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Methods

Species, sampling method and location affect eel eDNA detection in a turbid lowland lake

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Abstract

Biodiversity is declining in freshwater ecosystems, increasing the need for efficient monitoring tools. Environmental DNA (eDNA) is a powerful approach for detecting aquatic species, but sampling methodology can influence detection outcomes. This study evaluated eDNA sampling methods for detecting shortfin eel (*Anguilla australis*) and longfin eel (*Anguilla dieffenbachii*) in a turbid lowland lake in Aotearoa New Zealand. The sampling methods compared included active pump filtration, passive samplers, tow nets and sediment sampling. The spatial distribution of eDNA was assessed, and occupancy modelling was used to optimise sampling strategies. Active pump filtration of 3 L water samples produced the most consistent detections of eel eDNA. Passive samplers deployed for 13 h yielded the highest eel eDNA concentrations, although they exhibited high inter-replicate variability. Sediment samples and short-duration passive samplers performed poorly, and collection issues with tow-net sampling led to its exclusion from further analysis. Eel eDNA concentrations were higher nearshore, particularly near the lake's outflow, potentially reflecting eel activity. Detection rates were higher for *A. australis* than for *A. dieffenbachii*, likely due to species-specific differences in abundance, distribution and eDNA dynamics. Occupancy models showed detection probability was driven by sampling method for *A. australis* and by both sampling method and location for *A. dieffenbachii*. Pump filtration and long-duration passive sampling provided the highest detection probabilities, with three replicates per site sufficient for reliable detection, although only nearshore for *A. dieffenbachii*. These findings highlight the importance of sampling method and location when designing eDNA surveys for fish in lakes, with broader relevance across eDNA applications, including single-species detection and metabarcoding.

Key words: Droplet digital PCR, environmental DNA, freshwater eel, lake, occupancy modelling, sampling design

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Introduction

Freshwater ecosystems are some of the most threatened on the planet, with biodiversity declining faster than in terrestrial or marine systems (Dudgeon et al. 2006; Stendera et al. 2012; Piczak et al. 2023; Dudgeon and Strayer 2025).

Fish communities comprise a key part of this biodiversity, often contributing the highest economic, cultural and recreational ecosystem services (Lynch et al. 2016). In Aotearoa New Zealand, the native shortfin eel (*Anguilla australis*) and the endemic longfin eel (*Anguilla dieffenbachii*) are ecologically, culturally and economically significant species. As important apex predators, eels occupy varied freshwater habitats, with longfin eels preferring inland lakes, rivers and ponds, whereas shortfin eels mainly inhabit lower-elevation waterways (Glova et al. 1998). Known collectively as tuna to Indigenous Māori, eels hold cultural significance and feature in Māori mythology as guardians of waterways, as well as traditional food (Parai 2021). Eels also support fisheries across Aotearoa New Zealand and are exported globally (Jellyman 2007). Populations of *A. australis* and *A. dieffenbachii* have declined significantly due to habitat loss and the blocking of migration pathways through practices such as wetland drainage, river channelling, irrigation schemes and hydroelectric dams (Noth et al. 2008; Jellyman and Harding 2012). *Anguilla dieffenbachii* is especially affected and has an IUCN status of 'Endangered' (Pike et al. 2019). Reliable and efficient monitoring tools are essential to inform the ongoing management and conservation of these species.

Monitoring freshwater fish populations is critical for effective conservation and management; however, traditional survey methods such as fyke and gill netting, trapping and sonar surveys are labour-intensive, invasive and can rarely provide the monitoring resolution required for effective management (Thomson-Laing et al. 2023). As a result, environmental DNA (eDNA) is gaining popularity as a non-invasive monitoring tool for aquatic organisms that improves temporal and spatial resolution for biodiversity estimates (Rees et al. 2014; Beng and Corlett 2020; Blancher et al. 2022). In theory, if a target species is present, the rapid diffusion of DNA should enable its detection throughout the aquatic environment, not just at the source (Rees et al. 2015). However, eDNA detection is influenced by numerous environmental and biological variables. In the water column, DNA can degrade relatively quickly from UV exposure, enzymatic breakdown and microbial assimilation, with persistence varying due to environmental conditions such as turbidity, transport, temperature and hydrology (Joseph et al. 2022; Scriver et al. 2023). Therefore, despite increased sensitivity using eDNA methods, challenges with false negatives (type II error) still occur when eDNA fails to detect a species even though it is present, often due to insufficient sensitivity or limitations in the sampling methods used (Burian et al. 2021).

A range of eDNA collection techniques are used in freshwater environments. Active water filtration via syringe or pump has been the most applied (Rees et al. 2014; Rourke et al. 2022). More recently, tow-net sampling, which uses boat movement to filter water through fine-mesh nets, has also been used (Schabacker et al. 2020; von Ammon et al. 2020). Passive sampling involves placing filter membranes into water to accumulate eDNA over time, meaning this *in situ* method avoids handling large volumes of water, does not require filtration equipment and turbidity is less of a concern since particle content does not interfere with effective eDNA capture (Bessey et al. 2021). Sediment sampling can be more integrative over time and provide historical DNA signals, and some studies have found higher concentrations of fish DNA in sediment compared to water (Picard et al. 2023); however, it may be more spatially

heterogeneous (Turner et al. 2015; Thomson-Laing et al. 2024). Evaluating the performance of these approaches in parallel is therefore critical for the robust interpretation of eDNA-based biodiversity results, standardising eDNA sampling protocols and improving comparability across studies. These considerations are highly relevant to single-species detection, metabarcoding and metagenomic applications, where biases in DNA capture and sampling design can influence both taxon detectability and inferred community composition (e.g. Peixoto et al. 2021; Yao et al. 2022; Altermatt et al. 2023).

Understanding the spatial distribution of eDNA within lentic systems is equally important for determining which species are present. In lakes, eDNA concentration is rarely uniform, and assessing how eDNA varies between pelagic and nearshore zones can help refine sampling designs to better capture spatial patterns in species occupancy (Hervé et al. 2022). Integrating these data with occupancy modelling can determine species-specific detection probabilities (Burian et al. 2021) and inform more targeted monitoring strategies.

Globally, more than 99% of lakes have a surface area less than 1 km^2 (Verpoorter et al. 2014), making small lakes highly representative of freshwater ecosystems worldwide. However, these systems are often characterised by high suspended solids and turbidity associated with eutrophication, which can pose significant challenges for successful eDNA sampling and detection (Kumar et al. 2022). This study therefore aimed to test the following objectives in a small, turbid lake system:

i. Quantitatively compare eDNA yield, detection consistency and total DNA concentration among different eDNA sampling methods.
ii. Assess the spatial distribution of eel eDNA across pelagic and nearshore zones.
iii. Compare eDNA detections with traditional fyke-net survey data.
iv. Apply occupancy models to estimate detection probabilities and inform future sampling strategies for freshwater eel monitoring.

The outcomes of this study will assist in optimising eDNA sampling designs in small, turbid lake systems and improve the ability to target fish with heterogeneous eDNA distribution.

Methods

Study lake

Samples were taken from Lake Moāwhitu (40°48'33"S, 173°48'41"E), which is located on Rangitoto ki te Tonga (D'Urville Island, Marlborough Sounds, Aotearoa New Zealand; Fig. 1). The lake has a surface area of 0.35 km^2, a mean depth of 8 m and a maximum depth of 14 m (Fig. 1; Parai 2021). Lake Moāwhitu is a low-lying, turbid lake heavily enriched with nitrogen and phosphorus and classified as hypertrophic (Parai 2021). Sampling was undertaken between 5 and 7 November 2024 at up to 10 sites using traditional fish survey methods and four different methods to collect water and sediment eDNA. The lake was partitioned into a spatial grid of sampling sites, with shallow sites < 4 m (S1, S2, S4–S6) considered nearshore and deeper sites 4–13.4 m (S3, S7–S10) considered pelagic (Fig. 1).

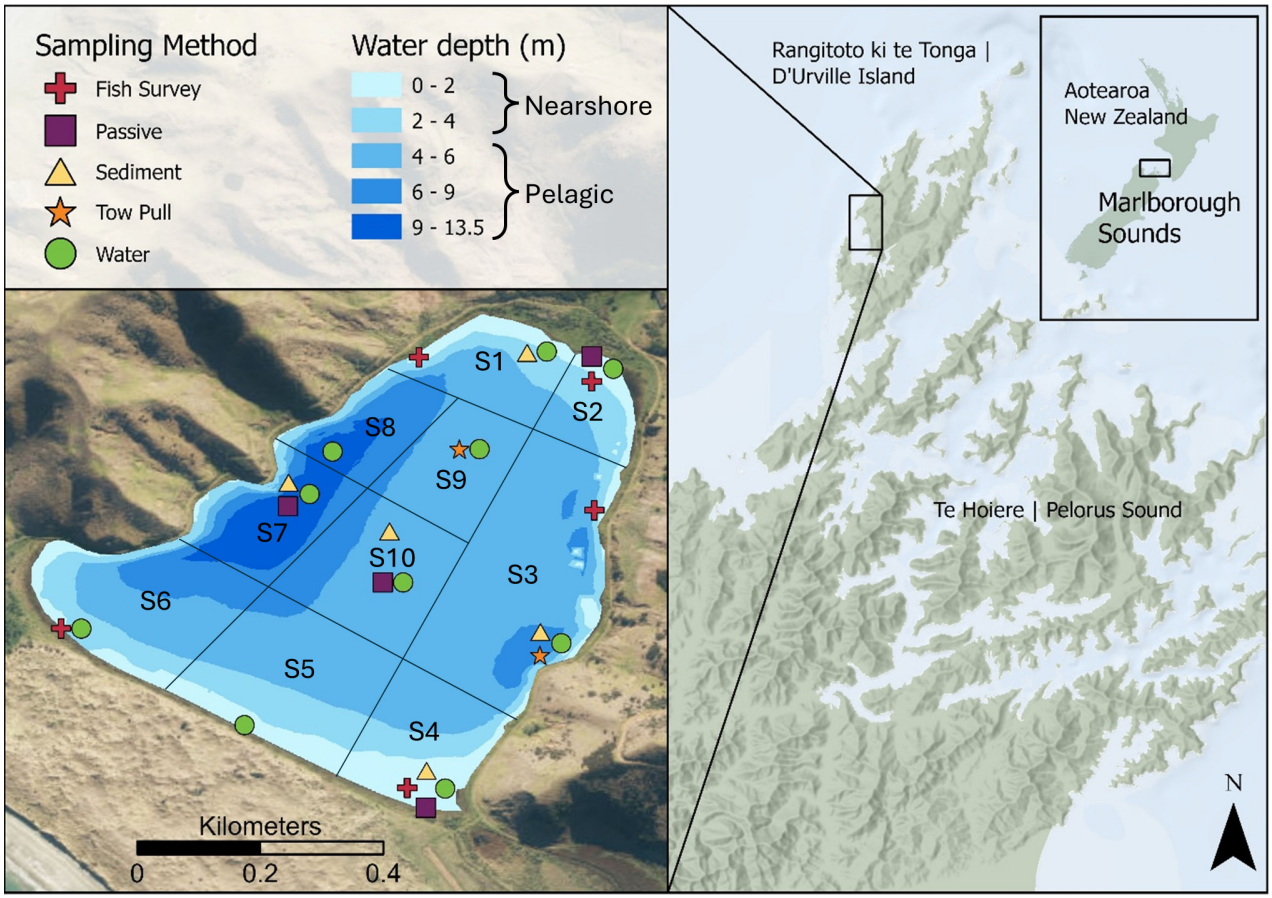

Figure 1. Location of Lake Moāwhitu within Aotearoa New Zealand, sampling sites and lake bathymetry (metres). Sampling sites (S1–S10) and sampling methods at each site are shown. Sites < 4 m depth (S1, S2, S4–S6) are classified as nearshore, and sites 4–13.4 m (S3, S7–S10) as pelagic.

Fish surveys

Anguilla dieffenbachii and *A. australis* were caught using fyke nets, which were set overnight (approximately 12 h) at five locations around the edge of the lake (Fig. 1). Eels captured in the nets were identified to species level, and total net weight was taken using hanging scales. Eels were then released back into the lake. Eels that escaped during capture were recorded as "unknown eels" and did not have an associated weight. Fish capture was carried out in accordance with the approved animal ethics application (AEC-2024-CAW-03; NMIT Animal Ethics Committee).

Environmental DNA sampling methods

During eDNA sample collection, disposable gloves were worn, and equipment, including tweezers, scissors and filter holders, was decontaminated using 10% bleach and ethanol between samples. All water filters and sediment samples were placed in labelled, sterile tubes and frozen (−20 °C) before DNA extraction. The following sampling methods were applied:

- Active water filtration ("Water") samples were collected using a battery-powered handheld pump (Grover Scientific; Australia). Water samples were collected in triplicate, consisting of three independent 3 L samples,

at 10 locations around the lake (Fig. 1). Water was filtered through 20 µm nylon filters (Millipore).

- Tow-net ("Tow") filter samples were collected from surface water along 10–20 m horizontal transects with a cruising-speed net (von Ammon et al. 2020) equipped with a modified cod-end with 20 µm nylon filter inserts (Pochon et al. 2023). Nets were pulled behind a boat at approximately 5 knots. Triplicate samples were taken at S3, with only one replicate at S9 and no sampling conducted at a third site due to challenges with the sampling method (i.e. clogged filters and trapped water in the filter device).
- Passive samplers (20 µm nylon filters attached to a stationary pole for nearshore sites or suspended from a buoy for pelagic sites) were immersed in water at four sites around the lake. At each site, there were three replicate filters, which were submerged for either 2 or 13 h ("Passive2" and "Passive13", respectively) before collection. These deployment durations were selected to represent a short submersion period and a practical day/overnight deployment, based on previous studies showing effective eDNA accumulation across exposure times ranging from approximately 0.5 to 72 h (Chen et al. 2022).
- A Ponar grab was used to collect surface sediment samples ("Sediment") from five sites in triplicate around the lake (Fig. 2). Approximately 10 g of the top 0.5 cm of sediment from the surface of the Ponar grab was carefully sampled using a sterile spatula.

DNA extraction

All molecular analyses were performed in controlled, designated sterile laboratories, ensuring a separate and sequential workflow to minimise cross-contamination. Prior to DNA extraction and PCR setup, the room was sterilised by UV for a minimum of 15 min, and all equipment and surfaces were sterilised with 10% bleach and ethanol. The PCR and template rooms were equipped with laminar flow cabinets to reduce contamination.

DNA was extracted from water filters using a PowerSoil Pro extraction kit (QIAGEN) following the manufacturer's protocol and automated QIAcube processing (QIAGEN). Extraction blanks containing the extraction kit buffers were used for every 24 samples. Sediment samples (6 g) were extracted using alkaline buffer extraction with ethanol precipitation, followed by a PowerSoil Pro kit (QIAGEN) (Thomson-Laing et al. 2022). Extracted total DNA concentration (ng/µL) was analysed in each sample using the nucleic acid application on a spectrophotometer (NanoDrop™ One, Thermo Scientific).

Droplet digital polymerase chain reaction (ddPCR)

Target eel species were amplified using previously designed droplet digital polymerase chain reaction (ddPCR) primers and probes (Thomson-Laing et al. 2021). The forward primer (A.aust16S-F: 5'–CCC AAA AGC AGC CAC CTG–3'), reverse primer (A.aust16S-R: 5'–AGG GGG TGG GGA GTT TAT TA–3') and PrimeTime probe (A.aust16S-P: 5'–/56-FAM/AAA GAA AGC/ZEN/GTT AAA GCT CCG A/3IABkFQ/–3') were used to target the mitochondrial 16S ribosomal RNA gene of *A. australis*. The forward primer (A.dieffCytB-F: 5'–GAT TCT TCG CAT TCC ACT

TCT TA–3'), a reverse primer (A.dieffCytB-R: 5'–GGA CTT TGT CTG CGT CAG AGT TT–3') and a PrimeTime probe (A.dieffCytB-P: 5'–/5-HEX/TCC TAC ATG/ZEN/ AAA CAG GAT CAA GCA ATC CA/3IABkFQ/–3') were used to target the mitochondrial *cytochrome b* (*cytb*) gene of *A. dieffenbachii* (Thomson-Laing et al. 2021).

The assays were run in multiplex, and each ddPCR reaction mix was 22 µL, containing 5 µL of sample DNA, 1 µL of each primer (forward and reverse) and probe (FAM and HEX) and 11 µL of ddPCR Supermix for Probes (No dUTP; Bio-Rad). One positive control, containing 1 µL of *A. australis* and *A. dieffenbachii* tissue DNA, and one negative control with MQ water were included in each ddPCR run. The following cycling protocol was used: 95 °C for 10 min to denature DNA, followed by 45 cycles of 94 °C (30 s) and 59 °C (1 min) and a final 98 °C for 10 min (Thomson-Laing et al. 2021). Following PCR amplification, the plate was placed into a QX200 for each droplet to be read using a two-colour (FAM/HEX) detection system (Bio-Rad). For each run, the baseline threshold for separating positive and negative droplets was manually chosen above the cluster of negative droplets to accommodate fluctuations in positive droplets. The sample was classed as positive if at least two replicates had one or more droplets, or one replicate had two or more droplets.

Data and statistical analysis

RStudio version 4.2.1 (RStudio Team 2021; R Core Team 2023) was used for statistical analysis and plotting graphs using the '*ggplot2*' package (Wickham 2016). A Shapiro–Wilk test was conducted to test the normality of the eel DNA concentration (copies/µL), as well as total eDNA concentrations (ng/µL), between sampling methods and location (i.e. nearshore or pelagic). Due to the violation of normality assumptions ($p < 0.05$), non-parametric tests (Kruskal–Wallis) were used to compare both eel and total DNA concentrations between sampling methods and between species. For sampling methods deployed in both pelagic and nearshore locations (i.e. Passive13, Passive2 and Water), an aligned rank transform (ART) ANOVA was used to test for the main effects of sampling method, in-lake location and their interaction on eel eDNA concentrations ('ARTool' package; Wobbrock et al. 2011).

Due to the consistency of eel eDNA concentrations recovered using Water samples and the spatial spread of this sampling method, the spatial distribution of eel DNA captured using Water samples was visualised with maps created using ArcGIS (ESRI 2024).

Occupancy modelling

RStudio version 4.2.1 was used to perform occupancy modelling. Occupancy modelling was used to assess eel eDNA distribution in Lake Moāwhitu, evaluate the influence of sampling method and in-lake location on detection probability and simulate best-practice sampling guidelines. Occupancy modelling uses presence–absence data to estimate eDNA distribution across space and time while accounting for imperfect detectability. Fish survey methods were not included in the models due to a lack of site replication.

Assessed occupancy (Ψ) and detection probabilities (p) for *A. australis* and *A. dieffenbachii* eDNA were undertaken using the '*unmarked*' package (Fiske

and Chandler 2011). DNA concentration data were converted to binary presence (1) and absence (0) values. Single-season occupancy models were developed for each species using data from 10 sites (Fig. 1; five nearshore, five pelagic) and four sampling methods (Sediment, Water, Passive13, Passive2). Tow samples were excluded due to insufficient replication.

Each site (n = 10) was treated as a sampling unit, and all methods and replicate samples (n = 3 per method) within a site were treated as independent repeated surveys. Consequently, the number of repeated surveys per site ranged from 3 to 12, depending on the number of methods used at each site. For each species, the following were derived:

- $\Psi_{naïve}$ = naïve occupancy, calculated as the proportion of sites with at least one positive eDNA detection.
- Ψ = model-estimated occupancy, probability that a site is occupied by eel eDNA.
- p = probability of detecting eel eDNA in a single site replicate, given that the site is occupied.

The basic assumptions of single-season occupancy models were considered in this study. The closed-system assumption was met because sites were only visited once, ensuring there was no change in occupancy status during the survey. Sites and replicates were assumed to be independent due to distinct spatial separation between sites, and replicate samples were taken separately. However, some non-independence between water samples cannot be ruled out due to potential eDNA transport in the water column. The assumption of no false-positive detections was addressed using species-specific assays with a high degree of specificity, ddPCR technical replication and implementation of laboratory negative controls to minimise the potential for false positives.

For each species, a set of *a priori* single-season occupancy models was fitted to test (1) whether site location (nearshore vs pelagic) influenced occupancy (ψ) and (2) whether sampling method or site location influenced detection probability (p). The sampling method was included only as an observation-level covariate on detection probability (p), while site location was included as both a site-level covariate on occupancy (ψ) and an observation-level covariate on detection probability (p). The following models were tested:

- ψ(.), p(.)
- ψ(.), p(Sampling method)
- ψ(.), p(Site location)
- ψ(Site location), p(.)
- ψ(Site location), p(Site location)
- ψ(Site location), p(Sampling method)
- ψ(.), p(Site location + Sampling)
- ψ(Site location), p(Site location + Sampling Method)

Occupancy (ψ) and detection probability (p) were estimated on the logit scale, and predictions were back-transformed to the probability scale (0–1) with 95% confidence intervals using the *predict()* function in "unmarked". All fitted models were ranked according to Akaike information criterion (AIC) values

provided in the model outputs, with Akaike weights calculated. Covariates that impacted model fit were identified, and models with lower AIC values and fewer predictors were favoured.

Occupancy simulations

RStudio version 4.2.1 was used to perform occupancy simulations. Detection probability estimates derived from the best-performing occupancy models were used to simulate detection histories and to determine the optimised sampling design for each eel species in Lake Moāwhitu. Simulations were run using these pre-determined detection probabilities across sampling methods for *A. australis* and across sampling methods and site locations (i.e. nearshore and pelagic) for *A. dieffenbachii*.

Two simulation approaches were defined:

1. Assuming constant occupancy (ψ = 1, i.e. eel eDNA is omnipresent), the combination of sites and replicates representing the minimum effective sampling design was determined for each sampling method (*A. australis*) and for each sampling method and site location (*A. dieffenbachii*).
2. Across a range of eDNA occupancies (ψ = 0–1), the minimum sampling effort required to achieve reliable detection was determined for each sampling method or site location, given the estimated detection probabilities.

For the first approach, simulations were conducted using a constant occupancy (ψ = 1) and empirical detection probabilities for sampling method or site location to determine the optimal balance between the number of replicates and the number of sites. For each predefined sampling scenario (2–6 replicates, up to 50 sample sites), 10,000 detection histories were run as described in Guillera-Arroita et al. (2010). Minimum meaningful sampling effort was defined as the lowest effort at which the estimated probability of empty histories ≈ 0% (no empty histories observed in 10,000 simulations) and estimation error was low (MSE < 0.05).

For the second approach, simulations were run across a range of eel eDNA occupancies. The minimum meaningful number of replicates identified for each species in the first approach (three replicates for *A. australis*, three replicates for nearshore sites and six replicates for pelagic sites for *A. dieffenbachii*) was used. Using empirical detection probabilities for each sampling method or site location, 10,000 detection histories were generated for occupancies ranging from 0.1 to 1. From these simulations, the minimum number of sites required to achieve 99% confidence of detecting eel eDNA in at least one replicate was determined for each occupancy level.

Results

Fish surveys

The five fish surveys confirmed the presence of *A. dieffenbachii* and *A. australis* in Lake Moāwhitu, with *A. australis* and *A. dieffenbachii* caught in 100% and 80% of surveys, respectively. A total of 14 *A. dieffenbachii* and 12

A. australis individuals were captured, with the highest number of *A. australis* at site 3 (five eels) and the highest number of *A. dieffenbachii* at site 6 (six eels; Table 1).

Table 1. Fyke-net captures of freshwater eel species at five different sampling sites in Lake Moāwhitu. NA = not available. "Unknown" eels escaped from capture before species-level morphological identification was carried out.

		Anguilla australis	*Anguilla dieffenbachii*	Unknown eel
Site 1	Number	1	0	0
	Weight (kg)	0.35	0	0
Site 2	Number	3	5	3
	Weight (kg)	2.35	3.69	NA
Site 3	Number	5	1	0
	Weight (kg)	3.09	0.47	0
Site 4	Number	2	2	0
	Weight (kg)	1.75	1.43	0
Site 6	Number	1	6	1
	Weight	0.45	5.55	NA
Total	**Number**	**12**	**14**	**4**
	Weight (kg)	**7.99**	**11.14**	NA
	No. sites detected	**5**	**4**	**2**

Total eDNA concentrations

There was a significant difference in total DNA concentration (ng/μL) between the sampling methods (x^2 = 58.38, df = 4, p < 0.001; Suppl. material 1). Water (23.19 ± 1.03 ng/μL) and Tow methods (26.52 ± 0.98 ng/μL) yielded the highest overall eDNA concentrations (ng/μL). However, the Water samples exhibited the greatest variability (11–43 ng/μL). Sediment samples exhibited lower concentrations (5.34 ± 1.0 ng/μL). Both passive sampling methods resulted in the lowest concentrations (< 3.89 ng/μL).

Targeted eel eDNA concentrations

Eel eDNA concentrations varied greatly between samples (0–3892 copies/μL DNA, summed across species), depending on the eDNA sampling method and location (pelagic or nearshore; Fig. 2). No eDNA from either species (*A. australis* and *A. dieffenbachii*) was detected in any negative controls.

Eel eDNA concentrations differed significantly between methods (Kruskal–Wallis; x^2 = 33.94, df = 4, p < 0.001), with the Passive13 method yielding significantly higher eel eDNA concentrations (mean ± standard error: 217.90 ± 163.3 copies/μL) compared to all other methods (p < 0.001; Fig. 2A). Although the Water method did not accumulate as much eel eDNA (0.30 ± 0.08 copies/μL) as the Passive13 method, its lower variability between sites suggests it offers more stable and repeatable results (Fig. 2A). Tow samples resulted in a similar eel eDNA concentration to Water samples, albeit with sample collection challenges (0.27 ± 0.1 copies/μL). Other

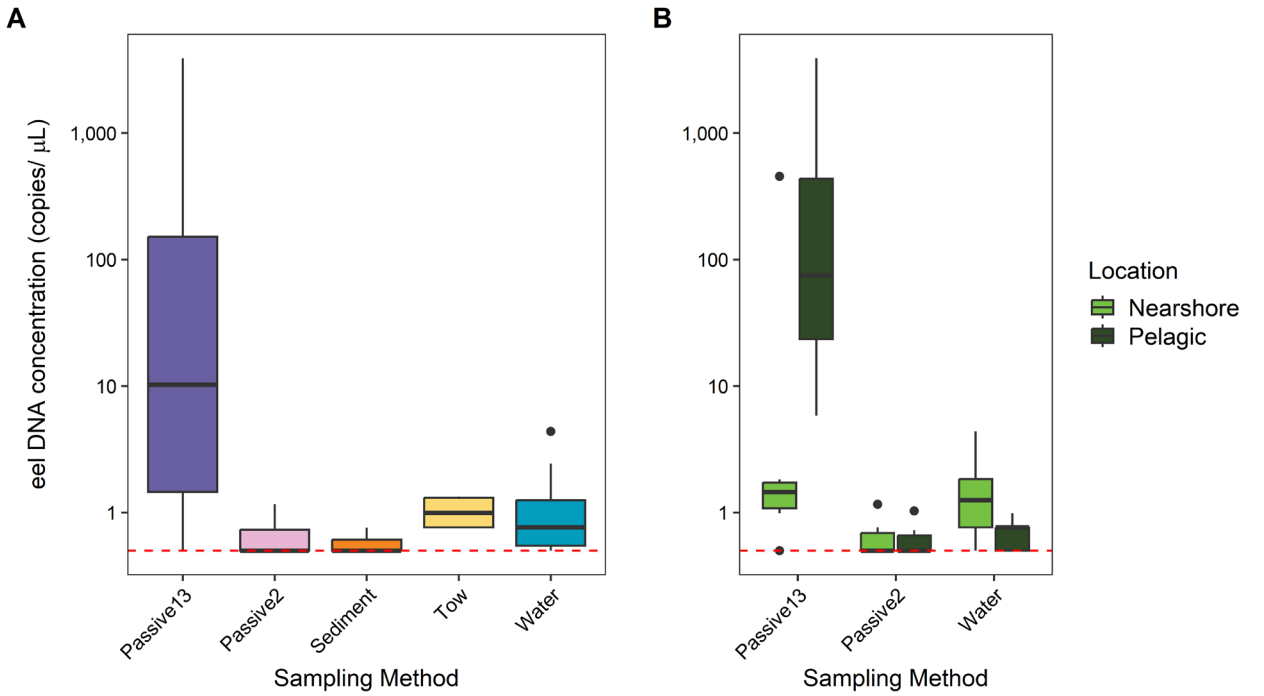

Figure 2. Comparison of total eel DNA concentration (summed copies/µL for *Anguilla dieffenbachii* and *Anguilla australis*) between **A** All sampling methods and **B** Sampling methods at different locations (nearshore and pelagic). Values are plotted on a logarithmic (10) scale. The red dashed line indicates the ddPCR no-detection value. Box plots show the median (centre line), interquartile range (boxes; 25th–75th percentiles) and whiskers extending to 1.5× the interquartile range.

sampling methods, including Passive2 and Sediment, generally resulted in lower eel eDNA concentrations (≤1 copies/µL).

When comparing sampling methods applied in two different locations (nearshore and pelagic; Fig. 2B), there were significant effects of location and sampling method, as well as a significant interaction between location and sampling method (ART-ANOVA, Location × Sampling Method interaction: $F_{2,48}$ = 6.69, p = 0.0027). *Post hoc* comparisons revealed location-dependent differences in eel eDNA concentrations that varied among sampling methods. For both Passive2 (Tukey-adjusted p = 0.041) and Water (p < 0.001) methods, eel eDNA concentrations were significantly higher in nearshore compared to pelagic sites. In contrast, eel eDNA concentrations did not differ between Passive13 samples in nearshore and pelagic sites (p = 0.46). The absence of a detectable difference in Passive13 sampling between locations may reflect the high variability among replicates (397.7 ± 322.8 copies/µL), with eDNA concentrations spanning several orders of magnitude, particularly in nearshore samples. Higher eel eDNA concentrations captured with Passive13 methods compared to other sampling methods were evident in nearshore but not pelagic sites.

Spatial distribution of eDNA using active pump filtration

Using the Water method, *Anguilla australis* was detected at 100% of the sites compared to *A. dieffenbachii*, which was detected at 50% (Fig. 3). Overall, significantly more DNA was detected for *A. australis* (0.51 ± 0.14 copies/µL) compared to *A. dieffenbachii* (0.089 ± 0.03 copies/µL; x^2 = 12.99, df = 1, p < 0.001). The highest abundance of eDNA from both species was found around the

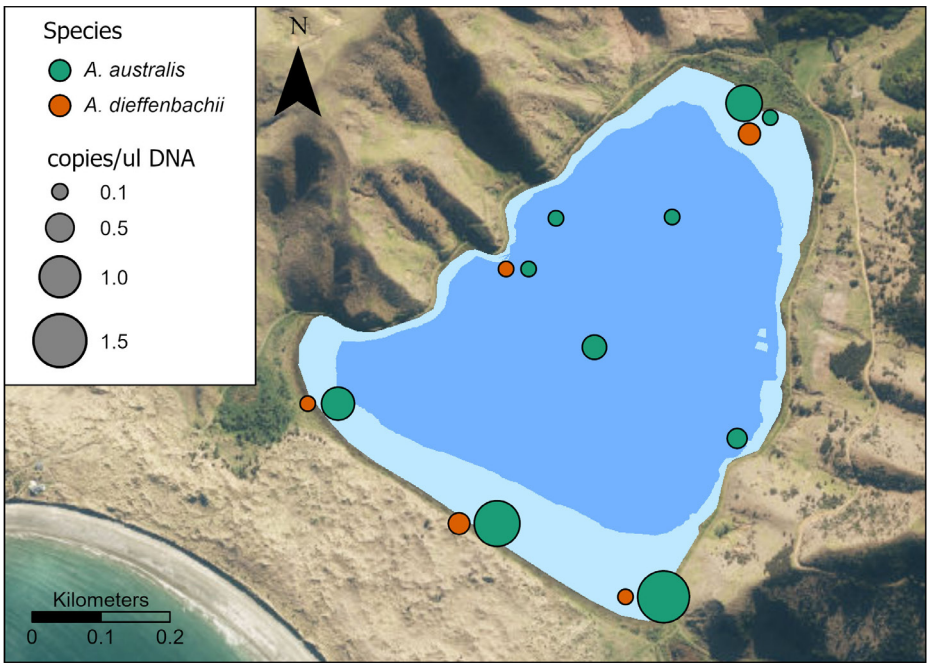

Figure 3. Spatial distribution of *Anguilla australis* and *Anguilla dieffenbachii* environmental DNA (eDNA) concentrations at Lake Moāwhitu derived from 3 L active pump filtration samples (Water). Values are means calculated from three replicates at each site. Dark blue indicates the pelagic zone (> 4 m depth), and light blue indicates the nearshore zone (< 4 m depth).

nearshore, with *A. dieffenbachii* only detected at one pelagic site. For *A. australis*, concentrations were highest along the southern lake edge (> 0.8 copies/μL) and lower in the pelagic sites (< 0.4 copies/μL; Fig. 3).

Other than the positive detection of eels using eDNA methods alongside the successful capture of eels using traditional fyke-net methods (Fig. 4A), there were no evident patterns between the eel eDNA concentrations measured and the total site weight of eels captured at the same site (linear regression: $r^2 < 0.2$, $p > 0.05$; Fig. 4B).

Occupancy and detection probabilities for eDNA

The best-fitting occupancy models, run separately for each species, assumed constant occupancy and included 'sampling method' as a sample covariate for *A. australis* or both 'sampling method' and 'site location' as sample covariates for *A. dieffenbachii* (Table 2). These models were ranked highest for each species, respectively.

Naïve estimates of eel occupancy were 1.0 for *A. australis* and 0.7 for *A. dieffenbachii* (Table 3). Because naïve occupancy was already extremely high, the model-derived occupancy estimates (ψ (95% CI): 0.99 (0–1)) had very low precision and were not considered interpretable. As a result, only detection probability outputs were considered from the models (Table 3).

For *A. australis*, detection probability varied among sampling methods, with the highest probability associated with the Passive13 method ($p = 0.92$), followed by Water sampling ($p = 0.67$), Passive2 ($p = 0.33$) and Sediment sampling ($p = 0.27$; Table 3). Detection probabilities exceeded 0.25 across all methods for this species.

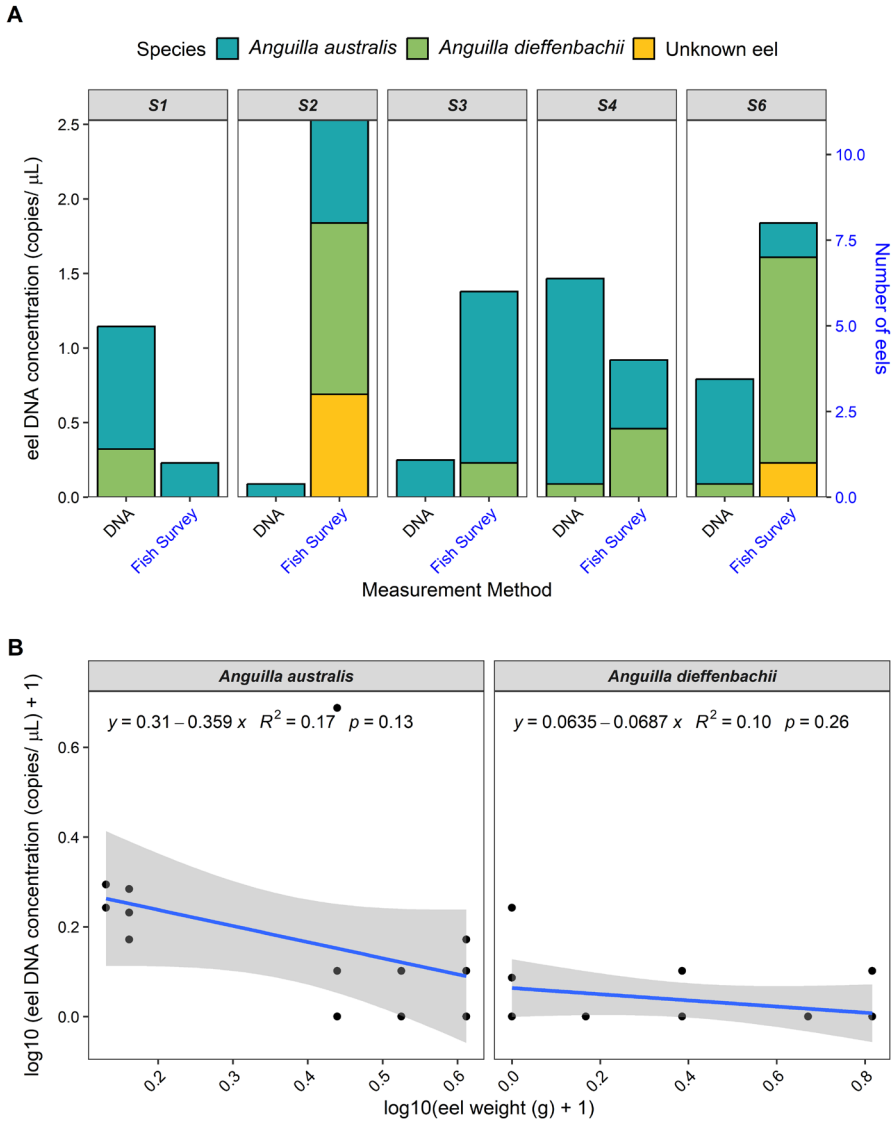

Figure 4. A Eel environmental DNA (eDNA) concentrations at sites where available fish survey data were collected, shown alongside corresponding eel counts and **B** Log10-transformed linear relationship between eel eDNA concentration and total eel weight at paired sites.

For *A. dieffenbachii*, detection probability varied with both sampling method and site location. Passive13 and Water methods generally yielded the highest detection probabilities, particularly in nearshore sites (p = 0.27 and 0.37, respectively), whereas detection probabilities for all sampling methods were consistently lower in pelagic locations (p < 0.1). Sediment sampling produced extremely low detection probabilities at both nearshore and pelagic sites (p < 0.001). Across all methods and locations, detection probabilities for *A. dieffenbachii* were lower than those observed for *A. australis* (p < 0.37; Table 3).

Occupancy simulations

To estimate minimum sampling effort, simulations were run to determine the minimum number of sampling sites required across a range of replicate numbers (2–6) to detect eel eDNA in a system where occupancy = 1 (i.e. eDNA of the species

Table 2. Ranking of the four fitted models for *Anguilla australis* and *Anguilla dieffenbachii* on Akaike information criterion (AIC) values. The covariates considered in each model are noted in brackets. Occupancy (ψ) and detection probability (*p*).

Species	Fitted occupancy model	AIC	ΔAIC	Model weight
Anguilla australis	ψ(.), *p*(Sampling method)	87.75	<0.001	0.531
	ψ(.), *p*(Site location + Sampling Method)	89.75	1.995	0.196
	ψ(Site location), *p*(Sampling method)	89.75	1.997	0.196
	ψ(Site location), *p*(Site location + Sampling Method)	91.75	3.994	0.072
	ψ(.), *p*(.)	98.48	10.727	0.002
	ψ(.), *p*(Site location)	100.45	12.698	0.001
	ψ(Site location), *p*(.)	100.48	12.727	0.001
	ψ(Site location), *p*(Site location)	102.45	14.697	0.000
Anguilla dieffenbachii	ψ(.), *p*(Site location + Sampling Method)	57.50	<0.001	0.329
	ψ(.), *p*(Site location)	58.00	0.502	0.256
	ψ(Site location), *p*(Site location + Sampling Method)	59.50	2.000	0.121
	ψ(Site location), *p*(Site location)	60.00	2.502	0.094
	ψ(.), *p*(Sampling Method)	60.29	2.793	0.082
	ψ(.), *p*(.)	61.11	3.605	0.054
	ψ(Site location), *p*(Sampling Method)	61.58	4.074	0.043
	ψ(Site location), *p*(.)	63.11	5.604	0.020

Table 3. Occupancy (ψ) and detection probability (*p*) results from the best-performing fitted model (ψ(.), *p*(Sampling method)) for *Anguilla australis* and (ψ(.), *p*(Site location + Sampling Method)) for *Anguilla dieffenbachii* based on Akaike Information Criterion (AIC) values.

Species	Ψ_{naiive}	Sampling method	Site location	*p* (95% CI)
Anguilla australis	1	Passive13	*NA*	0.916 (0.587–0.988)
		Water	*NA*	0.667 (0.483–0.810)
		Passive2	*NA*	0.333 (0.131–0.624)
		Sediment	*NA*	0.267 (0.104–0.533)
Anguilla dieffenbachii	0.7	Passive13	Nearshore	0.270 (0.068–0.653)
			Pelagic	0.063 (0.009–0.342)
		Water	Nearshore	0.370 (0.177–0.616)
			Pelagic	0.097 (0.023–0.327)
		Passive2	Nearshore	0.138 (0.019–0.570)
			Pelagic	0.028 (0.002–0.262)
		Sediment	Nearshore	<0.00001 (0–1)
			Pelagic	<0.00001 (0–1)

is everywhere). Detection probabilities were derived from the best-performing occupancy models, which included sampling covariates and constant occupancy.

For *A. australis*, two and four eDNA replicates minimised the number of sampling sites required across all methods if occupancy = 1 (Table 4). For example, the Passive13 method (highest detection probability for *A. australis*; $p = 0.9$) required only two sites with two replicates, whereas Sediment sampling (lowest detection probability for *A. australis*; $p = 0.27$) required six sites sampled with four replicates (Table 4).

Table 4. Minimum sampling effort required to detect eel environmental DNA (eDNA) under a scenario of complete occupancy (ψ = 1), based on detection probabilities derived from top-ranked occupancy models for each eel species. Values represent the minimum number of sampling sites and replicates per site required to achieve reliable detection, defined as no empty detection histories and an MSE < 0.05 estimate error. Total sampling effort is calculated as the product of sites and replicates.

Species	Sampling method	Site location	Detection probability	Number of sites	Number of replicates	Total sampling effort
Anguilla australis	Passive13	NA	0.917	2	2	4
	Water	NA	0.667	3	3	6
	Passive2	NA	0.333	6	3	18
	Sediment	NA	0.267	6	4	24
Anguilla dieffenbachii	Passive13	Nearshore	0.270	12	2	24
		Pelagic	0.063	23	5	115
	Water	Nearshore	0.370	6	3	18
		Pelagic	0.097	12	6	72
	Passive2	Nearshore	0.133	18	3	54
		Pelagic	0.028	43	6	258
	Sediment	Nearshore	<0.00001	<50	*NA*	*NA*
		Pelagic	<0.00001	<50	*NA*	*NA*

For *A. dieffenbachii*, between two and three eDNA replicates were required to minimise the number of sites if sampling sites were nearshore, with replicate numbers depending on sampling method. Between five and six eDNA replicates were required to minimise sampling effort at pelagic sites. The corresponding number of sites was much greater across all methods and locations compared to *A. australis*, reflecting the lower detection probability for *A. dieffenbachii*. For example, Water sampling in a nearshore site (p = 0.37) required six sites with three replicates, whereas all other site–method combinations required more than 12 sites. Detection probabilities in pelagic sites were consistently low across all methods (p < 0.1), corresponding with a high number of sites (≥ 12 sites) and corresponding total sampling effort (≥ 72 samples). Methods with very low detection probability (i.e. Sediment; < 0.00001) required an unmanageable amount of sampling effort, exceeding 50 sampling sites irrespective of replication.

Three replicate samples represented the most efficient sampling approach for *A. australis* and nearshore sampling for *A. dieffenbachii*. For pelagic sampling for *A. dieffenbachii*, six replicates were more efficient. Simulating the required number of sites with three replicates or six replicates across a range of occupancies (0.1–1) indicated that, as species occupancy declines, the number of required sites increases. For example, for Water sampling at occupancies < 0.4, more than 10 sites with three replicates are required for *A. australis*; at occupancies < 0.5, more than 10 nearshore sites with three replicates are required for *A. dieffenbachii*; and at occupancies < 0.8, more than 10 pelagic sites with six replicates are required (Fig. 5).

Considering the naïve occupancies for *A. australis* and *A. dieffenbachii* in Lake Moāwhitu (ψ = 1 and ψ = 0.7, respectively), these simulations support that the most efficient sampling strategy for *A. australis* would be 1–2 sites, with three replicates using either Passive13 or Water sampling to provide 99%

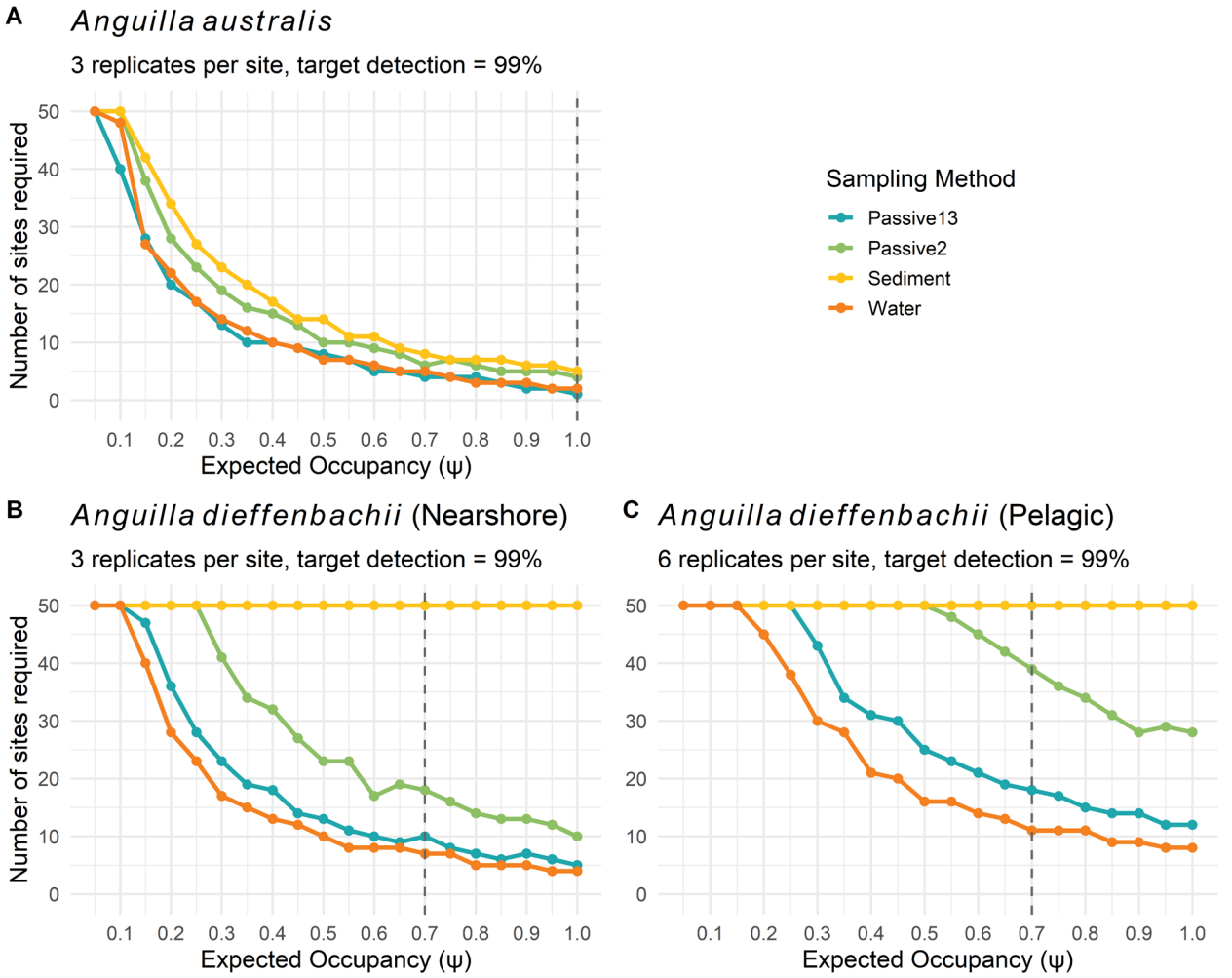

Figure 5. Minimum recommended number of sites for environmental DNA (eDNA) sample collection **A** Three replicate samples for *Anguilla australis*, **B** Three replicate samples for *Anguilla dieffenbachii* (nearshore) and **C** Six replicate samples for *A. dieffenbachii* (pelagic) to detect the presence of eel eDNA with 99% confidence. Points indicate modelled minimum site numbers at different predicted occupancy values for each sampling method. The dotted line indicates the estimated occupancy of each eel species in Lake Moāwhitu.

confidence of detection (Fig. 5A). For *A. dieffenbachii*, the most efficient sampling strategy would be Water sampling at seven nearshore sampling sites with three replicates to provide 99% confidence of detection (Fig. 5B). This is a much lower sampling effort than the most efficient pelagic sampling, which would require Water sampling at 11 sites with six replicates (Fig. 5C).

Discussion

This study investigated how sampling method, in-lake location and species abundance influenced the detection and concentration of eel eDNA in a turbid, lowland lake. This study found that active water filtration and longer-duration passive samplers produced the highest and most consistent eDNA detections in systems with omnipresent target eDNA. In the studied lake, eel eDNA was spatially patchy, with higher concentrations in nearshore habitats, and detection differed markedly between *A. australis* and *A. dieffenbachii*, despite both occupancy estimates being close to 1 (i.e. the target eDNA is inferred to be present

at all sampled sites after accounting for imperfect detection). Occupancy modelling determined that the most efficient sampling design for eel eDNA in Lake Moāwhitu (i.e. a lake with high eel presence) was species-specific, and detection probabilities varied with both method and site location.

Sampling method impact on eel eDNA detection

Sampling method strongly affected the detection of eel eDNA. Sampling method has been widely established as a critical factor in the successful eDNA detection of fish (e.g. Nevers et al. 2018; Stevens et al. 2024). In this study, passive samplers deployed for 13 h accumulated the highest amount of eel DNA, specifically for *A. australis*, despite capturing relatively low total DNA. This confirms that passive sampling is an effective method for capturing fish eDNA in lakes, as seen in other aquatic studies (Bessey et al. 2021; Chen et al. 2024; Cananzi et al. 2025). However, deploying the passive samplers for only 2 h yielded very low levels of eel DNA, demonstrating that deployment length was critical. Other research has also found significant links between longer deployment durations of passive samplers and higher eDNA yields (von Ammon et al. 2025). There was high variation among replicate passive samples, suggesting this method is less consistent and potentially unreliable with less sampling effort, a finding that has also been found for fish eDNA in a marine environment (Thompson et al. 2025). This variability was retained in the statistical analyses and contributed to greater within-method variance. The passive samplers were directly exposed to the water column, creating the possibility for direct contact with an eel. This may explain the highly variable results; for instance, the highest concentration of eel eDNA was 3000-fold the method average. Further investigation across a broader range of deployment durations, lake sizes and water quality conditions would help to better determine the potential and reliability of passive samplers in lakes.

Active pump filtration of 3 L water samples captured a slightly lower concentration of eel DNA but performed more consistently across replicates and species. This further supports a wide base of research where active water filtration is the most widely used method of aquatic eDNA collection (Tsuji et al. 2019; Shu et al. 2020). In this study, 3 L was the maximum volume that could be filtered within a practical time frame, as filter clogging substantially slowed filtration beyond this point. To further increase eDNA detection, the active pump filtration method could filter larger volumes of water, which has been shown to capture more eDNA from the environment, especially from rare species (Sepulveda et al. 2019). The consistency in eDNA capture using active pump filtration would make it a preferable method for ensuring repeatable results across different sampling events.

Although the tow-net method had similar concentrations of eel DNA compared to water filtration, there were issues with the filters becoming quickly clogged and water being retained in the sampling device, which made sample recovery difficult. This tow-net method was developed for marine environments (von Ammon et al. 2020; Pochon et al. 2023), where high algal biomass and suspended solids are less of an issue. Consequently, this method may be less suitable for high-turbidity lake environments. Additional considerations include the requirement for a boat to deploy the tow-net method, which introduces

further logistical and health and safety constraints. Therefore, it may be more practical to use in larger, clearer, more spatially heterogeneous lake systems.

Sediment sampling yielded the lowest detection rates for eel DNA. This was especially notable for *A. dieffenbachii*, which was not detected at all in the sediment samples. Unlike in water, where eDNA is more evenly dispersed, the detection of eDNA in sediment is more variable, influenced by transportation and the successful deposition and adsorption of eDNA to surface sediments (Huston et al. 2023). Therefore, eDNA may accumulate in localised areas, such as where carcasses or faeces have directly fallen onto the surface sediment, leading to uneven distribution of sediment eDNA. This pattern was evident in Lake Moāwhitu, where total DNA concentrations ranged from 0–15 ng/μL across five sites, and eel eDNA was detected in only a small subset of sediment samples. Sediment characteristics may further reduce eDNA detectability in this system. Picard et al. (2023) showed that low-density, organic-rich sediments are poor at preserving eDNA. The nutrient-rich sediments in hypertrophic Lake Moāwhitu likely support high microbial activity, which accelerates DNA degradation through enzymatic breakdown and microbial consumption of extracellular DNA as a nutrient source (Joseph et al. 2022).

It is important to consider that, in addition to sampling method, other factors such as filter size, type and sample volume can influence eDNA detection; however, assessing these was outside the scope of this study (Li et al. 2018). In this study, a large pore-size filter (20 μm) was used to enable greater water quantities to be filtered both through active pump filtration (i.e. 3 L) and surface tow methods. Turbid and algal-rich environments often require a larger pore size for effective water filtration (Holmes et al. 2024). The trade-off is that, while larger pore sizes allow greater water volumes to be processed, they may fail to retain fine particles or extracellular DNA, although fish eDNA has been successfully captured using 20 μm filters (Cooper et al. 2022). Future work comparing filter types and sizes across a range of lake conditions would help determine the optimal filtration strategies for lowland, turbid lake systems.

Spatial distribution of eel eDNA

Across all sampling methods, the concentration of eel DNA was impacted by site location, with a positive association with nearshore sites compared to pelagic sites. When focusing solely on water filtration, eDNA was unevenly distributed for both species, with greater eDNA concentrations and detection, especially for *A. dieffenbachii*, in nearshore areas. Littoral and nearshore zones have been found to have higher biodiversity and greater eDNA concentrations, particularly for fish species that spend more time in these habitats (Lawson Handley et al. 2019; Hervé et al. 2022). Limited water circulation and rapid deposition of eDNA from the water column in lakes can further contribute to patchiness by allowing eDNA particles to accumulate in sheltered areas (Eichmiller et al. 2014). The patchiness highlights that more than one sampling location within a lake is required for representative results when targeting fish eDNA (Lawson Handley et al. 2019; Picard et al. 2023; Yates et al. 2023).

For eels, this patchiness in eDNA may be linked to species-specific habitat use and movement patterns. For example, juvenile shortfin eels typically occur in higher densities near the shore, likely due to greater food availability and

their ability to exploit fluctuating water levels and newly inundated habitats (Jellyman 1989; Jellyman 1997). In Lake Moāwhitu, the highest concentrations of eel eDNA were found in the south of the lake, near the outflow to the associated wetland, which is directly linked to the ocean. This area serves as both an entry point for glass eels migrating into the lake and as a staging area for mature eels preparing to migrate downstream to spawn (Jellyman et al. 1999; Parai 2021). The outflow may also concentrate food resources, further attracting eels and contributing to elevated eDNA concentrations (Jellyman et al. 1999).

Species differences in eDNA detection

Positive detections of eel eDNA in Lake Moāwhitu were consistent with fish survey results, as eels were physically captured at all five nearshore survey sites. *Anguilla australis* was caught at 100% of sites, and *A. dieffenbachii* was captured at 80% of sites. This pattern aligns with the eDNA results, which showed consistently higher concentrations of *A. australis* eDNA compared to *A. dieffenbachii* eDNA across sampling methods. Studies have demonstrated that fish species presence, spatial distribution and habitat use strongly influence eDNA detection probability and concentration (Eichmiller et al. 2014). Consequently, even subtle differences in species distribution or habitat use within the lake may contribute to the more variable detection of *A. dieffenbachii*.

In addition to differences in spatial occupancy, species-specific eDNA shedding and degradation rates may further influence detectability. Species release different amounts of eDNA due to physiology, behaviour and environmental interactions (Stewart 2019). Experimental work on eels in Aotearoa New Zealand has shown that *A. australis* and *A. dieffenbachii* shed eDNA at different rates under different conditions, despite being closely related species (Che-Pelicier et al. 2025). These differences may be linked to species-specific differences, such as mucus characteristics (Tsutsui et al. 2016). While more ubiquitous distribution is the likely cause of higher *A. australis* eDNA detection in this study, species-specific shedding characteristics may further contribute to the observed differences in eDNA concentration between species (Klymus et al. 2015).

Optimising sampling effort

In this study, naïve occupancy of *A. australis* eDNA was extremely high (100%), while occupancy for *A. dieffenbachii* was moderately high (70%). This aligned with the presence and absence of *A. australis* and *A. dieffenbachii* in the nearshore fish surveys. This supports historical knowledge of Lake Moāwhitu, where eel populations are known to be persistent (Parai 2021). These occupancy values also suggest that, when present in Lake Moāwhitu, eel eDNA is generally well distributed. Despite this, detection probability was strongly species-dependent, with *A. australis* consistently achieving much higher detection probabilities than *A. dieffenbachii*. For example, when using the active filtration method, *A. australis* had nearly twice the likelihood of detection compared with *A. dieffenbachii*, highlighting the challenge of reliably detecting longfin eels despite moderately high occupancy. Detection probability for *A. australis* was strongly influenced by sampling method, whereas detection of *A. dieffenbachii* was influenced by both sampling method and site location. For both species,

long-duration passive samplers and water filtration produced the highest detection probabilities, and for *A. dieffenbachii*, nearshore sampling was necessary to achieve higher detection probabilities.

Using empirically derived detection probabilities, occupancy simulations provide practical guidance for designing efficient eel eDNA surveys in turbid, lowland lakes such as Lake Moāwhitu with high eel occupancy (> 70%). Simulation-based approaches have been widely used to optimise eDNA sampling designs by balancing detection probability, number of sites and replication effort (Guillera-Arroita et al. 2010; Burian et al. 2021; Fukaya et al. 2022; Steiner et al. 2022; Picard et al. 2023). For *A. australis*, the relatively high detection probability across methods meant that only two or three replicates per site were required, and the Passive13 and Water methods achieved reliable detection with as few as two sampling sites. Sediment and Passive2 methods were still feasible, although at much greater sampling effort (i.e. > 18 total samples).

In contrast, the lower detection probabilities for *A. dieffenbachii* required substantially greater sampling effort: Water sampling was the most effective method and still required three replicates per nearshore site at seven sites and six replicates per pelagic site at 11 sites to achieve robust detection. Other sampling methods with very low detection probabilities (e.g. Sediment and Passive2) were impractical for *A. dieffenbachii* detection, requiring unrealistically large sampling effort (i.e. > 50 total samples).

These simulations also allow sampling designs to be extrapolated to scenarios with different occupancy levels, using various eDNA sampling methods and sampling locations. As occupancy declines, the number of sites required increases disproportionately (Schmelzle and Kinziger 2016; Steiner et al. 2022). These findings can therefore be used to determine the appropriate number of replicates and sites needed to achieve a robust eDNA sampling design that accounts for both method- and species-specific detection probabilities, as well as varying occupancy. This approach is particularly useful for informing conservative survey designs when eel occupancy is unknown or expected to be low. For example, assuming a low-occupancy scenario (e.g. $\psi \approx 0.2$), a survey should prioritise nearshore water sampling with triplicate samples collected across 20–30 sites to achieve a high probability of detection or confidently infer absence given the estimated detection probabilities.

Similar simulation-based approaches have been successfully applied to guide eDNA sampling design, efficiency and detection likelihood in other systems and for other organisms (Lugg et al. 2018; Peixoto et al. 2023; Mace et al. 2025). A consistent finding across these studies is that species with patchier distributions and lower occupancy require substantially greater spatial replication and sampling effort to achieve reliable detection (Fukaya et al. 2022; Steiner et al. 2022; Peixoto et al. 2023; Picard et al. 2023). The results of this study follow this pattern, with *A. dieffenbachii* requiring greater effort than *A. australis* and pelagic habitats requiring more sampling effort than nearshore locations. Together, this highlights the strong species- and location-specific nature of eDNA detection probability and the importance of determining minimum sampling effort, particularly for species with heterogeneous eDNA distributions.

The spatial heterogeneity in eDNA observed in Lake Moāwhitu, a small, shallow lake, suggests that such approaches will be especially valuable in other lentic systems where eDNA distribution is also uneven, including large lakes,

reservoirs and wetlands with complex habitat structure (Eichmiller et al. 2014; Lawson Handley et al. 2019). While precise sampling effort and strategies are not directly transferable across all species and systems, they provide a useful baseline for designing eDNA surveys for similar organisms (i.e. fish) and in similar lowland, turbid lake environments. More broadly, robust sampling design is critical for improving downstream eDNA analyses, from single-species detection to metabarcoding and community-level assessments.

Conclusion

This study demonstrates that eel eDNA detection in a turbid, lowland lake is strongly influenced by sampling method, eel species and spatial variability within the lake. Active water filtration and long-duration (~13 h) passive samplers displayed the most reliable detection for both eel species, whereas sediment and short-duration passive methods performed poorly. Despite being closely related, *A. australis* consistently showed higher eDNA concentrations and higher detection probabilities and required substantially less sampling effort than *A. dieffenbachii*. Occupancy modelling and simulations indicated that 3–6 replicates per site were sufficient for high-confidence detection of eel eDNA, with the number of required sites varying according to sampling method as well as sampling location. Sampling survey design will therefore depend on species-specific detection probabilities, sampling method used and location sampled from as well as expected occupancy, as low-occupancy scenarios require greater sampling effort (i.e. upwards of 20–30 sites sampled in triplicate). These findings are likely to be applicable to eDNA surveys targeting fish in lowland, turbid lakes.

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Supplementary material 1

Comparison of total eDNA concentration (ng/µL) between sampling methods

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

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The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI.
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Author contributions

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.