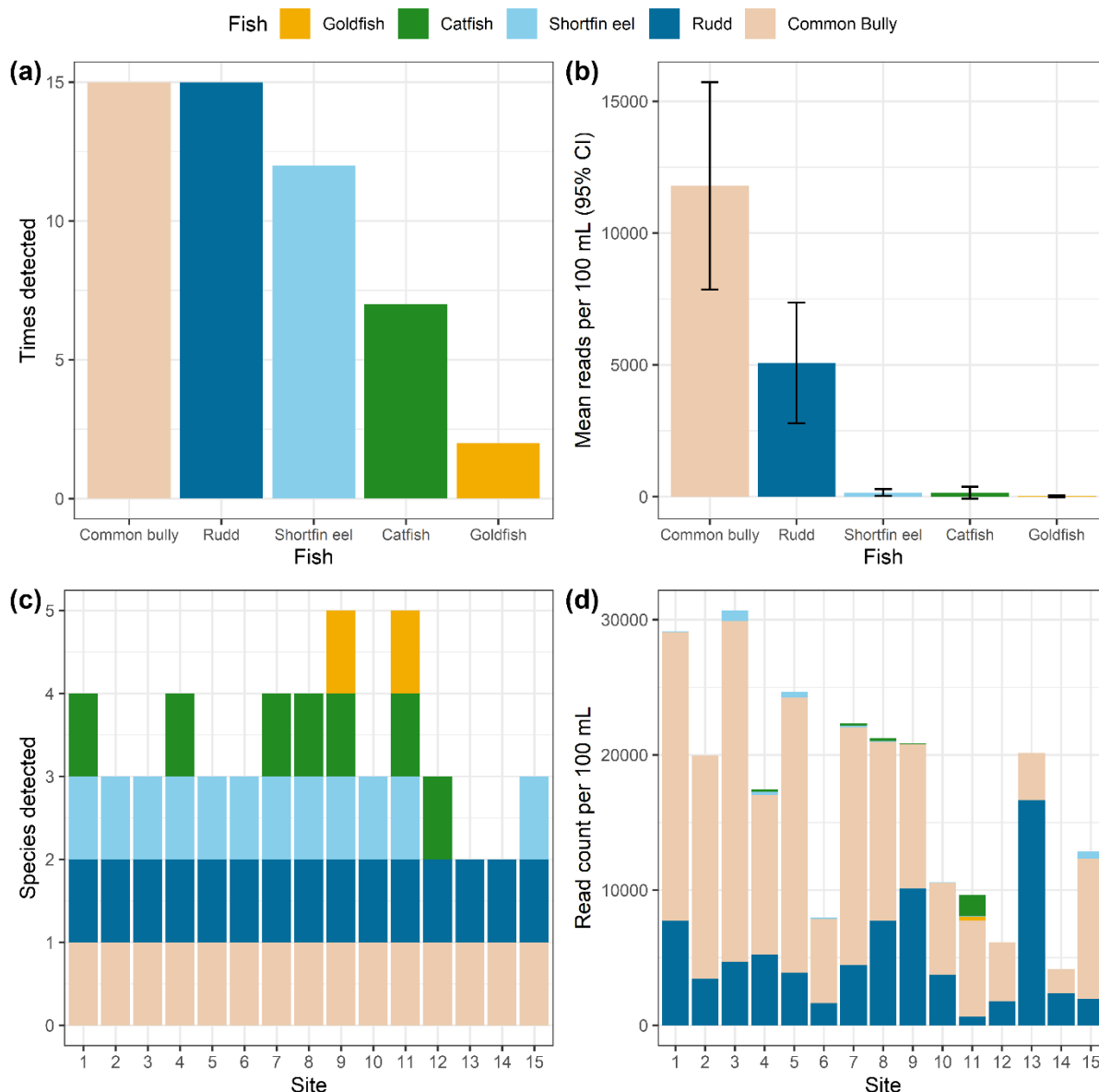


Figure 3. Fish species results from eDNA samples collected from Lake Puketi on the 11th March 2025. (a) Detections rates in 15 samples, (b) mean read counts per 100 mL (95% confidence interval), (c) the number and identity of fish species detected in each of the 15 samples, (d) the read counts per 100 mL and identity of fish species detected in each of the 15 samples.

Common bullies dominated read concentrations with 11,790 (7,856 – 15,724 95% CI) mean reads per 100 mL (Fig.3b). Rudd were also prominent in read concentrations with 5,073 (2,783 – 7,363 95% CI) mean reads per 100 mL (Fig.3b). Shortfin eels, catfish, and goldfish all had mean read concentrations lower than 200 reads per 100 mL (Fig.3b).

There were few obvious spatial patterns in eDNA detections. Shortfin eels were less likely to be detected in mid-lake (40%) samples when compared to near-shore (100%) samples (Fig.3c). Total read counts tended to be higher in near-shore samples when compared to the mid-lake samples (Fig.3d).

Four fish species were detected using eDNA in Lake Rotoiti (Fig.4). These included native common bullies and non-native rudd, brown bullhead catfish, and perch (*Perca fluviatilis*).

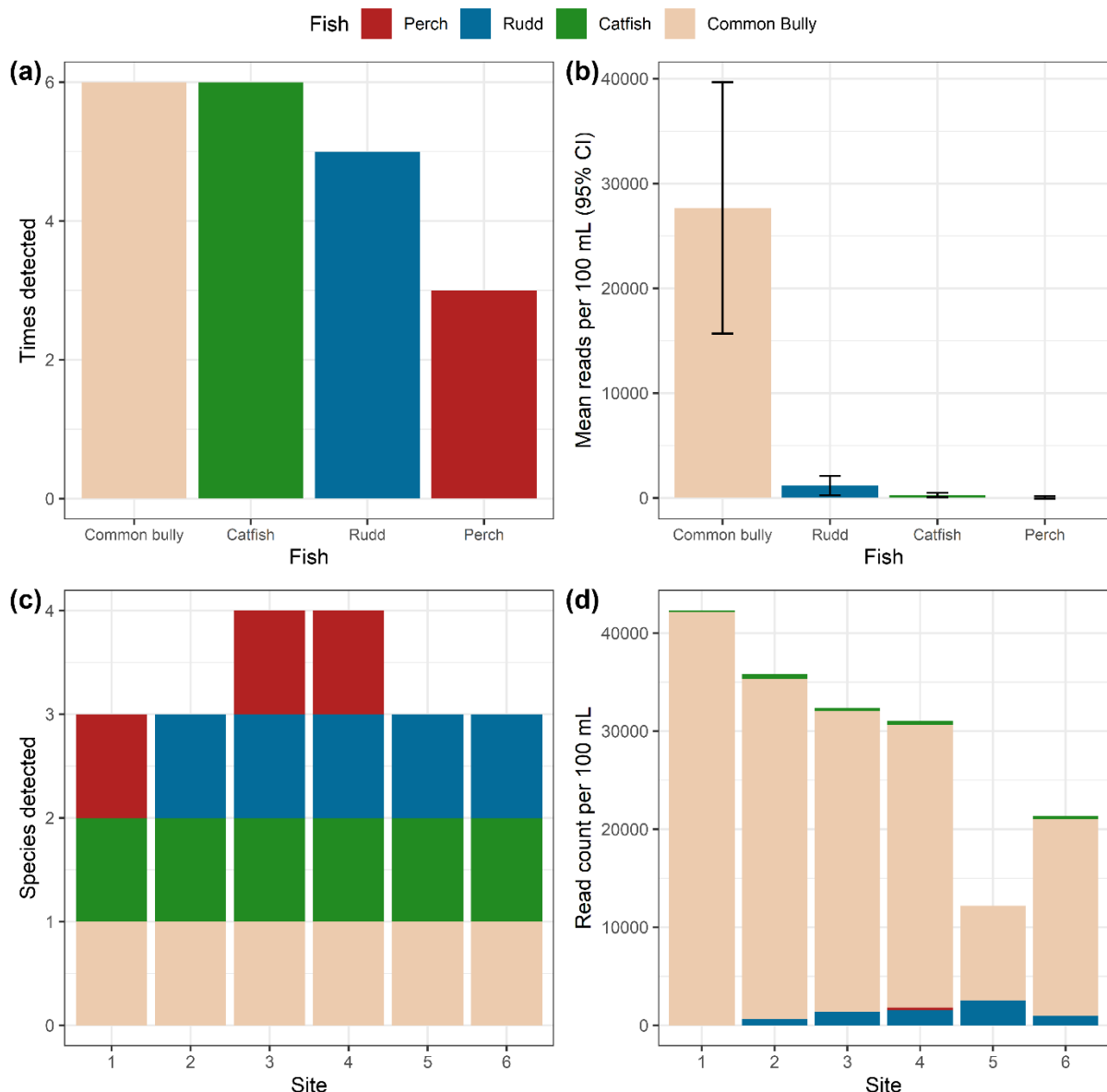


Figure 4. Fish species results from eDNA samples collected from Lake Rotoiti on the 11th March 2025. (a) Detections rates in 6 samples, (b) mean read counts per 100 mL (95% confidence interval), (c) the number and identity of fish species detected in each of the 6 samples, (d) the read counts per 100 mL and identity of fish species detected in each of the 6 samples.

Common bullies and catfish were detected in all samples (Fig.4a). Rudd were detected in 5 samples, and perch were only detected in 3 samples.

Common bullies dominated read concentrations with 27,677 (15,685 – 39,669, 95% CI) mean reads per 100 mL (Fig.4b). Rudd were less prominent in read concentrations with 1,177 (256 – 2,098, 95% CI) mean reads per 100 mL (Fig.4b). Catfish and perch both had mean read concentrations lower than 300 reads per 100 mL (Fig.4b).

There were few obvious spatial patterns in eDNA detections. Perch were detected in near-shore samples, but only one mid-lake sample was collected (Fig.4c). Total read counts were lowest at the mid-lake sample, despite rudd having its highest read concentration at this location (Fig.4d).

The field negative control samples showed the presence of species found in the lakes (common bully, rudd, shortfin eel), except for the detection of brown bullhead catfish (also found in eDNA samples, but not nets) and tench (*Tinca tinca*) in a single sample. The lab negative control samples showed no fish species, indicating that the species detected in the field may have been from airborne DNA or another source of contamination.

Traditional sampling

We recorded four fish species using traditional sampling on Lake Puketi. These included two native fish species previously recorded from the lake, common bullies and shortfin eels. We also recorded the presence of longfin eels (*A. dieffenbachii*). The sole non-native species caught was rudd.

Common bullies and rudd were recorded at all sites sampled (Fig.5a). Longfin eels were recorded at 2 sites. Rudd were only recorded at Site 4 (Fig.5b).

Common bullies dominated abundance at all sites sampled (Fig.5c). Rudd were conspicuously low in abundance at the site they were recorded, with only two individuals caught. In contrast, *Anguilla* spp. dominated biomass at all sites (Fig.5d), reflecting the large size of individuals caught. The mean length of longfin eels was 743 mm (630 – 856 mm, 95% CI) with a minimum length of 600 mm. The mean length of shortfins was 747 mm (704 – 790 mm, 95% CI) with a minimum length of 560 mm.

Considering abundances as catch per unit effort showed that on average, common bullies dominated abundances at the sites sampled (Fig.5e). In contrast, *Anguilla* spp. dominated biomass as catch per unit effort (Fig.5f). Despite their abundances, common bullies conspicuously contributed little to biomass CPUE, reflecting their small average body mass of 0.82 g (0.71 – 0.92 g, 95% CI) in Lake Puketi.

Only two fish species were recorded using traditional sampling on Lake Rotoiti. The native fish species was the common bully, and the sole non-native species caught was rudd.

Common bullies were recorded at all sites sampled (Fig.6a). Rudd were recorded at all sites except Site 3 (Fig.6b).

Common bullies dominated abundance at all sites sampled on Lake Rotoiti (Fig.6c). This native species was more abundant in Lake Rotoiti compared to Lake Puketi. Rudd were also more abundant in Lake Rotoiti, and dominated biomass at two of the sites (Fig.6d).

Considering abundance CPUE showed that on average, common bullies dominated abundances at the sites sampled (Fig.6e). Rudd dominated biomass CPUE, albeit with greater variability

(Fig.6f). The contribution of common bullies to biomass CPUE was greater than in Lake Puketi. Common bullies were larger in Lake Rotoiti with an average body mass of 2.67 g (2.16 – 3.17 g, 95% CI).

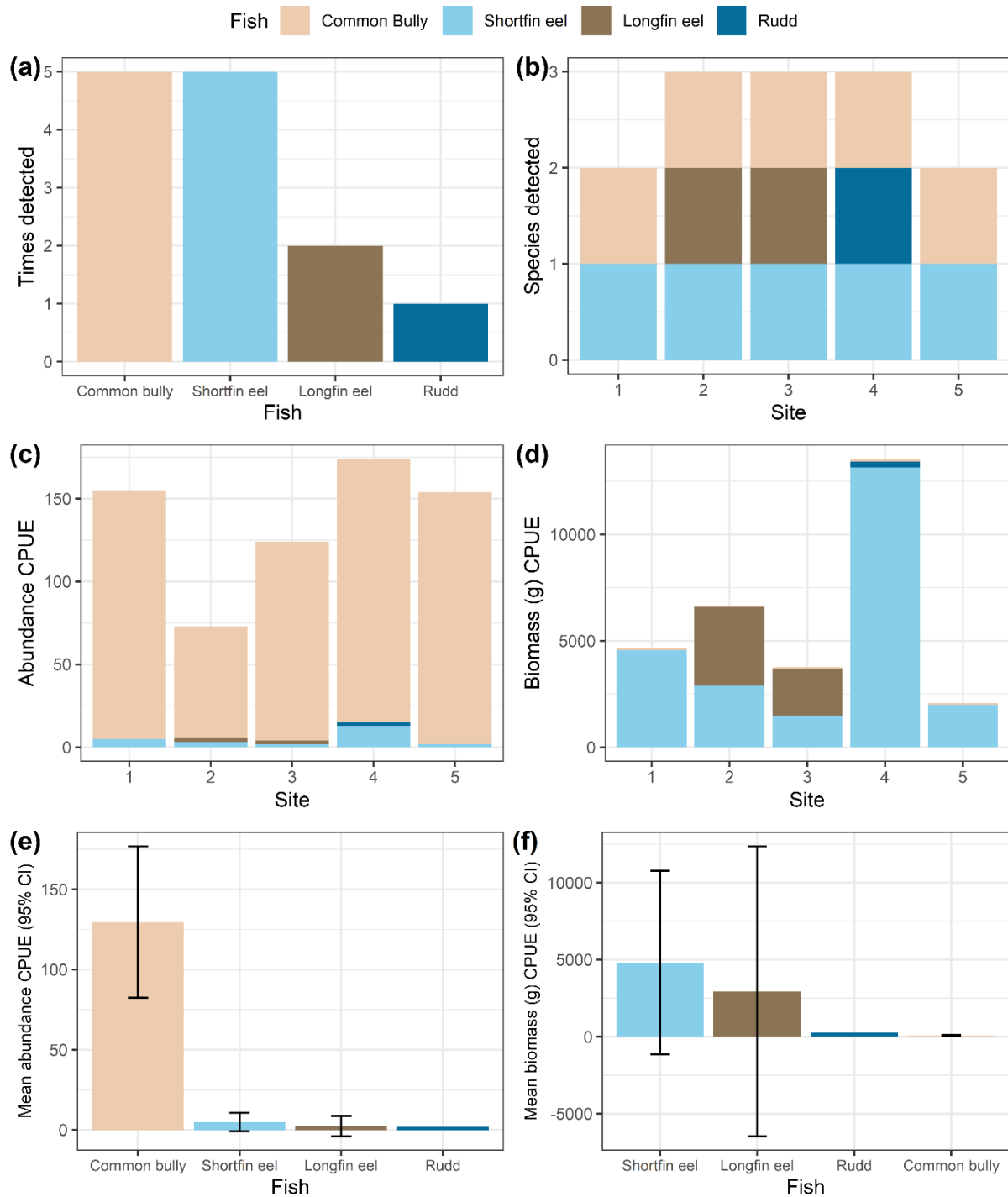


Figure 5. Fish species results from traditional sampling (fyke netting, Gee's minnow trapping) in Lake Puketi on the 11/12th March 2025. (a) Species detections rates at 5 sites; (b) the number and identity of fish species detected at each site; (c) the abundance Catch Per Unit Effort (CPUE) and identity of fish species detected at each site; (d) the biomass (g) CPUE and identity of fish species detected at each site; (e) mean abundance CPUE (95% confidence interval) for each fish species; (f) mean biomass (g) CPUE (95% CI) for each fish species.

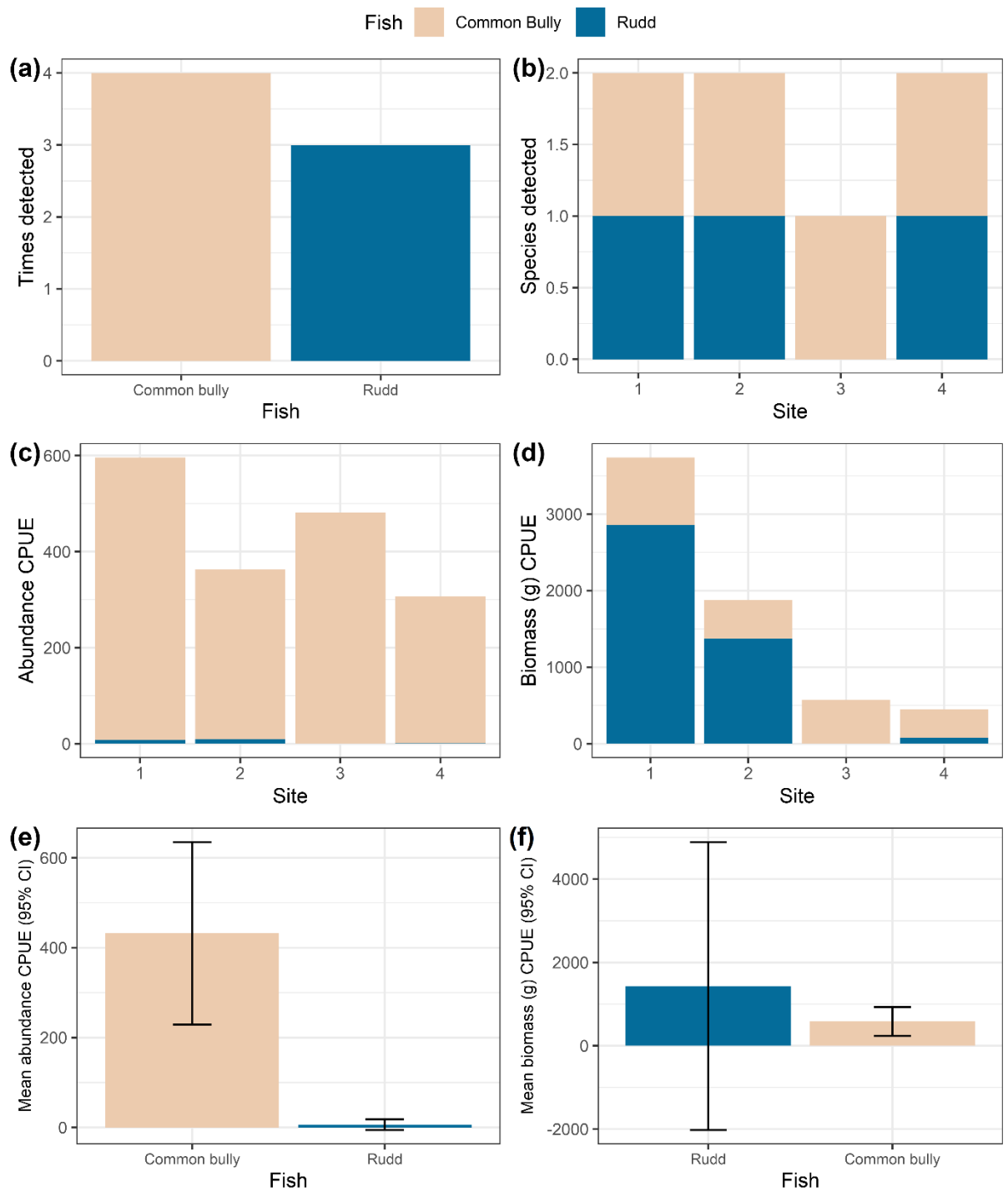


Figure 6. Fish species results from traditional sampling (fyke netting, Gee's minnow trapping) in Lake Rotoiti on the 11/12th March 2025. (a) Species detections rates at 4 sites; (b) the number and identity of fish species detected at each site; (c) the abundance Catch Per Unit Effort (CPUE) and identity of fish species detected at each site; (d) the biomass (g) CPUE and identity of fish species detected at each site; (e) mean abundance CPUE (95% confidence interval) for each fish species; (f) mean biomass (g) CPUE (95% CI) for each fish species.

Stable Isotope Analysis

We used stable isotopes of carbon and nitrogen to characterise the food webs of Lakes Puketi and Rotoiti. The Lake Puketi food web showed the eels as top predators with the highest $\delta^{15}\text{N}$ values (Fig.7). Common bully and rudd occupied intermediate trophic levels, with invertebrates demonstrating lower $\delta^{15}\text{N}$ values. Basal resource showed some variation in $\delta^{13}\text{C}$, with *Egeria densa* having a more enriched value than the other putative carbon sources (Fig.7).

The Lake Rotoiti food web differed with the absence of eels and overall, $\delta^{13}\text{C}$ values were more depleted than those observed in Lake Puketi. Common bully and rudd were the top predators with the highest $\delta^{15}\text{N}$ values, although a single sample from a leech (Hirudinea: *Richardsonianus*) also showed an elevated $\delta^{15}\text{N}$ value on parity with the two fish species (Fig.7). Several odonate species (*Aeshna brevistyla*, *Hemianax papuensis*, *Hemicordulia australiae*, and *Xanthocnemis zealandica*) occupied intermediate trophic levels. Invertebrate primary consumers and basal resources had overlapping $\delta^{15}\text{N}$ values, but benthic detritus was more depleted in $\delta^{13}\text{C}$ relative to the two macrophytes sampled (*Lemna* and *Eleocharis*).

In each lake we analysed the trophic diversity of the community (invertebrates and fish) using Bayesian standard ellipses (Fig.8). We found that the trophic niche area occupied by each community was similar (Fig.9), with no significant difference ($P=0.47$). We also considered the Layman metrics to further characterise different aspects of trophic diversity. Most of the metrics had similar values between the two lakes, but the packing of taxa was higher in the Lake Rotoiti food web (Fig.10). The Nearest Neighbour Distance (NND) was 40% lower and the Standard Deviation of NND 60% lower in Lake Rotoiti when compared to Lake Puketi (Fig.10).

We also estimated the trophic position of each fish species using Bayesian inference. We found that longfin eels occupied the highest trophic level in Lake Puketi (median = 4.75), followed by shortfin eels (median = 4.41). Common bully (median = 3.87) had a higher trophic position than rudd (median = 3.61) in this lake (Fig.11). In Lake Rotoiti, rudd (median = 4.16) and common bullies (median = 3.97) occupied a higher trophic level than in Lake Puketi. The trophic position of rudd was higher than that of common bullies in this lake (Fig.11).

Trophic position was influenced by body size, as evidenced by the positive relationship between individual fish body mass and $\delta^{15}\text{N}$ in both lakes (Fig.12). The relationship was stronger in Lake Puketi ($F_{1,18}=16.5$, $P<0.001$, $R^2=0.477$) when compared with Lake Rotoiti ($F_{1,18}=8.41$, $P<0.001$, $R^2=0.318$).

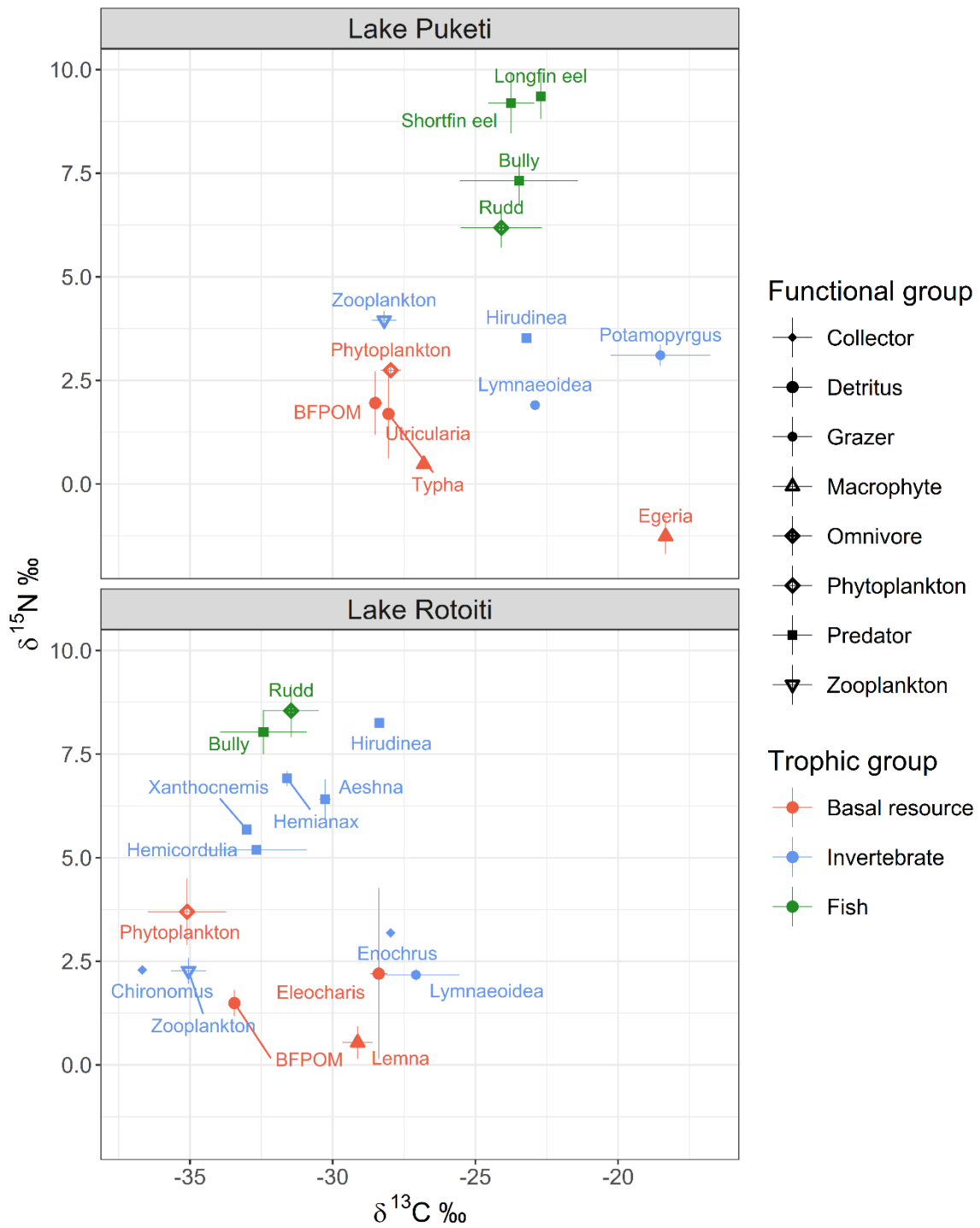


Figure 7. Stable isotope biplots of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) describing food webs of Lakes Puketi and Rotoiti. Error bars (± 1 standard deviation) are shown to indicate variation in stable isotope ratios for selected food web components. Symbols indicate functional groups (including invertebrate functional feeding groups), colours indicate broad trophic groups (basal resources, invertebrates, fish). Longfin eel, *Anguilla dieffenbachii*; Shortfin eel, *A. australis*; Bully, *Gobiomorphus cotidianus*; Rudd, *Scardinius erythrophthalmus*; BFPOM, benthic fine particulate organic matter. Rudd was characterised as an omnivore in both lakes.

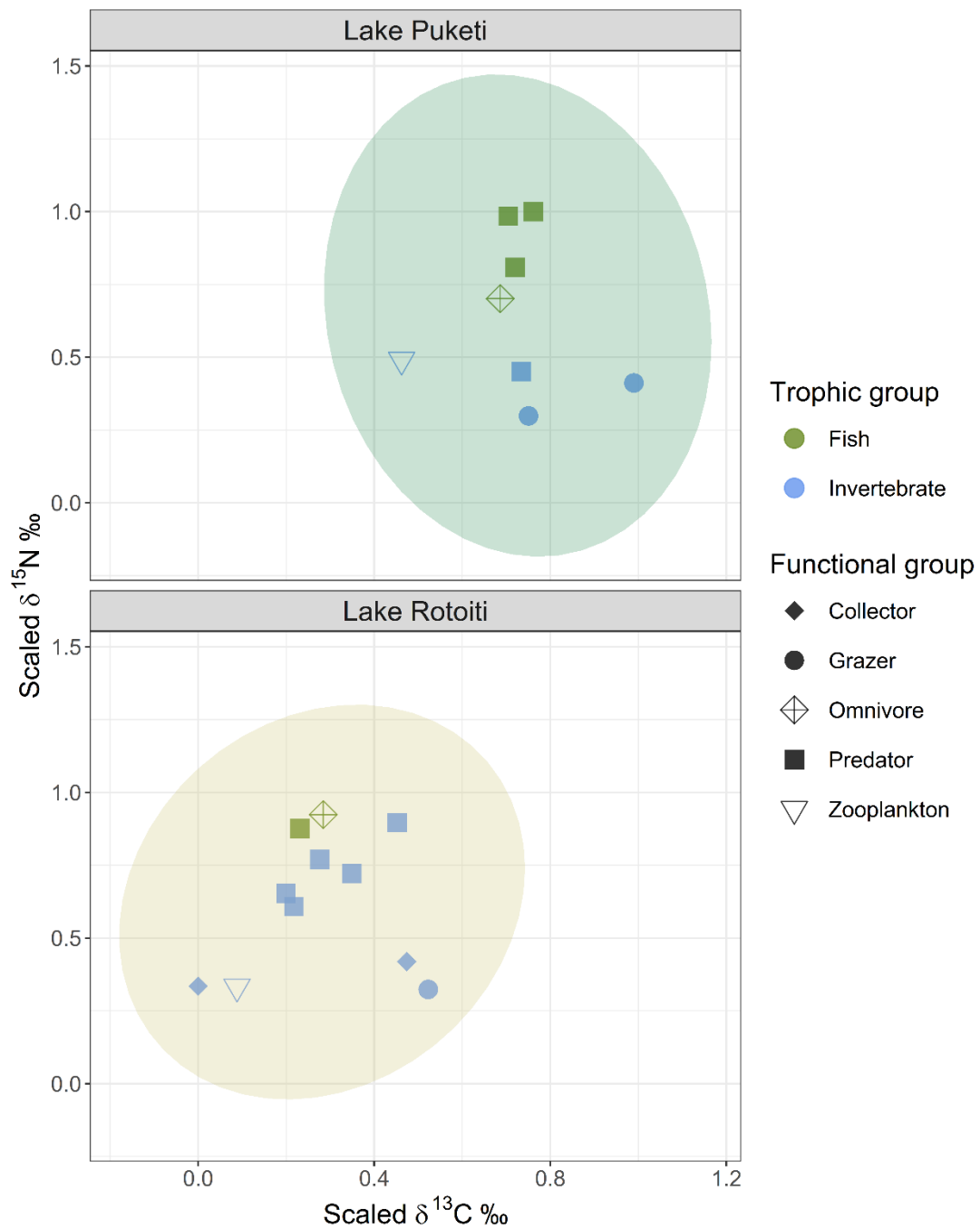


Figure 8. Stable isotope biplots of scaled $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) describing the community trophic niche for consumers (invertebrates and fish) in Lakes Puketi and Rotoiti. Each point represents mean values for a consumer taxon. Standard ellipses indicate the 95% confidence interval. Symbols indicate functional groups (including functional feeding groups), colours indicate broad trophic groups (invertebrates, fish). Rudd was characterised as an omnivore in both lakes.

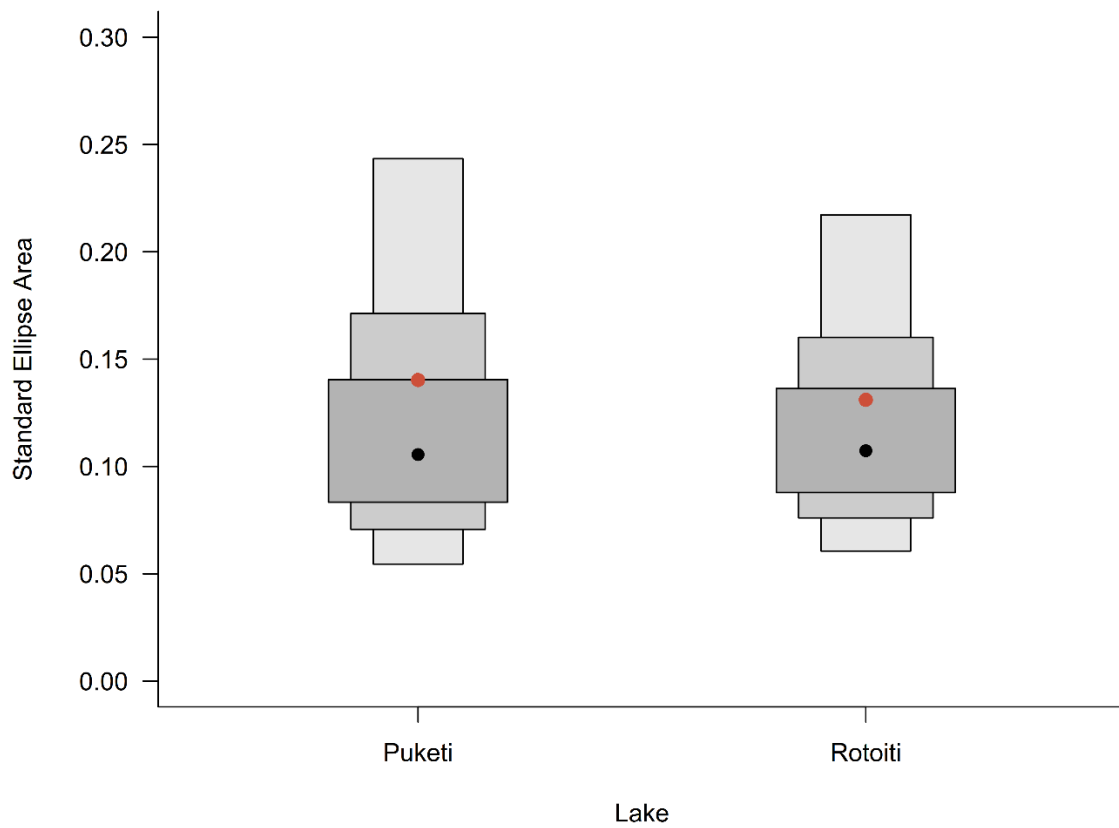


Figure 9. Boxplot describing the variation in the Bayesian Standard Ellipse Area (scaled $\% \text{ } ^2$) for consumers (invertebrates and fish) in Lakes Puketi and Rotoiti. Boxes indicate the 50%, 95% and 99% credible intervals. The black dot indicates the mode for the estimated values of Bayesian Standard Ellipse Area. The red dot indicates the maximum-likelihood (ML) estimated Standard Ellipse Area corrected for sample size (SEA-c).

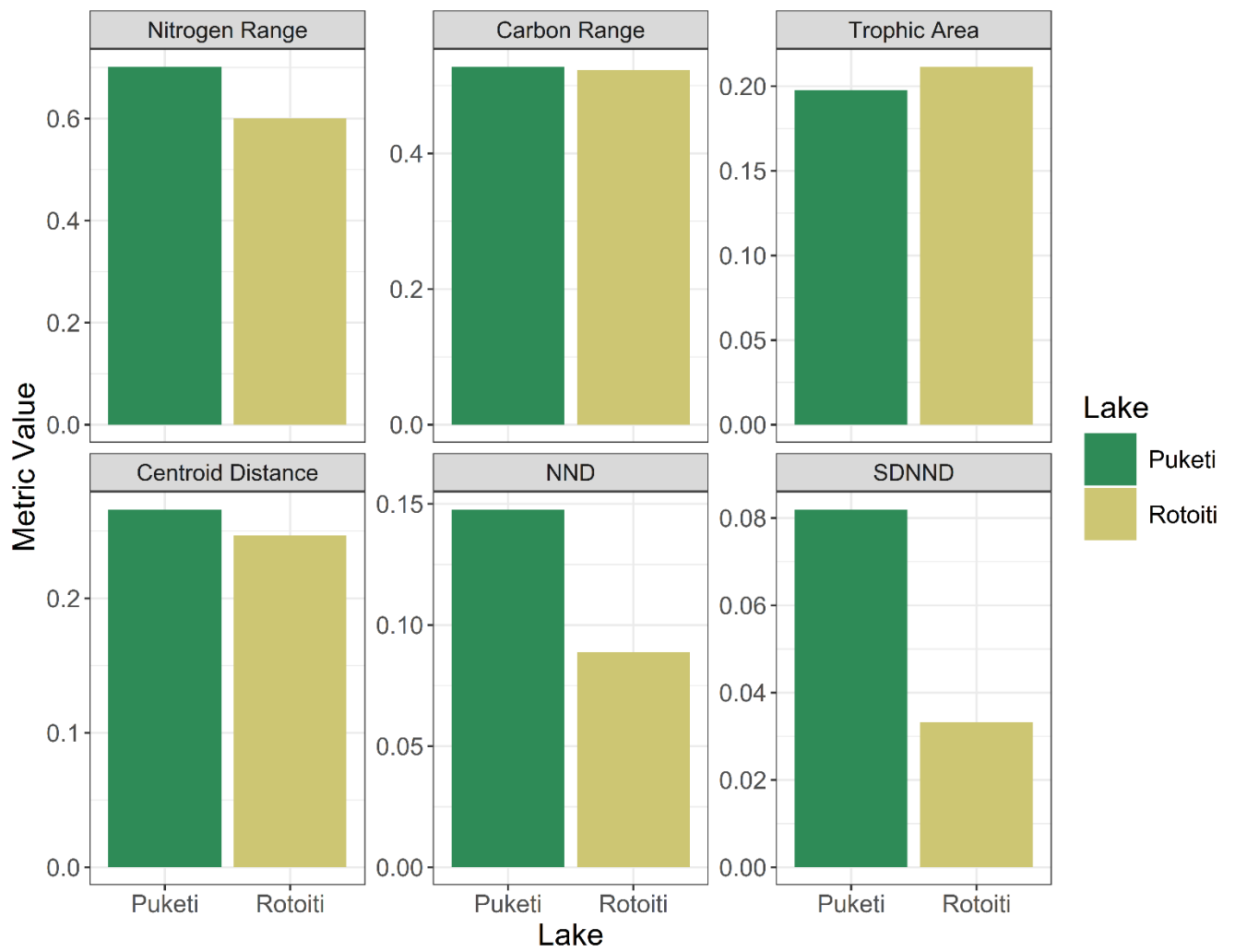


Figure 10. Barplots describing the six Layman metrics for consumers (invertebrates and fish) in Lakes Puketi and Rotoiti. Scaled data for consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) was used to calculate each metric. NND, Nearest Neighbour Distance; SDNND, Standard Deviation of Nearest Neighbour Distance.

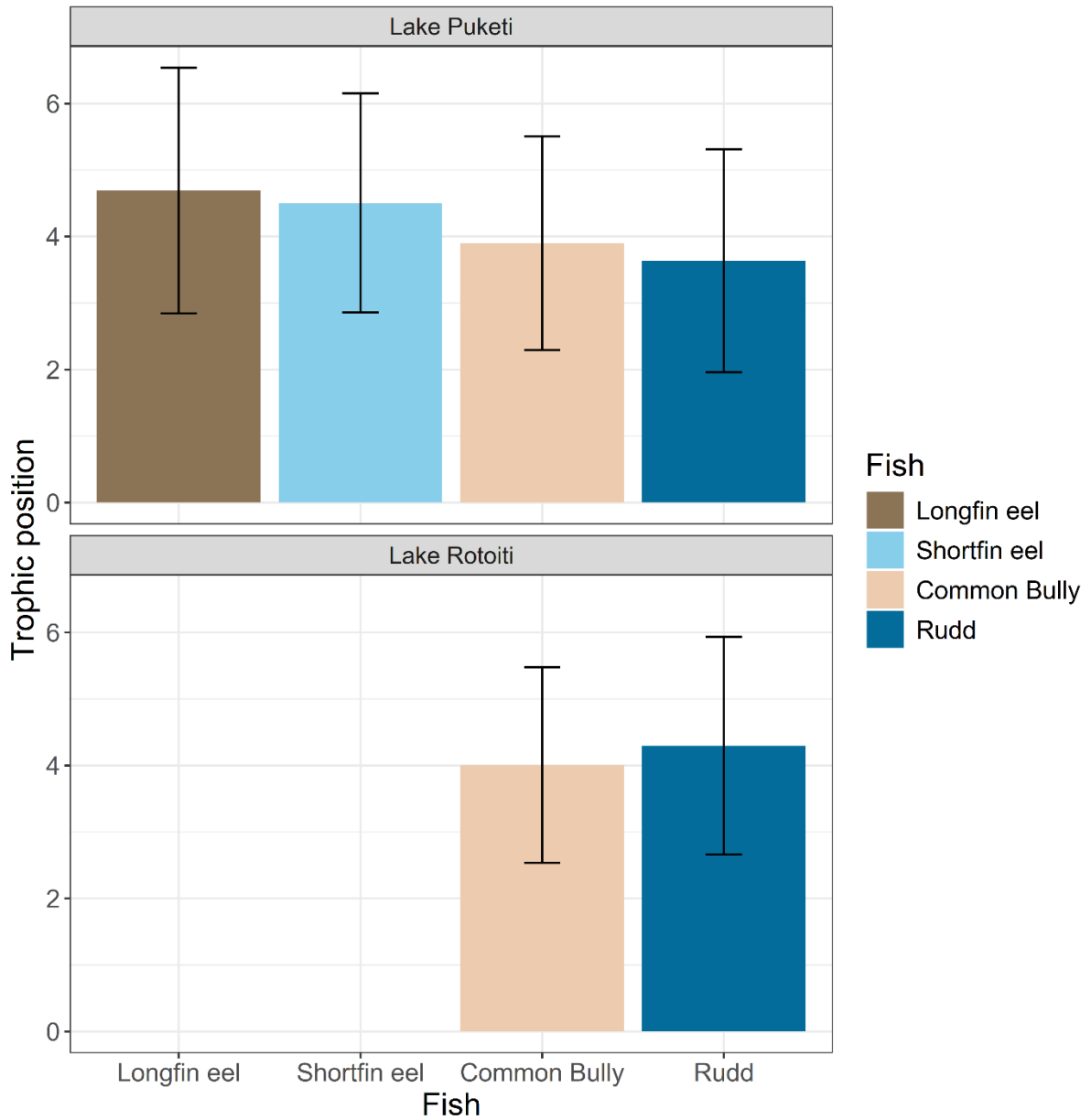


Figure 11. Median trophic position (\pm standard deviation) of fish in Lakes Puketi and Rotoiti estimated from stable isotopes using Bayesian inference. Longfin eel, *Anguilla dieffenbachii*; Shortfin eel, *A. australis*; Common bully, *Gobiomorphus cotidianus*; Rudd, *Scardinius erythrophthalmus*. See Fig.B1 for credible intervals.

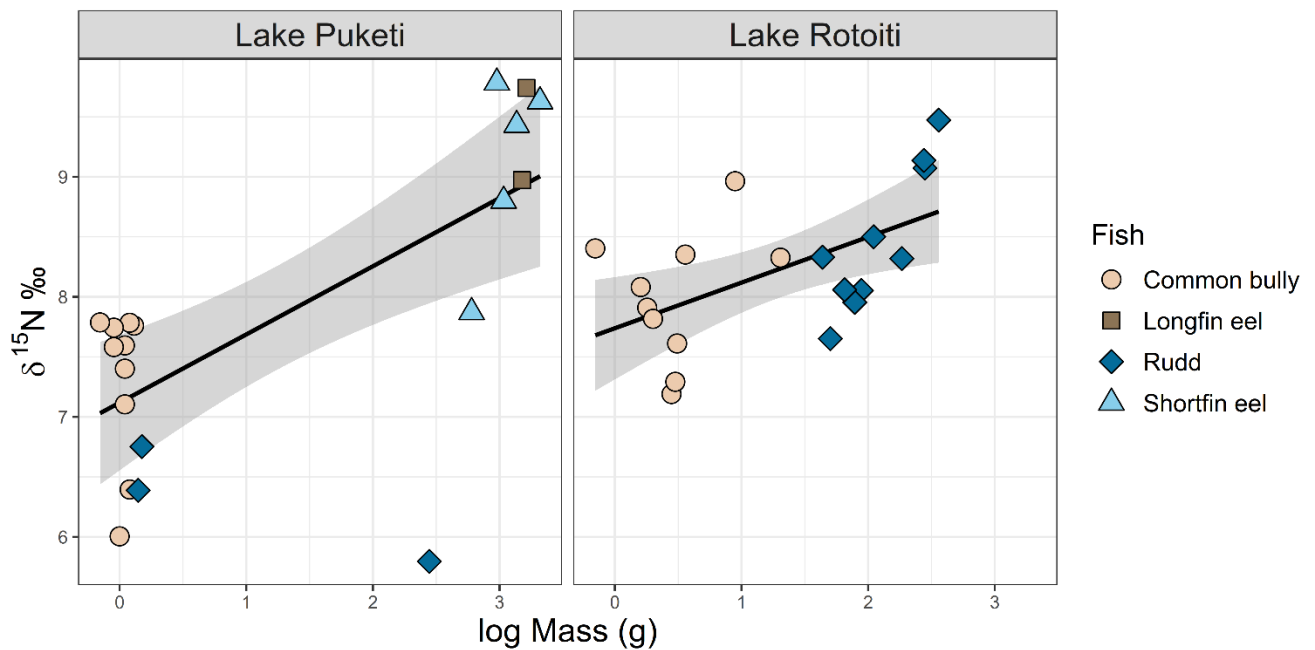


Figure 12. Linear regression relationships of log-transformed fish body mass with stable isotopes of $\delta^{15}\text{N}$ for individual fish collected from Lakes Puketi and Rotoiti. Longfin eel, *Anguilla dieffenbachii*; Shortfin eel, *A. australis*; Common bully, *Gobiomorphus cotidianus*; Rudd, *Scardinius erythrophthalmus*.

Discussion

Environmental DNA (eDNA) analysis is a rapidly evolving field with the potential to revolutionize how we monitor aquatic ecosystems (Altermatt et al. 2025). Our eDNA survey is the first of its kind in Lakes Puketi and Rotoiti, and it revealed the potential presence of several fish species previously unrecorded from these Waikato dune lakes. We detected five species in Lake Puketi using eDNA but only recorded three of these species in the traditional sampling (Table 1). Furthermore, although not detected by eDNA, we were able to confirm the presence of longfin eels in Lake Puketi. This combined survey potentially doubled the known fish species pool (6) in Lake Puketi.

Likewise, we found evidence for the presence of two additional species present in Lake Rotoiti using eDNA (Table 1). Our traditional sampling failed to record the presence of shortfin eel, indicating that this native species may have been extirpated from this smaller lake. eDNA for shortfin eel was detected in one sample from Lake Rotoiti (Table B1), but this failed to meet our requirement for species detection in ≥ 2 samples (McCull-Gausden et al. 2021). The abundances, body size, and biomass of common bullies from our traditional sampling indicate that native piscivorous shortfin eels are at least functionally extinct in Lake Rotoiti. Given the size structure of the eel populations in both lakes, it is likely that they are recruitment limited. A post-2016 algal bloom and resulting hypoxia might have caused a “fish kill” event in Lake Rotoiti, potentially eliminating or severely reducing the shortfin eel population (Willie Muir, pers. comm.).

The presence of additional non-native species (goldfish, catfish, and perch) in the eDNA samples from Lakes Puketi and Rotoiti was surprising, but plausible given their wide distribution in the Lower Waikato River Floodplain (Pingram et al. 2021). Catfish were recorded frequently in all waterbodies sampled using eDNA, suggesting their presence is highly likely. Goldfish and perch were detected in a single sample in each of the lakes where we have not recorded them (Table B1), further solidifying their potential presence in the regional species pool. However, without validating these detections with actual specimens caught using traditional methods there remains a possibility that these eDNA detections are due to contamination.

Table 1. Fish species detected in ≥ 2 eDNA samples and recorded from traditional sampling (fyke netting, Gee's minnow trapping) in Lakes Puketi and Rotoiti sampled on the 11/12th March 2025. Green indicates presence, red indicates absence. *New records

Status	Species	Lake Puketi		Lake Rotoiti	
		Traditional	eDNA	Traditional	eDNA
Native	Longfin eel*	Green	Red	Red	Red
	Shortfin eel	Green	Green	Red	Red
	Common Bully	Green	Green	Green	Green
Non-native	Rudd	Green	Green	Green	Green
	Catfish*	Red	Green	Red	Green
	Goldfish*	Red	Green	Red	Red
	Perch*	Red	Red	Red	Green

Contamination and sensitivity

Contamination is a significant concern at every stage of the eDNA workflow (Goldberg et al. 2016). Field contamination can include cross-contamination between sampling sites, but this was unlikely because we followed best practices with decontamination protocols and took care to sample in front of the boats. The boats used differed between the two lakes, further reducing the chance of cross-contamination. The introduction of foreign DNA was also possible, but we decontaminated surfaces and wore proper personal protective equipment (PPE) to prevent this. There is also a chance that contamination can occur from the sampling environment, such as airborne DNA or DNA present on the exterior of sampling containers. We collected field blanks (samples of sterile water taken to the field and treated like regular samples), but these did not indicate that the detection of three non-native species (goldfish, catfish, and perch) was due to this form of contamination. We also have laboratory blanks (samples of sterile water treated like regular samples in a tightly controlled environment), but those results have not been received yet.

Contamination can also occur in laboratory during the analysis of eDNA samples. Carry-over contamination from previous PCR reactions, contaminated reagents, and airborne DNA can contaminate lab surfaces and samples (Ficetola et al. 2016). There is also a potential for contamination during the bioinformatics pipeline. Errors or misannotations in reference databases used for sequence identification can lead to incorrect species assignments (Keck et al. 2023). In high-throughput sequencing, a small amount of signal from one sample can "bleed" into another, causing false positives in low-concentration samples (Brandt et al. 2021). However, these sources of contamination are highly unlikely as Wilderlab are a commercial laboratory with ISO 17025 (International Organization for Standardization) accreditation for DNA analysis and have extensive experience processing freshwater eDNA samples. If any detections of the three non-native species (goldfish, catfish, and perch) in our study are due to contamination, it is more likely that this occurred in the field.

We also witnessed a false negative result (failing to detect a species that is actually present) with our sampling of Lake Puketi. In our traditional sampling we were able to record several large longfin eels from two sites. This species was, however, undetected by eDNA. Several reasons exist for false negatives. If the target species are present in very low numbers, it might shed low concentrations of DNA that fall below the detection limit of the eDNA assay (Wilcox et al. 2013). It is likely that the longfin eels are present at low abundances, which could lead to false negatives despite their large body mass. Many organisms do not release DNA at a constant rate. Shedding can be influenced by factors like life stage, reproductive status, stress levels, and activity, further affecting detection (Jo et al. 2019). DNA will also degrade in the environment, due to various factors including ultraviolet light, temperature, pH, and microbial activity (Barnes et al. 2014). Another reason for false negatives is insufficient sequencing depth. Deeper sequencing generates more reads, increasing the chances of detecting DNA from species that are present at low concentrations in the environment (Shirazi et al. 2021). This latter problem is unlikely to have affected our analysis, because Wilderlab use an Illumina NextSeq platform, offering over 1,000,000 eDNA reads per sample on average (Shaun Wilkinson, pers. comm.).

Allometric relationships

However, one interesting feature of our combined sampling approach was the dominance of the smaller-bodied common bully in terms of abundance and sequence read counts. This was contrasted with the dominance of biomass by *Anguilla* spp. in Lake Puketi. The relationship between eDNA concentrations and the abundance or biomass of fish populations is an area of active research. This relationship is complex and influenced by various factors, including allometric scaling. Allometry is the study of how biological traits scale with body size. In the context of eDNA and fish, allometric relationships suggest that larger fish do not necessarily release proportionally more DNA than smaller fish. Several studies indicate that mass-specific eDNA production rate (eDNA released per unit of body mass) tends to decrease as body size increases (Yates et al. 2021a, Yates et al. 2021b).

Studies have shown that integrating allometric scaling into models can significantly improve the correlation between eDNA concentrations and fish abundance or biomass. The allometric scaling coefficient (b) for eDNA production in fish has been estimated to be around 0.64 to 0.92, indicating a non-linear relationship where larger fish produce relatively less eDNA per unit mass (Yates et al. 2023). This may be due to larger surface area to volume ratios. Smaller organisms have a larger surface area relative to their volume compared to larger organisms. Since DNA is often shed from external surfaces (e.g., skin, mucus), smaller fish might release more DNA per unit of their body mass (Yates et al. 2021a). Alternatively, metabolic rates and other physiological processes often scale allometrically with body size (Brown et al. 2004). For instance, larger animals tend to have lower metabolic rates per unit mass, which could influence the rate of cell turnover and thus DNA shedding.

If eDNA production scales allometrically, simply assuming a direct linear relationship between eDNA concentration and fish biomass can lead to inaccurate estimates. For example, a water sample with a high eDNA concentration might be due to a large number of small fish rather than a few large fish with the same total biomass. Other factors like stress, reproductive stage (e.g., spawning), and feeding rates can affect DNA shedding (Jo et al. 2019). Also, different fish species likely have varying rates of DNA release due to differences in their biology and behaviour (Yao et al. 2022).

Food webs

Despite their close proximity, Lakes Puketi and Rotoiti had notable differences in food web structure. Common bullies and rudd occupied higher trophic positions in Lake Rotoiti than observed in Lake Puketi. One reason for this difference could be the absence of large eels in Lake Rotoiti, thus enabling the bullies and rudd to occupy a higher trophic position due to less top-down control and competition from eels. We saw evidence for a positive relationship between body size and trophic position ($\delta^{15}\text{N}$). This result was consistent with other studies which have shown a positive relationship between species' trophic position and body size (Romanuk et al. 2011).

Furthermore, common bullies were significantly larger in Lake Rotoiti. The smaller mean body size of bullies in Lake Puketi may have been due to the presence of large, piscivorous eels. Other studies have shown such effects. In Europe, the presence of northern pike (*Esox lucius*) impose strong selection on threespine stickleback (*Gasterosteus aculeatus*), causing reduced body size among other demographic effects through consumptive effects (Heins et al. 2016). Non-consumptive, risk-induced stress may also reduce foraging efficiency and growth in prey species. Further analysis of our data could focus on abundance-body mass relationships. There is some evidence that the presence of piscivores may affect size spectra of fish communities, although it may be more relevant to the elevation (intercept) of the relationship as opposed to the slope (Mehner et al. 2015, Marin et al. 2023).

Rudd are typically herbivorous as they mature, and we saw evidence for this with a relatively low $\delta^{15}\text{N}$ value for a large-bodied individual in Lake Puketi. In contrast, the elevated trophic position of rudd in Lake Rotoiti may have been due to the absence of submerged macrophytes, meaning rudd were unable to occupy a facultative niche as a herbivore, and relied more on secondary production as an energy source. In contrast, Lake Puketi has extensive beds of submerged macrophytes, helping to explain how the large rudd caught was able to occupy its preferred niche as a facultative herbivore, thus contradicting the general pattern between body size and trophic position ($\delta^{15}\text{N}$).

Another notable feature of the Lake Rotoiti food web were the depleted values of $\delta^{13}\text{C}$ for both basal resources and consumers. Strongly depleted $\delta^{13}\text{C}$ values are typical of dystrophic lakes (Taipale et al. 2016). Dystrophic lakes typically have high refractory dissolved organic carbon (RDOC) inputs (Carpenter and Pace 1997). RDOC in lakes is a major component of coloured dissolved organic matter (CDOM), contributing to the brown water observed in some lakes. RDOC is a recalcitrant material highly resistant to degradation and can persist for long periods rather than being rapidly used by microorganisms. Lake Rotoiti visually possessed the traits of a dystrophic lake with brown water. Other Waikato lakes have been characterised as dystrophic, such as Lake Ngāhewa (Forsyth and McColl 1975).

I also used the Layman metrics (Layman et al. 2007) to examine differences in trophic diversity between the two lakes. In Lake Rotoiti, the Nearest Neighbour Distance (NND) was considerably smaller, indicating greater packing within a trophic niche-space of similar area to Lake Puketi. This pointed to greater redundancy, but also potentially greater competition, since there were a number of species (e.g., Odonata) occupying a similar trophic niche. The lower standard deviation of NND (SDNND) in Lake Rotoiti is less influenced by sample size and indicated a more even distribution of trophic niches, as opposed to those in Lake Puketi which were more divergent. These differences were driven in part by the relatively diverse odonate fauna of Lake

Rotoiti. Further understanding the factors that promote the presence of these charismatic aquatic insects will aid restoration efforts that seeks to increase biodiversity values.

Conclusion

In conclusion, our eDNA survey of Lakes Puketi and Rotoiti has significantly expanded our understanding of their fish communities. The survey revealed the potential presence of previously unrecorded non-native species in both lakes. Combined with the confirmed presence of longfin eels means that the fish species richness in Lake Puketi may be at least double that previously known. The stable isotope analyses highlighted the importance of the eels in Lake Puketi as top predators, in addition to the flexible trophic roles of common bullies and rudd which occupied higher trophic positions in Lake Rotoiti, where large eels were absent. While the eDNA results largely corroborated traditional sampling methods, they also highlighted the capacity of eDNA to detect species overlooked by traditional approaches. However, the detections of catfish, goldfish and perch warrant further validation due to the inherent risks of contamination throughout the eDNA workflow, despite the stringent protocols implemented. Furthermore, the failure to detect longfin eels in Lake Puketi underscores the challenges of false negatives in eDNA studies, potentially linked to low abundance, variable shedding rates, environmental degradation of DNA, or insufficient sequencing depth. The observed dominance of common bully eDNA signals despite the biomass dominance of the larger eels also emphasizes the complex, likely allometric, relationship between eDNA concentrations and fish abundance or biomass. This study underscores the power of eDNA as a complementary tool for biodiversity assessments in these valuable dune lake ecosystems, while also highlighting the critical need for careful consideration of potential biases, rigorous quality control measures, and further investigation into the factors influencing eDNA detection and quantification.

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allometric scaling. Environmental DNA 5:1105-1115. doi:
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Appendix A

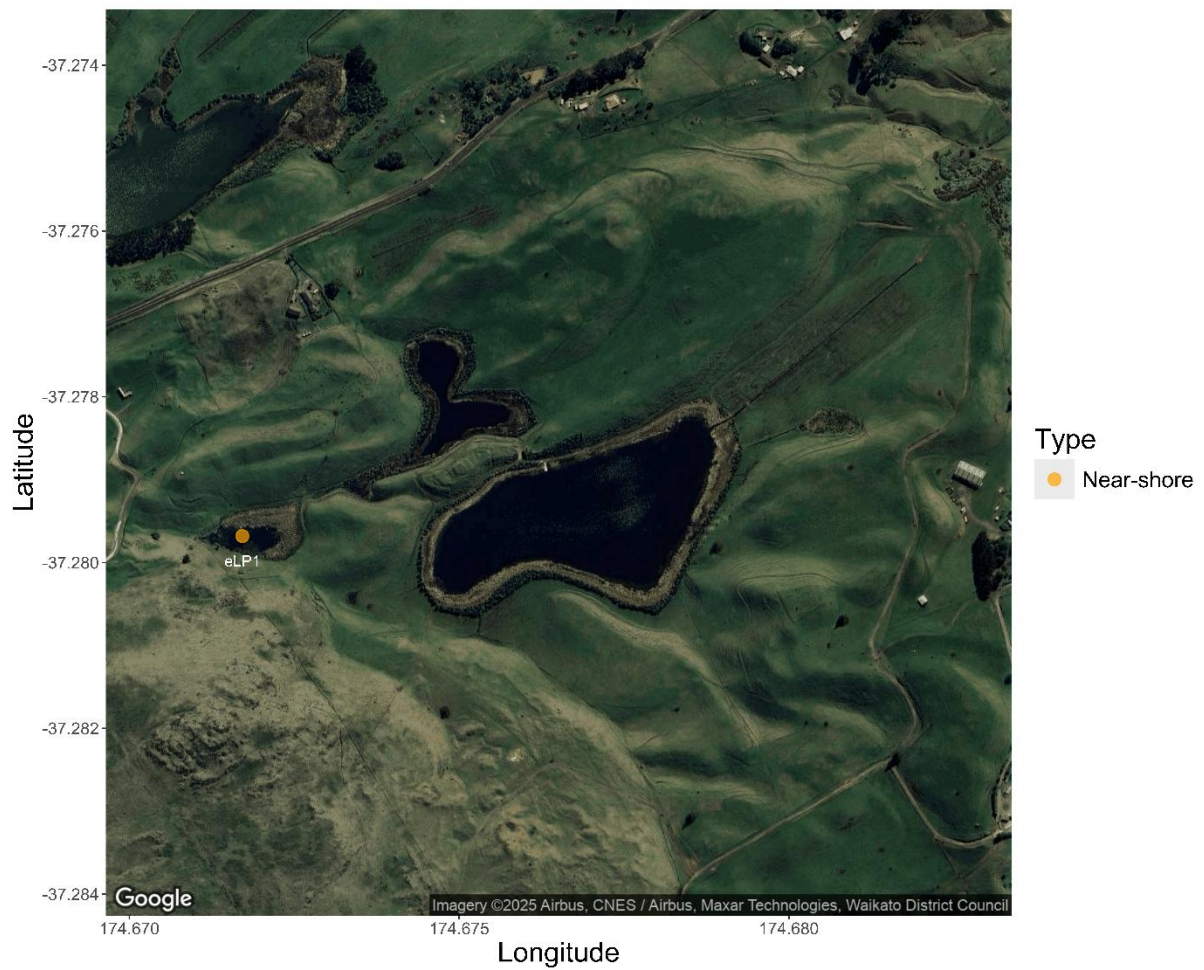


Figure A1. Approximate location of the composite eDNA sample collected from Little Lake Puketi on the 11th March 2025.

Table A1. Fish species detected in a single composite sample from Little Lake Puketi sampled on the 11th March 2025.

Common name	Species	Read count per 100 mL
Rudd	<i>Scardinius erythrophthalmus</i>	43.4
Common bully	<i>Gobiomorphus cotidianus</i>	18.0
Catfish	<i>Ameiurus nebulosus</i>	1.3

Appendix B

Table B1. Fish species detected in a single sample from the eDNA survey of Lakes Puketi and Rotoiti on the 11th March 2025. These species were excluded from further analysis to avoid any risk of false positives (McColl-Gausden et al. 2021).

Lake	Common name	Species	Read count per 100 mL
Puketi	Perch	<i>Perca fluviatilis</i>	2.6
Rotoiti	Goldfish	<i>Carassius auratus</i>	535.6
Rotoiti	Shortfin eel	<i>Anguilla australis</i>	205.0
Rotoiti	Giant bully	<i>Gobiomorphus gobioides</i>	76.8

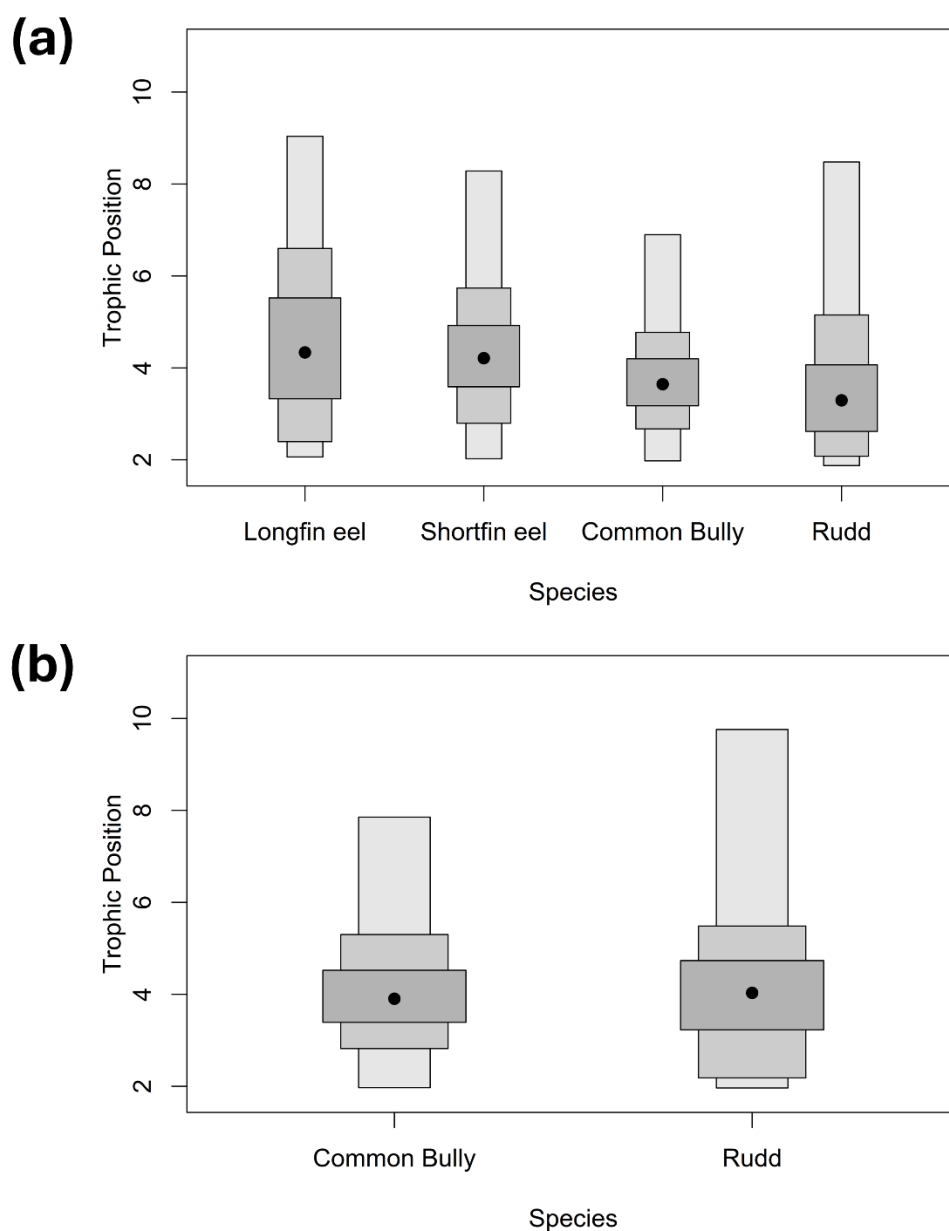


Figure B1. Boxplot describing the variation in the estimated trophic position of fish species in lakes a) Puketi and b) Rotoiti using Bayesian inference techniques. Boxes indicate the 50%, 95% and 99% credible intervals. The black dot indicates the mode for the estimated values of trophic position.