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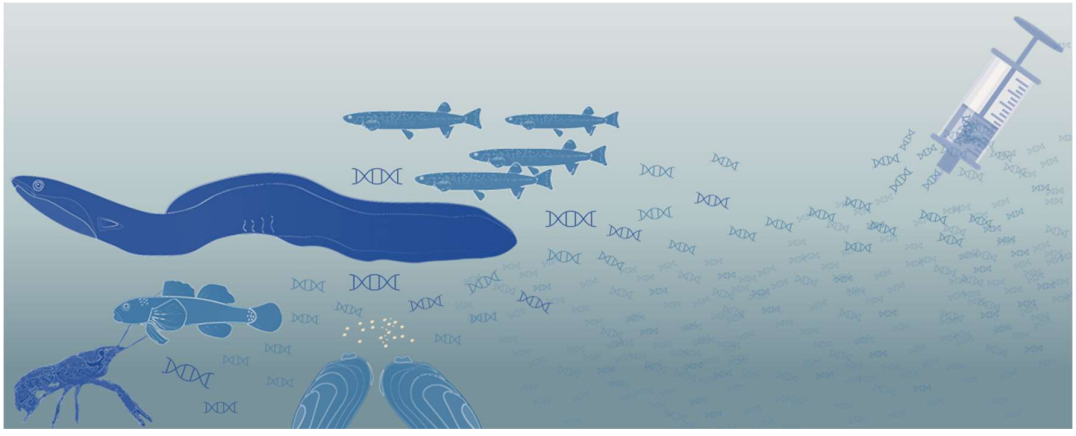
eDNA Guidelines and field protocols for lotic systems

December 2023



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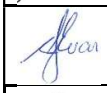	Formatting checked by:	Carole Evans
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Contents

Tables

Figures

Executive summary

The use of environmental DNA (eDNA) as a tool for biodiversity surveys is a rapidly advancing field with great potential. This manual is designed to provide comprehensive guidance on using eDNA to characterise aquatic populations and assess the ecological communities of waterways, particularly in Aotearoa-New Zealand.

Detecting species using eDNA methods, rather than using conventional sampling methods, can reduce impacts on sensitive species and increase the power of field surveys for detecting rare and elusive species. Moreover, eDNA offers the possibility of sampling systems that cannot be sampled using conventional techniques such as non-wadeable waters, and offers the potential to increase monitoring efficiency and reduce costs, primarily by reducing the need for expert staff and consultancy time. This scalability can significantly expand our knowledge of freshwater species' distributions, especially in regions with limited or no existing fish community records.

The eDNA guidelines for lotic systems have been developed with guidance from the Environmental DNA Working Group, a consortium of freshwater experts from regional councils, Ministry for the Environment, Department of Conservation, NIWA, University of Waikato and Wilderlab.

The primary objective of these guidelines is to establish a standardised national approach to eDNA monitoring in lotic systems, ensuring uniformity in effort and methods for similar applications. This manual primarily focuses on protocols for sampling fish and macroinvertebrates, where comprehensive reference libraries and validated field trials exist. As research and development in eDNA methods and applications progress, these guidelines are expected to evolve and adapt to reflect the latest advancements in the field.

Key guidance provided in this manual includes:

Optimal eDNA field sampling considerations: offering essential considerations for conducting optimal eDNA field sampling, including the general collection sequence, contamination prevention, replication (e.g., six sample replication), sample volume (1 L), and temporal, spatial and environmental factors to consider.

eDNA capture methods: Exploring various eDNA capture methods, including passive versus syringe sample filtration techniques and their relevance in eDNA sampling.

Results of eDNA sampling: Interpreting the results of eDNA sampling is crucial. This section discusses positive and negative detections, Wilderlab reporting results, and outlines eDNA positive criteria.

Prevalence index: Discusses the concept of the eDNA Prevalence Index. It outlines how this index is calculated and its potential suitability in assessing the presence and prevalence of fish and large macroinvertebrate species in environmental samples.

eDNA versus conventional electric fishing: Explores the advantages and disadvantages of using eDNA monitoring over traditional methods for assessing fish populations.

Using eDNA to examine the effectiveness of fish passage barrier remediation: Focusing on a practical application of eDNA, this section discusses how eDNA can be effectively employed to evaluate some aspects of the success of fish passage barrier remediation efforts alongside physical

monitoring methods. It provides insights into survey methods, protocols, and sampling frequency for this purpose.

Finally, we further discuss and summarise challenges related to eDNA as a potential source of false positives, presence versus viable populations, and confounding sources of eDNA.

Overall, eDNA monitoring is a new and rapidly developing field that is likely to become a mainstay of water quality ecosystem health monitoring into the future. As such it is expected that sampling methods and analyses will continually evolve through time, and it is envisaged that this manual will be reviewed and updated as required to reflect advancements in the technology and its application.

1 Background

The use of environmental DNA (eDNA) as a tool for biodiversity surveys is a fast-developing field. Despite its relative infancy, eDNA is proving itself useful for surveying a wide range of organisms (including animals, plants, fungi, viruses and microorganisms) – particularly for aquatic species where traditional capture-based methods may not detect rare species and can be time-consuming and expensive. In freshwater environments, eDNA can complement and inform traditional sampling methods as a cost-effective, first-pass look to direct further monitoring effort.

This manual will help direct future efforts to use eDNA to characterise aquatic populations, and to understand the ecological integrity of waterways. Advantages of eDNA sampling include:

- Enhanced detection of rare or endangered species to enable their distribution and habitats to be identified and protected;
- Enhanced and effective characterisation of aquatic communities;
- Early detection of biosecurity incursions and biodiversity assessments;
- Increased ability to monitor the outcomes of policy and management interventions (and so design or adjust policy to meet environmental goals) e.g., monitoring the effectiveness of intervention programmes such as fish passage barrier remediation.

It is also expected that appropriate application of eDNA will improve efficiency and reduce costs associated with monitoring (largely through a greatly reduced need for expert staff/consultancy time), thus increasing the scale at which the environment can be monitored and subsequently improving our knowledge of the aquatic species present in our regions. There are significant areas in Aotearoa-New Zealand where there are little or no current records relating to fish or macroinvertebrate communities, and eDNA could be a powerful tool to rapidly characterise the species present across large areas.

eDNA monitoring is a new and rapidly developing field, which is likely to become a mainstay of water quality ecosystem health monitoring into the future. As such, it is expected that sampling methods and analyses will continually evolve through time.

1.1 Aim of guidelines

The eDNA guidelines was developed with input from the Environmental DNA Working Group; a group including freshwater practitioners from most regional councils, Ministry for the Environment, Department of Conservation, NIWA, University of Waikato, and Wilderlab, which meets regularly to discuss application and development of eDNA technology.

The aim of the eDNA guidelines is to provide a standardised national approach to eDNA monitoring in lotic systems, such that all parties are using the same effort and the same methods for similar applications. This manual focuses on protocols for sampling fish and macroinvertebrates, which currently have the most complete reference libraries for accurate individual recognition and validated field trials. It is expected that increased certainty in the appropriate application of eDNA sampling will increase its use and value as a monitoring tool.

1.2 Updates

We recognise that eDNA methods and resources are still rapidly evolving. It is anticipated that this eDNA guidance for biomonitoring in lotic systems will be reviewed and updated as required to reflect advancements in the technology.

2 Environmental DNA: definitions and applications

2.1 Environmental DNA

Environmental DNA (eDNA) refers to a collection of genetic material sourced from living organisms and their remnants present in the environment (Bruce et al. 2021). In environmental samples, a major portion of the DNA consists of single-cell microorganisms such as bacteria, and protists. However, eDNA samples also comprise genomic material from multicellular organisms, which can take on the form of entire microscopic organisms like zooplankton and meiofauna, or as remnants and traces of larger organisms like vertebrates, invertebrates, and plants (Pont et al. 2018, Rodriguez-Ezpelata et al. 2021). These genetic traces, referred to as macrobial (or extra organismal) eDNA, include reproductive material like gametes and various other components such as tissue fragments, epithelial cells, or secretions produced or expelled by the organism (Barnes and Turner 2016). Macrobial eDNA can persist in the environment for varying periods, sometimes ranging from hours to days (Sansom and Sassoubre 2017). By collecting and analysing eDNA, detection and monitoring of species can be possible, even when the organisms themselves are not physically present in the environmental samples (Barnes and Turner 2016, Sansom and Sassoubre 2017).

2.1.1 DNA barcoding and metabarcoding

DNA molecules contain species-specific genetic information, and short DNA fragments called DNA barcodes which can be used to identify species or higher taxa based on their level of variability. For eDNA barcoding, conventional polymerase chain reaction (cPCR) is used to detect the presence of a species (Jerde et al. 2011, Thomsen et al. 2012). These barcodes typically consist of a hypervariable region, allowing one barcode to be used for multiple species within a taxonomic group. Ideally, a DNA barcode should have enough variation to differentiate closely related species (variable at an interspecific level) while remaining relatively consistent within a species (conserved at an intraspecific level). Well recognised standard barcoding genes are commonly used to identify animals (Hebert et al. 2003), plants (Hollingsworth 2011), fungi (Schoch et al. 2012), and protists (Pawlowski et al. 2012).

While DNA barcoding uses species-specific primers to detect the DNA fragments of a single species, metabarcoding differs in that it analyses whole communities of species. Metabarcoding can be defined as the use of general or universal PCR primers on mixed DNA samples from any origin followed by high-throughput next-generation sequencing (NGS) to determine the species composition of the sample (Deiner et al. 2017). Community samples can be obtained from environmental or bulk samples, where the latter consists of a mixture of whole organisms from the environment. The number of different metabarcodes in a sample can be high and depends on the barcoding gene's specificity and the diversity of species present in the environment (Palowski et al. 2020). As an improved non-invasive aquatic biodiversity monitoring approach, eDNA metabarcoding is becoming increasingly prevalent for monitoring the biodiversity of aquatic ecosystems (Bista et al. 2017, Valdivia-Carrillo et al. 2021). The main challenge of typical metabarcoding research is to assign metabarcodes to species or higher taxonomic categories. The accuracy of the taxonomic assignment relies on the completeness of the barcoding reference database. Gaps in these databases represent a significant limitation in interpreting ecological metabarcoding data (Weigand et al. 2019).

2.2 Detection methods

2.2.1 Single-species detection methods

Single-species detection is commonly used in the detection and monitoring of rare and endangered species, management of biological invasions (Harper et al. 2017, Holderegger et al. 2019), and for detecting parasites and pathogens (Krieg et al. 2019). A specific advantage of the single-species method is that the amount of DNA can be quantified relatively accurately using qPCR (quantitative (real-time) PCR) and ddPCR (droplet digital PCR). Despite this quantitative information, the amount of DNA does not necessarily reflect organismal abundance. For example, DNA concentrations may be higher for organisms in close vicinity, and due to factors, such as spawning and decomposition, which can inflate DNA concentrations relative to population density/biomass. Nevertheless, numerous studies have shown that these selective single-species methods are highly efficient in detecting DNA traces of invasive and endangered species in water (Jerde et al. 2011, Mächler et al. 2014, Bass et al. 2015). The characteristics of using eDNA for single-species detection of aquatic species have been reviewed by Goldberg et al. (2016), and many others, and falls out of scope of these guidelines.

2.2.2 Multiple species detection methods

Multi-species detection is a common application of eDNA. In this case, the eDNA metabarcoding method is used to provide information about the composition, structure, and diversity of a community of organisms. This method is run by high-throughput sequencing technologies that generate millions of DNA sequences and potentially allow identification of all species present in a sample, including those that are rare and inconspicuous. Metabarcoding has been shown to detect communities as completely as traditional methods based on electric fishing (Hänfling et al. 2016, David et al. 2021) or kick-net sampling (Fernández et al. 2018, Mächler et al. 2019). Most eDNA-based multi species surveys in aquatic ecosystems use either water or sediment sampling.

2.3 Limitations of eDNA

Presence/absence information obtained from eDNA barcoding and metabarcoding can be valuable in conservation and biodiversity efforts, enabling the monitoring of populations at large spatial scales and identifying critical habitats for species of concern (Voros et al. 2017, Weltz et al. 2017). Additionally, eDNA can play a critical role in detecting the first occurrence of invasive species or confirming the presence of native species previously believed to be at low population densities or even extinct (Stoeckle et al. 2017, Trebitz et al. 2017). While eDNA can offer valuable insights, it is essential to recognise its constraints and the fact that eDNA-based tools in general are not intended to completely replace established or conventional methods but instead are an evolving technology to complement existing physical methods (Hering et al. 2018, Harper et al, 2019, Beng and Corlett 2020). Some of the principal limitations of eDNA sampling are summarised below, with further discussion of challenges for eDNA sampling summarised in Section 13.

2.3.1 Presence/absence versus abundance

While eDNA can effectively detect species presence, it does not provide detailed information about species abundance, behaviour, population size structure, or ecological interactions. Instead, it provides a snapshot of species occurrence (Goldberg et al. 2020). Presence/absence can be misleading when eDNA is present in the environment without the living target organisms or when eDNA is not detected despite the presence of the target organism (Song et al. 2017). An example of the former is the deposition of faecal material from birds. While an example of the latter is when there is too little DNA being released from animals that may be too small or rare in the system being

sampled. Data on the abundance and size structure of populations provides more robust information on the effectiveness of management interventions and on potential factors affecting populations and communities.

2.3.2 PCR concentration versus abundance

Quantitative PCR (qPCR) and droplet digital PCR (ddPCR) can estimate eDNA concentration (read abundance), but eDNA concentration may not directly correlate with species density or biomass. The relationship between eDNA concentration and population size will vary among species and ecosystems, challenging the assumption that read abundance correlates with genic or taxon abundance, or that there is a constant copy number to individual relationship (Minamoto et al. 2017). For example, eDNA abundance may depend on animal biomass, temperature affecting both rate of release and DNA breakdown, species specific rates of DNA release, spawning events swamping a sample with gamete DNA, species specific metabolic rates or activity levels. Relative metabarcoding reads may be swamped by abundant DNA arising from a single species e.g., cattle in a in stream downregulating other eDNA in abundance as a result (Rourke et al. 2021).

2.3.3 Dead versus living

Standard eDNA methods do not differentiate between DNA from live and dead organisms. Both live and dead organisms shed DNA into the environment, contributing to the eDNA pool. The contribution of dead organisms to the eDNA pool can vary considerably in different environments, particularly in warm conditions causing faster degradation rates, where carcasses do not persist long (Tsuji et al. 2017). Additionally, predator species can excrete DNA of prey items within environments that do not contain living prey organisms. For example, birds that prey on fish in one location and excrete their remains in another can lead to the detection of fish DNA in environments where these fish do not actually reside.

2.3.4 Persistence in the environment

eDNA results may not provide immediate real-time information about species presence due to various factors, including site characteristics, eDNA transport, and the time required for sample processing and laboratory analysis, making it less suitable for urgent monitoring needs. The degradation of eDNA in the environment can limit the scope of eDNA studies, with only small segments of genetic material remaining, particularly in warm conditions (Strickler et al. 2015, Goldberg et al. 2018, Harrison et al. 2019, Moushomi et al. 2019, Murakami et al. 2019). Conversely, where DNA persists, transport has been observed to range from several to 100 km contingent upon environmental and hydraulic conditions (Pont et al. 2018). As such, extended transport of DNA can affect inferences about fine-scale spatiotemporal trends in species and communities (Taberlet et al. 2012, Eichmiller et al. 2016, Goldberg et al. 2016, Deiner et al. 2017, Hering et al. 2018).

3 eDNA in freshwater systems

The eDNA present in freshwater ecosystems comes from both microbial and macrobial (extra-organismal) organisms, including small-sized animals like zooplankton and benthic meiofauna. Spatial and temporal interpretations based on the detection of DNA can be complex and varies depending on its source. For microbial and meiofaunal components, the DNA extracted from environmental samples is more directly linked to the biology, occurrence, and ecology of living organisms since entire individuals are present in the eDNA samples (Pawlowski et al. 2020). In contrast, for macrobial organisms, their DNA originates from cellular remains suspended in water or attached to particles in the sediment. Here, the detectability of macrobial eDNA is influenced by environmental and biological factors not directly related to the organisms themselves. These factors can be categorised into three main groups: production, degradation, and transportation (Pawlowski et al. 2020):

Production

Production is the shedding of DNA into the environment and largely depends on the abundance and density of a taxon and its biological and physiological features. Fish, for example, are known to release large amounts of DNA into the environment, while arthropods release much less DNA, probably due to their exoskeleton. The amount of DNA released also depends on species-specific metabolic rates and can change during a species' life-cycle, for example, increases during the breeding season due to the release of gametes (Maruyama et al. 2014, Bylemans et al. 2016, Figure 3-1). The volume of DNA excreted by each individual within a species can also be influenced by biomass. While eDNA production generally rises with individual mass (Maruyama et al. 2014), larger individuals often emit fewer eDNA particles than a similar biomass of smaller members of the same species (Maruyama et al. 2014, Mizumoto et al. 2018, Takeuchi et al. 2019). Therefore, in environments with identical densities of individuals but differing biomasses, one would expect variations in eDNA particle concentration (Yates et al. 2020). Consequently, the variation in production of eDNA from different species can vary extensively in space and time making quantitative interpretation of eDNA difficult (Pawlowski et al. 2020).

Degradation

Degradation of eDNA depends on various physicochemical and biological factors, including temperature, dissolved oxygen, pH, ions, and microbial activity (Strickler et al. 2015, Barnes and Turner 2016). Several studies show that macrobial eDNA persists longer in colder and more alkaline conditions (Goldberg et al. 2015). Bacterial activity is also hypothesised to have a strong impact on eDNA degradation, often in relation with physicochemical parameters such as temperature or demands for phosphorus. The consequence of eDNA degradation is the reduced number of molecules that can be detected. It has been shown that macrobial eDNA generally does not last more than 14 – 60 days in the water column (Goldberg et al. 2015). However, degradation can also lead to some chemical modifications of DNA molecules that can hamper the correct identification of species in eDNA data. Degradation of eDNA is an important factor that needs to be considered after sampling and sample processing. For example, samples need to be stored and handled in ways that ensure the eDNA is preserved (Pawlowski et al. 2020).

Transport

Transport of macrobial eDNA refers to the passive movement of intra-, extra-cellular or particle—bound DNA in the environment (e.g., by flow), such that the eDNA can be sampled at a different place than where it was produced (Pawlowski et al. 2020). It has been estimated that eDNA can be

transported over at least ten kilometres in streams (Deiner and Altermatt 2014, Civade et al. 2016), and up to several hundred kilometres in large rivers, with travelling time estimated at 41.7 hours for 100 km, depending on flow rate (Pont et al. 2018). As eDNA can potentially be transported over long distances, its analysis provides biodiversity information at broad spatial scales and integrates information at the scale of sub-catchment or catchment levels (Deiner et al. 2015). Conversely, transportation can impede fine-scale interpretation of locations where a species actually occurs. Transport of macrobial eDNA is also driven by the movement of other species via what they eat and sub-sequentially excrete (Pawlowski et al. 2020, Figure 3-1).

Detectability

Detectability is a function of the combination of production, degradation, and transport, but also depends on the sampling design and protocols. For example, the proximity of a sampled site to species' habitats, the volume of sampled material, the number of replicates, and the filtration method (e.g., filter pore size). Successful detection also depends on molecular protocols, specifically efficiency of DNA extraction methods and the specificity of PCR primers (Pawlowski et al. 2020). Laboratory methods for detecting eDNA in water samples is, however, out of the scope of this guidance manual.

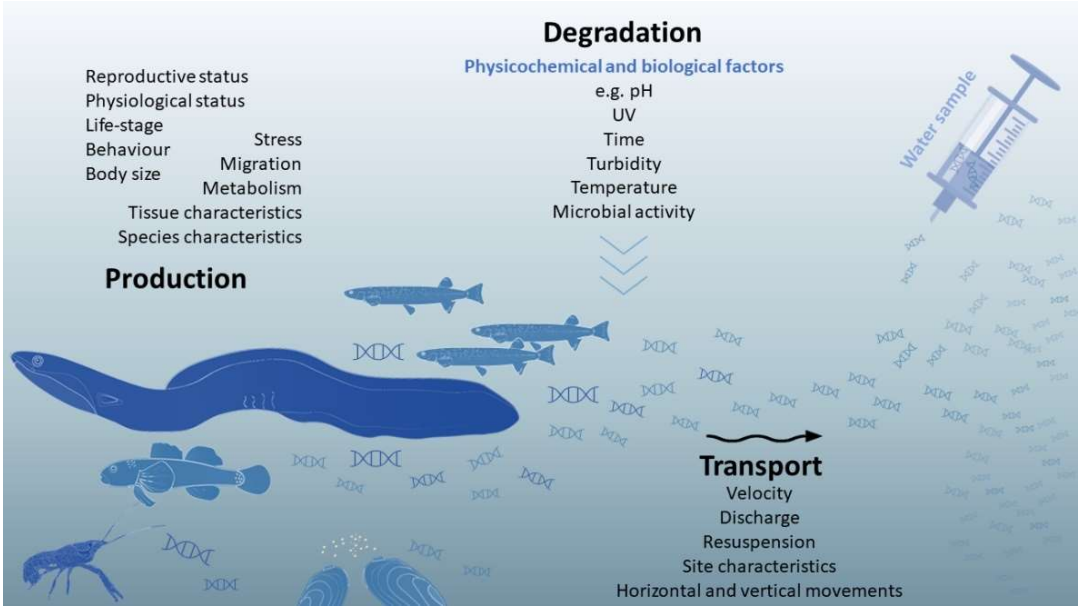


Figure 3-1: eDNA detection is dependent on a variety of factors including the production of eDNA (e.g., organism life-stage), degradation processes (e.g., temperature, UV, pH), and the transportation by flow in waterbodies.

3.1 Detectability in lotic ecosystems (running water bodies)

In moving water bodies, such as streams and rivers, the spatial distribution of eDNA differs from standing ecosystems like lakes and ponds. This is generally due to the unidirectional flow of water, which transports eDNA through the system and is influenced by discharge (Deiner and Altermatt 2014, Deiner et al. 2015, Carraro et al. 2018). As a result of transportation and degradation processes, eDNA approaches in running water bodies are less suitable for very localised biodiversity

assessments at point scales. However, they offer the potential to infer potentially catchment-level properties (Deiner et al. 2015, Altermatt et al. 2020, Carraro et al. 2020). As a guiding principle, the greater the flow rate, the larger the upstream area represented by a sample. This may vary from a few hundred metres in slow-flowing lowland streams and rivers to tens of kilometres in fast-flowing systems (Pont et al. 2018, Seymour et al. 2018). Rivers present specific challenges for detecting macrobial eDNA due to their larger size, higher flow rates, and greater volume of water. Traditional sampling from the shore may not provide a representative sample, necessitating adjustments in the sampling strategy. For instance, sampling in the middle of the river may be required (Adrian-Kalchauser and Burkhardt-Holm 2016). In interpreting eDNA data, factors like transport distance and deposition velocity of DNA-containing particles need to be considered. These factors can contribute to the dispersal of eDNA over larger distances, affecting its detection patterns (Deiner and Altermatt 2014). In streams, eDNA can also be influenced by surrounding land-use practices, and input from soils and leaves falling into the water may introduce terrestrial signals and hinder PCR-based sampling through inhibition by humic acids (Mansfeldt et al. 2020).

Furthermore, depending on environmental conditions, eDNA can degrade at variable rates (Jerde et al. 2016, Shogren et al. 2017). Furthermore, within channel and subsurface interactions may trap eDNA in substrate interstices (hyporheic zones), where DNA then can bind to sediments upon contact (Turner et al. 2014, Shogren et al. 2017). In the case of microbial DNA, transport and interactions with sediments has been well characterised and studied (Droppo et al. 2009), but macrobial eDNA poses additional challenges because the DNA is characterised by polydispersal particles of variable size, either as free DNA, organelles, cells, and/or pieces of tissue (Turner et al. 2014). Each particle, differing in material origin and size, will have potentially different transport and retention rates, as well as propensity for cohesion and/or buoyancy and resuspension (Jerde et al. 2016).

3.2 eDNA sampling to detect fish

Fish are suitable for detection using eDNA because they shed substantial amounts of DNA into the water, their genomic data are well represented bioinformatics databases, and traditional monitoring methods (especially electric fishing) are resource-intense, invasive, and logistically challenging in large water bodies. The use of eDNA for fish species detection is becoming more and more commonly applied as an alternative to electric fishing and other traditional sampling methods (e.g., netting and trapping). Fish eDNA is not only found in the water column but is also present in sediments, where it can persist for longer timeframes (Turner et al. 2014). Several studies indicate that benthic species are only detectable in samples collected in proximity to the specific habitat, for both lentic and lotic systems (Adrian-Kalchhauser and Burkhardt-Holm 2016, Hänfling et al. 2016). In large rivers, fish eDNA can be transported downstream over hundreds of kilometres (Pont et al. 2018).

3.3 eDNA sampling to detect macroinvertebrates

The source of DNA of invertebrates (like other aquatic organisms) in the eDNA sample may vary widely, including faeces, mucus, and gametes. It has been shown, especially for lotic systems, that macroinvertebrate eDNA may also be transported over large distances (Deiner and Altermatt 2014), which may complicate comparison to classic sampling that is often very localised (kick-net sampling). Together with a relatively high level of uncertainty in classic sampling approaches, this makes

comparisons to eDNA samples challenging. Many studies have undertaken eDNA sampling to survey insects, but the results of classical and molecular approaches were not always congruent (Fernández et al. 2018, Mächler et al. 2019). The biggest advantage of macroinvertebrate eDNA studies is the ability to sample across a much larger taxonomic range, including groups such as Diptera that are difficult to identify morphologically.

3.4 The use of eDNA for implementation of the National Policy Statement for Freshwater Management (NPSFM)

The National Policy Statement for Freshwater Management (NPSFM) mandates comprehensive monitoring and reporting of freshwater ecosystems. Because the use of presence/absence-based metrics like MCI and Fish IBI have been mandated, there is an inherent attraction to using multi-species eDNA for NPSFM monitoring and reporting due to its ability to easily detect the presence/absence of a wide range of species.

It is essential to recognise that the other mandated macroinvertebrate indices (i.e. QMCI and ASPM) require abundance data for their calculation. Also, David et al. (2021) found Fish IBI estimates using eDNA samples were consistently higher than those estimated using the 150 m electrofishing methodology. David et al. (2021) suggested that at the survey sites, eDNA monitoring sampled a wider spatial scale, comprising DNA from both within the sampled reach and from an unknown distance upstream.

While eDNA is valuable, it may not be an ideal tool for early detection of deterioration in ecosystem health or monitoring for adaptive management because it does not measure factors such as abundance, size structure, or overall health of aquatic organisms that are more sensitive indicators of ecosystem change and response to management interventions. Nonetheless, responsive indicators within eDNA, such as bacterial and microeukaryotic communities, can act as effective sentinels for ecosystem health when their patterns of natural variability are well-understood, as demonstrated by Glasl et al. (2017).

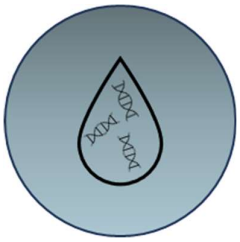
However, single or multi-species eDNA may serve as an effective tool for New Zealand regional Councils to address some reporting and surveillance monitoring as required by the NPSFM, including but not limited to:

- Early detection and monitoring of invasive species, allowing for effective early management and/ or eradication interventions (relevant to Section 3.13.4 of NPSFM (2020)).
- Assessment of threatened species, allowing councils to delineate threatened species' habitats (relevant to Section 3.8.3c of NPSFM (2020)).
- Temporal monitoring using eDNA can help in tracking changes over time (relevant to Section 3.30 of NPSFM (2020)).

4 Simplified eDNA sampling process for multi-species detection

The eDNA sampling process in lotic systems is an efficient and non-invasive tool for detecting multiple species. In short, samples are collected directly from rivers and streams, with the detection of species' eDNA influenced by various physical and environmental factors. In the laboratory, DNA is extracted, amplified, and sequenced, with specialised primers targeting specific taxonomic groups. Results are cross-referenced with genetic databases to identify species where species specific genomic data are available and resolved by the primers (Table 4-1).

Table 4-1: Simplified summary of the eDNA sampling process including, sample collection, laboratory analysis and reporting of results.

Sample collection	
	Water samples are collected directly from a stream or river and processed using either filtration, precipitation, or centrifugation. The detection of a species' eDNA depends on several factors. These include the species' ecology (e.g., secretion levels, size, migration patterns) and environmental factors (e.g., water flow rate, salinity, temperature, turbidity).
At the laboratory	
	In the laboratory, DNA is extracted from the collected samples and then amplified using specialized techniques. Primers are employed to target specific taxonomic groups, allowing for detection ranging from broad groups (e.g., Eukaryotes) to narrower categories (e.g., fish or specific mollusc families). The choice of primers involves trade-offs between breadth and sensitivity, particularly for rare species. Amplified DNA sequences are analysed using high-throughput sequencing platforms.
Results	
	The obtained DNA sequences are cross-referenced with genetic reference databases to identify the range of species present. Only species registered in the database can be identified at the species level. To improve the reference database for missing species, a barcoding campaign can be incorporated into the project. Species that cannot be identified are classified at a higher taxonomic level, such as family or genus.
Reporting	
	Reporting provides the species identified within each sample as well as key findings and summary graphics.

5 Optimal eDNA field sampling considerations

5.1 General sampling collection sequence

When preparing for fieldwork, especially when visiting multiple sites concurrently, careful planning is essential. It is important to establish a systematic sequence to maintain consistency and accuracy, and to avoid cross-contamination of samples. Here are some key principles to consider:

Sample downstream to upstream

Start sampling at the site farthest downstream and proceed sequentially upstream to minimise contamination from upstream sites (**see Section 5.4.2 for further detail**).

eDNA collection method

Ideally, to avoid contamination, sampling should not involve entering the water body directly. Because this is rarely feasible, there are several options to consider:

- Ensure that all materials (such as waders and sampling equipment) that come into contact with the water are thoroughly decontaminated or between different sites if multiple sites are sampled in the same day.
- Minimise physical entry into the water (particularly for small streams) by sampling from the water's edge using a water grab system. For example, a single use or decontaminated cup attached to the end of a decoy retrieval stick (Figure 5-1). When conducting grab sampling, it is important to decontaminate both the grab system and the sample cup between replicates and between different sample sites. Alternatively, a bucket can be used to collect the total volume of water needed for all replicates at a site, with the bucket decontaminated between sites (Figure 5-1).
- In the case of larger water bodies, use a decontaminated boat.

Regardless of the chosen approach, it's critical to:

- **Collect samples upstream of where the sampler enters the water** to prevent physical disturbance or contamination of the sampled water.
- **Face upstream during sampling reduces the risk of contamination** and ensures a controlled process.
- **Standing side-by-side when multiple individuals are involved in** sampling, to prevent cross-contamination from each other's gear.

Please refer to the 'Contamination Prevention' Section 5.2 within this document for comprehensive guidance on minimising the risk of contamination during eDNA collection.

Figure 5-1: Water collection using a decoy retrieval stick. A & B, collecting approximately 5 litres of water for processing. C, use of a pottle or smaller container is also possible for easier retrieval of a water sample from the thalweg (i.e., the deepest or main flow of the stream).

Replicates

In the context of eDNA sampling, replicates refer to the practice of collecting multiple samples from the same or similar locations to increase the reliability and robustness of eDNA detection and analysis. Replicates can also help to increase the reproducibility of results and minimise the influence of random variations or errors. It is strongly advised to incorporate replicates into your eDNA sampling protocol. **For detailed guidelines on optimal replicates, refer to Section 5.3**.

For high replicate sampling, ensure **ALL** replicate samples are taken from:

1. the same sampling spot and;
2. at the same depth in the thalweg (i.e., deepest or main flow) of the stream.

Sample labelling

Properly label each collected sample with a unique identifier, corresponding to its sampling location. This labelling system is crucial for accurate data analysis and interpretation.

Record site information

Document detailed site-specific information for each sampling location, including GPS coordinates, and any signs of contamination, pollution, turbidity and flow. Environmental conditions (e.g., water temperature, flow rate), can be reported too, but it is important to ensure equipment like water quality and velocity meters are used downstream of the sampling location to avoid contaminating the sample. Remember to record **GPS coordinates at the sampling location**, not the carpark, particularly when parked near confluences of streams.

Reporting of metadata in eDNA studies should be consistent for reliability and replicability. Some criteria are more relevant than others depending on the nature of the study, and researchers should take care to report all relevant information (**see examples of critical and additional information and the example site sample form**).

Example Site Sampling Form

eDNA Site Sampling Form

Sample ID: _____
Date and Time: _____
Site GPS Coordinates: _____

Collected by:_____
Location and Site: _____
Filtration method: Active/Passive

Sample Photos:
[ ] Photo of Site Included
[ ] Photo of Sample Included

Site characteristics:
Weather conditions (Fine/Overcast/Rain)
Water flow (baseflow/ low/ mid/ high)
Turbidity (Clear/ low turbidity/ high turbidity)

Collection Method:
[ ] Wading (In-stream)
[ ] Grab Sample
[ ] Other: _____

Potential Contamination sources:
E.g., swimmers upstream, urban/ agri runoff

Sample Replicates (Ensure all six replicates taken at the same sampling spot)
Number of Replicates: ___
Sample Volume: _____ L/ per replicate _____ Total
Comments:

Sampling spot details:
Depth of Water Body: _____ m
Depth at Sample Collection: _____ m
Habitat sample collection: (run/riffle/pool, other_____)
Instream location (thalweg/near thalweg/other_____)

Water Quality Metadata:
- Water Temperature: _____ °C
- pH: _____
- Flow Rate: _____ m^3/s
- Turbidity: _____ NTU
- Dissolved Oxygen: _____ mg/L

Additional Information / Observations:

Remember to adhere to the specific sampling protocols and guidelines required for your eDNA sample.

Critical information as per the example site form.

Sample ID	A unique identifier for each sample.
Date and time	Date and time of sample collection.
Location and site	Detailed information about the sampling site location (including sampling point within cross-section of the stream. Include GPS coordinates.
Sample photos	Visual documentation of the sampling site and conditions.
Sample volume	The quantity of water or sample recovered during collection.
Collection method	Information about how the sample was collected, whether it was taken through wading (in-stream sampling) or a grab sample.
Sample replicates	The number of replicates taken for each sample.

Additional information

Water quality metadata	Include related water quality data, such as water temperature, pH, flow rate (velocity/discharge), turbidity/visual clarity, dissolved oxygen levels. Ensure water quality meters are placed downstream of the sampling location to avoid contamination
Sampling water depth	Water depth of water body and depth at which sample was taken**.**

Sample preservation

Samples are collected using sterile equipment after which they are preserved to maintain the integrity of the genetic material. Preservation helps prevent degradation of DNA and the growth of bacteria that could contaminate or alter the eDNA.

Adherence to protocols

Ensure that all sampling personnel are well-trained and strictly adhere to established eDNA sampling protocols. Training can help maintain consistency and data reliability. It is important to note that when using Wilderlab eDNA sampling kits, it is essential to follow the instructions provided on the Wilderlab forms or any other relevant guidance specific to the survey (See sections 7 and 8 for detailed protocols on active syringe sampling and passive sampling). Consistent reporting of these details not only ensures the reliability and replicability of research but also contributes to the broader understanding of eDNA ecology, which is vital for the advancement of this field. For detailed protocols for using Wilderlab syringe sampling kits and Wilderlab passive sampling kits **see Section 7 and 8 below.**

5.2 Contamination prevention

Macrobial eDNA is typically present at very low concentrations, making it highly susceptible to contamination. To ensure the reliability of eDNA monitoring, maintaining a clean field collection protocol is crucial. Contamination risks can arise from various factors throughout the sampling process, including environmental sources, equipment, and cross-contamination between samples (De Brauwer et al. 2022).

5.2.1 Equipment decontamination

Thorough decontamination of all equipment and field gear between sampling sites and sample replicates within sites is essential to guarantee the independence of samples (Goldberg et al. 2016). Consider using **single-use equipment** and containers for sampling whenever possible. If reusable supplies (e.g., grab bottles or containers) are employed, they must be cleaned and sterilised between each sampling event, as well as before and after use. The preferred method for decontaminating equipment is to use a solution of >3% sodium hypochlorite to eliminate any traces of DNA/RNA. However, for metallic objects that could be damaged by bleach, soaking them in hot soapy water followed by thorough rinsing with distilled or deionised water is advisable (De Brauwer et al. 2022). **As per section 5.1**, it is important to ensure equipment like water quality meters are placed downstream of the sampling location to prevent the eDNA sample from potentially becoming contaminated.

5.2.2 Minimising wader contamination

When full decontamination of waders is not possible between sites (e.g., sodium hypochlorite may damage neoprene waders), several alternative strategies can be effective.

- Disposable wader or boot covers that can be easily discarded between site visits, reducing the risk of cross contamination. Ensure covers are not slippery.
- Multiple sets of waders designated for different sites on the same day.
- Assigning different people to enter each site.

5.2.3 eDNA collection

As mentioned in Section 5.1, if possible, minimise physical entry into the water by sampling from the water's edge or, in the case of larger water bodies, use a decontaminated boat.

It is vital that samples are collected **upstream of where the sampler enters the water or the boat** to prevent physical disturbance or contamination of the sampled water. Furthermore, **facing upstream during sampling reduces the risk of contamination**. And last **standing side-by-side when multiple individuals are involved** in sampling, to prevent cross-contamination from each other's gear.

5.2.4 Controls

The biggest concern for eDNA studies is false positive and false negative records. A false positive is the detection of an eDNA signal when the organism and/or its DNA is not present in the environment, while false negatives are the lack of an eDNA signal when the organism and/or its DNA is present in the environment. False positives can occur due to contamination at the sampling, extraction, and sequencing step. False negatives can occur due to failing extraction, PCR or sequencing steps or subsampling effects. False negatives can also occur because the genetic library or the statistical method are at fault. Ensuring the accuracy of genetic libraries and the robustness of

statistical approaches is crucial. To exclude false positives/false negatives, or to at least know about their occurrence, controls and replication are an important part of all eDNA studies. Furthermore, it is advisable to ground truth species presence to confirm positive eDNA detection.

Field and laboratory negative controls

In many studies, field "control" samples are collected by filtering a quantity of distilled water. There is, however, reason to question the need for these samples and the value of the information obtained. While filtering a sample of distilled water from a bottle verifies the general cleanliness of the field equipment (e.g., the filter assembly), it cannot measure any contamination that may occur in the process of collecting a sample from a waterbody. Alternatively, laboratory "controls" are collected. For example, Wilderlab includes at least one internal negative control with every sequencing run. Internal negative controls are conducted by running a standard volume (200 ul) of blank DNA/RNA shield on the extraction platform alongside the standard samples and treating it as any other eDNA sample from there.

Additional precautions to minimise contamination risks should include:

- Wearing personal protective gear, including gloves.
- Employing pre-sterilised equipment whenever feasible.
- Avoiding any contact with potential sources of contamination, such as other biological materials, during sampling.
- Incorporating negative controls into the workflow (field or laboratory based), especially when equipment is being decontaminated and reused.
- Utilising dedicated decontaminated containers for transporting eDNA kits.

By following these contamination prevention measures, you can enhance the accuracy and reliability of eDNA monitoring efforts.

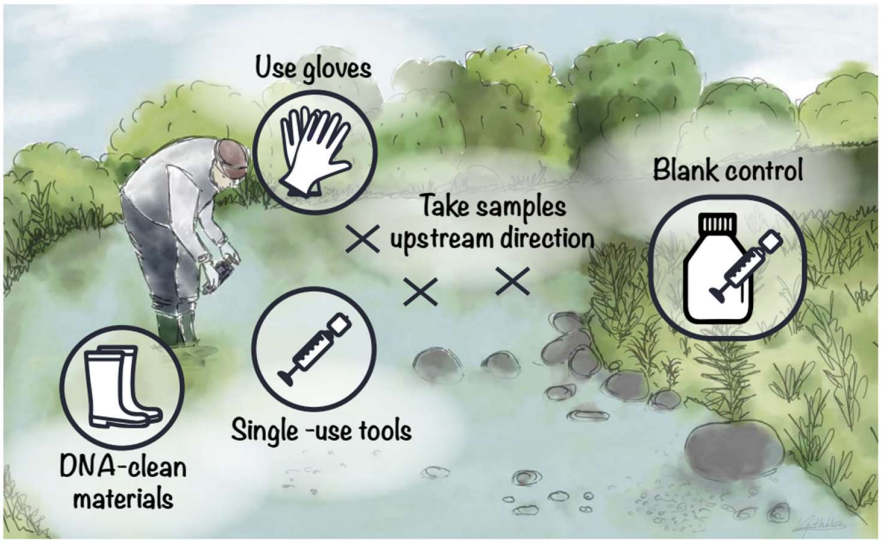


Figure 5-2: Measures to avoid contamination during sampling in lotic environments. Sourced from Pawlowski et al. 2020).

5.3 Replication and sample volume

5.3.1 Replication

To increase the optimal detection rate of taxa and increase confidence in the data, replicate sampling, i.e., the number of independently collected samples at the same sampling point and study site is critical (Rees et al. 2014, Shaw et al. 2016, Takashi et al. 2023). This is particularly crucial due to the inherent randomness of sampling and the possibility of eDNA molecules being unevenly distributed in the environment (Hunter et al. 2015). Consequently, heterogeneity between samples is often expected (Schmidt et al. 2013, Furlan et al. 2016, Shogren et al. 2017), primarily introduced during the water sampling process. Hence, a high risk of false negatives and/or unrepresentative samples is a major challenge in eDNA biomonitoring.

In early aquatic eDNA studies conducted from 2012 to 2014, it was common to have a limited number of replicates per sampling point, typically ranging from 1 to 3 replicates (but see Thomsen et al. 2012). However, after 2014, there was a notable shift in the methodology, with more studies opting for four or more replicates per sampling point, helping researchers obtain more reliable, accurate, and robust data, especially in the context of biodiversity monitoring and ecological research. For example, based on Smith et al (in prep), Orchard (2023) found that six replicates were necessary to reliably detect shortjaw kōkopu (*Galaxias postvectis*) in West Coast streams using the Wilderlab active syringe method.

5.3.2 Sample volume

To what extent replication is required to accurately capture local biodiversity may depend on the sample volume (Cantera et al. 2019) and the concentration of taxa (Furlan et al. 2016). Increased sample quantities may be necessary at locations characterised by challenging filtration conditions, such as high turbidity or PCR inhibition, (i.e., where PCR is suppressed due to the presence of contaminants or other factors that interfere with the amplification process). In such situations, increasing the number of collected samples could enhance the likelihood of capturing eDNA in the sampled water.

The volume of water filtered in published studies ranges from as little as 15 ml to over 100 L, but the most common volumes are between 500 ml and 5 L (McClenaghan et al. 2020). There is little consensus on the minimum viable filtration volume, which will depend to some extent on other factors such as:

- The sampling strategy employed, including number of sampling replicates and spatial representativeness of each sample.
- Practical constraints, such as turbidity of the water, which causes filters to clog.
- The size of the waterbody, which affects the concentration of eDNA in the water and, therefore, detection probability (Cantera et al. 2019, McClenaghan et al. 2020).

For any given sampling system, the volume of water filtered tends to correlate positively with the amount of DNA recovered and detection probability of rare species (McClenaghan et al. 2020). However, the relationship is by no means linear, and community composition can be well recovered even when relatively small volumes of water are filtered (Mächler et al. 2016, Muha et al. 2019, Xing et al. 2022). Indeed, a review conducted by Willoughby et al. (2016) found that the water volume sampled did not significantly influence species detectability. However, the number of replicate

samples taken at a site did have an impact, consistent with the findings of Furlan et al. (2016), Schmidt et al. (2013), and Shogren et al. (2017).

5.3.3 Optimal replication and sample volume validation trials

In Aotearoa-New Zealand, eDNA metabarcoding validation trials have shown that **six sample replicates at a single site give optimal detection rates for fish, macroinvertebrates, and other important taxa**. This method is now widely used as the standardised sampling protocol (for use in both active and passive samplers) by regional councils for characterising local aquatic communities around Aotearoa-New Zealand. Moreover, there were no significant differences between the standard 1 L samples and the enhanced 2 L sampling method ($p > 0.10$). Consequently, both sampling volumes were considered equally effective replicates for detectability (Box 1). For detailed information on active and passive sampling approaches see Sections 7 and 8.

Box 1 New Zealand Regional Council Six Sample Replication Trial (Smith et al. in prep)

Introduction

During the summer of 2020/2021, Aotearoa-New Zealand regional councils collaborated on a high replicate, nationwide eDNA trial. The primary goals for this trial were to determine the optimal sample replication number and filtration volume for monitoring fish and macroinvertebrate populations to explore the potential application of eDNA alongside regular biomonitoring activities. This study also aimed to explore how eDNA could be used to gain a wider understanding of biological communities and better contribute to the current knowledge of ecological health in riverways.

Methods

Each council contributed data for between 1-5 well known monitoring sites, resulting in 51 rivers being sampled across the country (see Figure 5-1). These sites spanned a wide range of habitats and hydrological characteristics. At each site, 16 replicate eDNA syringe samples were collected at a single time point using Wilderlab's eDNA active sampler mini kits. Eight of these were 'standard' 1 L filtered samples and the other 8 comprised of 2 x 1 L samples pooled to produce a single 'boosted' sample. The samples were processed using Wilderlab's freshwater assay panel comprising 11 metabarcoding assays.

Figure 5-3: Map of 51 sample sites (blue dots) included in the high replicate study collected between December 2020 and May 2021. White boundary lines mark regions.(Sourced from Wilderlab)

Results

This study resulted in a comprehensive national eDNA dataset with 816 samples detecting 153,492 unique sequences and identifying 2,364 unique species. ANOVA testing found no significant difference in proportional richness between the standard 1 L versus the boosted 2 L sampling method (p = > 0.10). Consequently, both 1 and 2 L samples were considered as effective sample volumes for replicate analysis (n = 816).

Box 1. (cont.) New Zealand Regional Council Six Sample Replication Trial

Species accumulation curves were used to visualise how the range in detected species proportional richness (proportion of the total number of detected species relative to the total species richness for a given sample size) changed as the number of replicates increased (Figure 9.2). Across both fish and macroinvertebrate groups, on average, the point of diminishing returns (the point on the curve where the addition of more replicates results in a diminishing increase in the number of detected species) was found to be between 5 and 7 sample replicates.

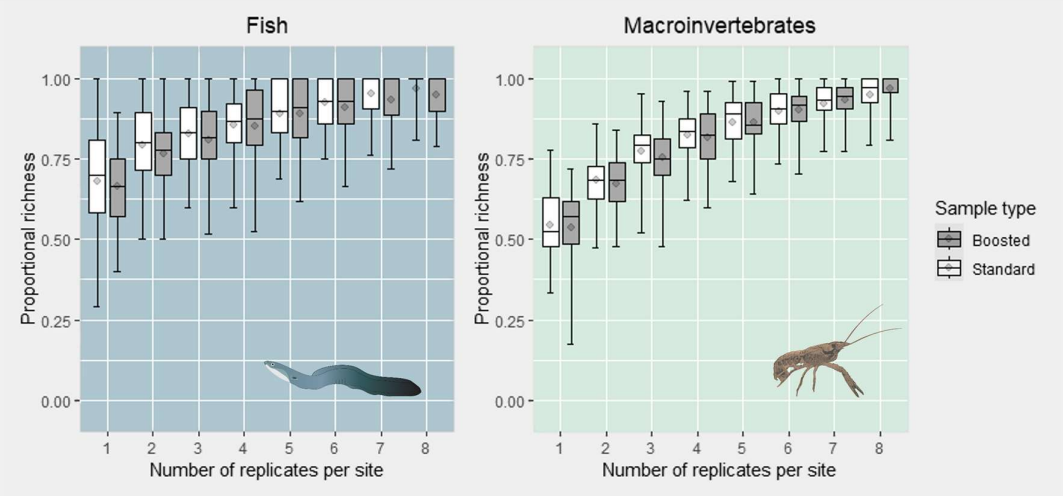


Figure 5-4: Species accumulation curves comparing cumulative proportion species richness in boosted and standard sample types for both fish (left) and macroinvertebrates (right). Figure by Michele Melchior.

Table 5-1. Mean species/taxa proportional richness (%) by stream order and elevation and replication difference for the four focused groups.

Group	Standard		Stream order				Elevation			
	n = 51		1-3, n = 23		4-8, n = 28		>100m, n = 13		<100m, n = 38	
	1 rep	6 reps	1 rep	6 reps	1 rep	6 reps	1 rep	6 reps	1 reps	6 reps
Fish	64.0	89.5	65.3	90.6	64.1	89.5	70.4	91.6	61.9	88.8
Macroinvertebrates	46.7	80.4	45.8	81.0	47.5	79.9	45.7	79.7	47.0	80.6

Note: Data shown from standard method using 6 replicates by stream order (small 1-3 and large 4-8) and elevation (high >100m and low <100m).

Conclusion

Six sample replicates at volumes of 1 L at a single site give optimal detection rates for fish, macroinvertebrates, and other important taxa. This method is recommended for use as the standardised sampling protocol for characterising local aquatic communities around Aotearoa-New Zealand.

5.4 Temporal and spatial considerations

For eDNA sampling in streams, it is essential to consider various temporal and spatial factors that can influence the detectability of environmental DNA. These factors encompass elements affecting eDNA degradation, transport within streams, and characteristics of the source organisms. When selecting environmental covariates for analysis, it is crucial to prioritise those that can be efficiently measured in the field.

5.4.1 When to sample

Seasonal considerations

Seasonal variations significantly impact eDNA detection. Detection rates tend to decrease during the winter months (Baker et al. in prep), with adverse effects on detection rates caused by dilution (by higher flow rates in rivers), lower activity in fish, and filter clogging, especially in turbid conditions. To optimise detection rates, it is advisable to conduct sampling during the warmer summer months between **December and March** or, if summer sampling is not feasible, during periods of low rainfall, when flows are low and stable, representing 'normal conditions' between spring and autumn. For studies focusing on specific species, sampling at various times throughout the year may provide more comprehensive data. For example, data indicate increased detection during the breeding season for shortjaw kōkopu in May to June, (Orchard 2023) and lamprey (*Geotria australis*) between November and January (Baker et al. in prep).

Avoid sampling after heavy rain events

Sampling should be performed when streams are **at or near base flow, not discoloured, and have received minimal rainfall in the past 2 -3 days** (no more than 10 mm). Heavy rainfall can lead to challenges in eDNA detection, particularly when sediments are present in turbid streams following rain events. DNA bound to sediment possesses the ability to remain detectable for significantly longer durations compared to DNA present in the water column. The presence of sediment-bound DNA in turbid streams after rainfall can lead to uncertainty about whether the eDNA detected corresponds to actual living organisms (true positives) or if it represents DNA washed in from the sediment (potentially leading to false positives) (Turner et al. 2015). Understanding the potential for this uncertainty is crucial for the interpretation of eDNA results.

Understanding species behaviour and reproduction

Some freshwater species exhibit unique behaviours, such as seasonal migration or distinctive breeding seasons, which can influence eDNA detectability. It is essential to have a comprehensive understanding of the behaviour of organisms in the sampled ecosystems. For example, species with external fertilisation release significant DNA during breeding seasons, affecting sampling results. When conducting multiple-species detection (eDNA metabarcoding), it is advisable to exclude sampling during the breeding seasons of dominant species to avoid skewed results.

Extra monitoring

If **extra monitoring is being done for sampling fish or invertebrates using nets and traps**, this should be **performed after eDNA sampling** to avoid potential contamination and false positives/negatives.

These considerations underscore the importance of establishing a solid understanding of the sample site, the species' phenology and eDNA degradation rates within specific environments. This

knowledge serves as a basis for developing effective sampling strategies and ensuring accurate result interpretation.

5.4.2 Where to sample

Selecting the appropriate sampling location is crucial to ensure the representativeness of your eDNA sample. Here are some considerations:

Sample reach

To maintain the sample's ecosystem representation, it is advisable, where feasible to exclude/consider areas near stormwater drains, farm access points, picnic areas, or popular swimming spots that could affect eDNA results and introduce unwanted contamination to the site. However, if these factors are unavoidable or are relevant to your specific research question, they can be included but should be considered when interpreting results. Also consider upstream tributaries, any connected lentic waterbodies, and changes in elevation.

Sample site

Whenever possible, gather eDNA samples from habitats with fast-flowing, well-mixed water. Focus on areas within or near the thalweg (i.e., the deepest or main flow) of the stream or river, as opposed to stagnant pools, which are less well-mixed and pose a higher contamination risk. These areas may contain inhibitors and facilitate eDNA binding to sediments, complicating eDNA detection and amplification, thus impacting the accuracy of identifying present versus past presence of organisms (Williams et al. 2017). Additionally, sampling close to the confluence of tributaries (downstream) should be avoided and sites located further downstream where the two systems are fully mixed.

Sample points

For high-replicate sampling (e.g., six replicates as outlined in Box 1), **ensure that all six eDNA sample replicates are collected from the same reach, habitat, and sample point location**. Remember to sample upstream towards an incoming flow of water to avoid contamination. This consistency is essential for accurate data collection.

Although some studies suggest sampling across the width of a stream (Bruce et al. 2021), biodiversity monitoring results by Sakata et al. (2020) found that aqueous eDNA metabarcoding showed consistencies between three different sampling positions at a single site. There was no significant difference in fish species composition between sampling positions across the stream despite the difference in velocity and sediment particle size distribution between the thalweg (deepest and main flow of stream) and edge of the stream.

5.4.3 Environmental effects on eDNA

When evaluating the factors influencing eDNA detectability in flowing waterbodies, it's crucial to consider various environmental characteristics. These factors play a role in eDNA degradation and include:

pH Levels

Low pH levels are typically associated with faster degradation. Acidic conditions can lead to DNA degradation, particularly when there's an abundance of positively charged enzymes indicative of low

pH. DNA preservation buffers are typically alkaline to counteract this effect (Strickler et al. 2015, Seymour et al. 2018).

Oxygen

Oxygen levels can impact degradation rates, with oxygen saturation decreasing as water temperature rises. DNA remains stable in anoxic conditions but degrades rapidly through hydrolysis in oxygenated environments (Barnes et al. 2021).

Velocity/discharge

Flow rate and discharge can significantly influence eDNA sampling and analysis by affecting the dilution of DNA, the collection of representative samples, the transport, and the degradation rate. Higher flows and discharge may also represent a larger upstream area in a sample, which may vary from a few hundred meters in slow-flowing lowland streams and rivers to tens of kilometres in fast-flowing systems (Pont et al. 2018, Seymour et al. 2018). Analysis of discharge (m^3s^1) data (hourly averages) associated with passive eDNA data collected from regional councils across New Zealand did not reveal any significant relationships between flow rate and macroinvertebrate and fish richness, nor total eDNA count (see Appendix A, Figure A1 – Figure A3). However, it is important to note that data is limited. In contrast, through monthly sampling of three sites in two streams across 10 months, Baker et al. (in prep) found a significant negative relationship between lamprey DNA concentration and discharge.

Water Temperature

Warmer water temperatures accelerate eDNA degradation due to increased microbial activity, a significant driver of eDNA breakdown. However, the higher eDNA production rate in warmer waters often balances out the faster degradation (Zulkefli et al. 2019). We examined temperature data (°C) as hourly averages, in conjunction with passive eDNA data collected from regional councils across Aotearoa - New Zealand. No significant relationships between temperature and both macroinvertebrate and fish richness, as well as total eDNA count were found (see Appendix A, Figure A4 – Figure A6).

Turbidity

Sediments in lotic environments introduce complexities in eDNA sampling and detection, potentially clogging filters used in the sampling process. DNA bound to particulates can also remain detectable for longer periods compared to DNA solely present in the water column. When collecting water samples from a turbid stream, especially after a rainfall event introducing sediments, there's a possibility of obtaining higher sequence counts. This adds uncertainty regarding the timing of eDNA detection, as DNA from different time periods may still be detectable in the sample (Turner et al. 2015). Turbidity (NTU) data (hourly averages) associated with passive eDNA data collected from regional councils across Aotearoa - New Zealand revealed no significant relationships of turbidity and macroinvertebrate and fish richness. However, a marginally positive relationship between turbidity and total eDNA count was observed (macroinvertebrates and fish, see Appendix A, Figure A7 – Figure A9). As with the other environmental data, the sample size used in this analysis was limited to nine sites. This could potentially impact the applicability of these findings and underscore the need for additional research in this area.

5.4.4 Landowner permission

Obtaining landowner permission is a requirement as well as an ethical and practical necessity to conduct responsible and successful eDNA research while respecting property rights, cultural sensitivities, and environmental considerations. Furthermore, there is a chance that samples might inadvertently detect human DNA, which might be culturally sensitive for some traditional owner groups (Handsley-Davis et al. 2021). Honest and clear communication with landowners is essential to avoid conflict and legal implications. In Aotearoa-New Zealand, research practices should consider the Wai 262 principles (Waitangi Tribunal 2012). It is strongly recommended that project outcomes are shared with landowners. The understanding of implications surrounding this issue is evolving and it is likely that new regulations will be developed in the future. It is the responsibility of project leaders to stay informed and conform to the most recent legislation on this matter. If potential concerns exist, it is advisable to consult with experts at the start of a new eDNA project (De Brauwer et al. 2022).

5.4.5 Ethics

eDNA sampling is a non-destructive method (for large organisms), which eliminates the need for most of the human or animal ethic approvals typical for other methods. At the time of writing, we are not aware of any institutions that require ethics approval for sampling eDNA from water samples. We do, however, strongly recommend consulting up-to-date regulations before commencing any new eDNA research project.

SUMMARY OF KEY FIELD CONSIDERATIONS

Sampling Scheme	Collect samples in or near thalweg, facing upstream. Collect samples upstream of where the sampler enters the water, in or near the thalweg, facing upstream.
Contamination control	Whenever possible, use single-use equipment for sampling, including gloves and syringes. If reusable supplies like containers are necessary, they must be cleaned and sterilized with bleach between each sampling event.
Sample replication	Based on Aotearoa-New Zealand eDNA validation trials, it is recommended to collect six sample replicates at a single site, all taken from the same location. This approach yields optimal detection rates for fish, invertebrates, and other significant taxonomic groups.
Temporal and spatial considerations	Sample in warmer months during base flows in summer (Dec – March) Sample six replicates at one sample site, and sample point within the stream. Remember to sample upstream of where you entered the stream and face upstream while sampling.
Environmental factors	Factors to consider include those influencing eDNA degradation (e.g., water temperature, pH, or solar radiation), transport within streams (e.g., water velocity, discharge, or channel complexity), and inherent characteristics of the source organisms (e.g., life stage).
Landowner permission	Ensure clear communication with landowners to avoid conflicts and legal issues. Research practices should align with the Wai 262 principles (Waitangi Tribunal 2012).
Ethics	eDNA sampling is a non-destructive method, eliminating the need for human or animal ethics approvals. Currently, there is no requirement for ethics approval when sampling eDNA from water samples.

6 eDNA capture methods

eDNA collection is one of the most challenging steps in eDNA-based approaches. The two principle methods for sampling eDNA from freshwater include filtration, and precipitation, both of which are characterised by individual operational steps and each can process different volumes of water (Li et al. 2018, Harper et al. 2018). This section will only focus only on filtration as an eDNA capture method.

6.1 Filtration

Filtration involves passing water through a fine porous membrane. Cellular and subcellular material is captured on the membrane and preserved ready for DNA extraction. Filtration can process much higher sample volumes when compared to precipitation (Li et al. 2018). Although early studies (e.g., Ficetola et al. 2008) used ethanol precipitation as the primary capture method for eDNA, there is now broad consensus that filtration is a more effective approach for detection of aquatic species in most environments and is the most prevalent and recommended method (Tsuji et al. 2019). Henceforth in this guide, we primarily focus on filtration strategies.

A wide range of methods are used for filtration-based capture of eDNA from water, including different filter membrane materials, pore sizes, and filtration mechanisms, different transportation, storage and preservation methods, and different DNA extraction protocols (McColl-Gausden et al. 2020).

6.1.1 Off-site filtration

Many published studies describe collecting water in sealed containers and transporting it to a clean laboratory for filtration using vacuum pumps (Jerde et al. 2011, 2013, Hänfling et al. 2016). This approach is attractive for speed and simplicity in the field. However, since eDNA degrades quickly, the water must either be kept refrigerated during transportation, preserved (e.g., using benzalkonium chloride), or filtered on the same day as collection or frozen for storage. This can be impractical and expensive, especially for large-scale sampling campaigns.

6.1.2 On-site filtration

The alternative to laboratory filtration is to perform filtration on-site, either manually with syringes or hand pumps, or with the aid of a powered pump (vacuum or peristaltic).

Active (or manual) filtration

Active filtration represents an inexpensive and universally applicable solution, but it can be hard physical work and time consuming depending on the targeted volume of water per sample, the particle load in the water, and the number of sites to be completed. Filtration with vacuum or peristaltic pumps makes it easier to process larger volumes of water, but may be unfeasible in situations where sampling is carried out by multiple field teams in parallel, or for use in remote areas where it is not possible to carry in the equipment and power supply needed. A cordless drill with a peristaltic pump head is an effective means to actively pump large volumes of water in remote locations and this method is used widely in Canada and North America (Laramie et al. 2012; Figure 6-1). Sampling equipment is an area of rapid innovation, and portable, fully integrated eDNA sampling systems have also been developed (e.g., Thomas et al. 2018).

Figure 6-1: Peristaltic pump head fixed to an 18 volt battery powered drill to actively pump water through a glass microfibre filter.

Syringe filtration method

Over recent years there has been a significant increase in studies using syringes to manually force water through a filter. Syringes are easy to use in the field for both professionals and non-experts with minimal contamination risk when used with enclosed filters, although they are commonly used for sampling smaller water volumes (≤1 L; Lugg et al. 2018). Because it is easy to use, the syringe method seems to be the method of choice in citizen science initiatives (Miya et al. 2022), and is one of the methods used by organisations and councils in Aotearoa-New Zealand (e.g., Wilderlab Ltd Syringe kits see Section 7). Note that as filtration pressure increases (whether using pump or syringe filtration), there is some evidence of reduced DNA retention on the filter membrane, as more molecules are forced through. However, this effect seems to be offset by the benefits of processing higher water volumes (Thomas et al. 2018).

6.1.3 Passive filtration

Passive eDNA sampling appears to be a simple solution to overcome the challenges associated with conventional methods. Passive eDNA sampling can be defined as the use of natural or artificial filters that can collect eDNA passively, without human intervention and eliminates the need to collect and filter water. The two main benefits of eliminating the filtration step are: 1) reduced time spent physically sampling and 2) no requirement for expensive equipment or power. However, field time and travel costs do increase due to passive samplers needing to be deployed for a 24-hour period, necessitating two site visits - one to set and one to collect the samples. The time spent filtering water could be used to deploy increasing numbers of passive replicate samples, which in turn would enable large-scale sampling and increased replication (Bessey et al. 2022).

The most important difference between active and passive eDNA sampling methods is that the latter cannot gather information about the water volume from which eDNA is captured, prohibiting a quantitative assessment of eDNA concentration in the sampled environment. This limitation of

passive sampling can be a critical disadvantage for studies that focus on the quantitative properties of eDNA (Chen et al. 2022). Passive filters immersed directly in water have been used successfully to collect eDNA from aquatic systems in Aotearoa-New Zealand and abroad (Bessey et al. 2021; see **Box 2**, Section 8).

Given that many regional and unitary councils have core sites for ecology, water quality and hydrology, with continuous stage and flow data, in the future it may be beneficial to conduct trials involving passive sampling throughout the year at hydrology sites to evaluate potential seasonal variations influenced by climate and flow conditions.

6.1.4 Pore size

The size of the filter used to collect eDNA and the volume of water sampled can influence both the quantity and quality of DNA extracted from environmental samples. Additionally, the source of DNA, whether it is extracellular or cellular, may determine the most suitable filter pore size (Taberlet et al. 2012). Some studies have indicated that smaller pore sizes can retain larger amounts of eDNA, as well as smaller eDNA particles (Shaw et al. 2017). Both Eichmiller et al. (2016) and Liang and Keeley (2013) found that smaller pore sizes, typically in the range of 0.2-0.6 µm, facilitated the extraction of more eDNA. However, Li et al. (2018) reported that pore size had no impact on eDNA yield or species detectability in their study, although they only tested filter pore sizes between 0.45 µm and 1.2 µm.

Despite the emerging consensus in the literature suggesting that smaller pore sizes are generally not detrimental and may even improve eDNA quantification, there are reasons to consider using filters with larger pore sizes. There exists an inherent trade-off between filter pore size and the volume of water that can pass through the filter (Mächler et al. 2016, Minamoto et al. 2016). Smaller pores tend to become clogged more easily, especially in turbid waters, which limits the amount of water that can be filtered for a given sample (Li et al. 2018). In practice, the choice of pore size should be influenced by the specific conditions of the study area. For instance, in very turbid environments, such as highly modified sites, larger pore sizes may be necessary since they are less prone to clogging, allowing a larger volume of water to be processed (although a recent study showed that 1.2 µm pore size filters outperformed 5 µm pore size filters, even at smaller volumes in turbid wetlands, Bird et al. in prep) Conversely, in clearer, faster-flowing streams, it may be preferable to use smaller pore sizes to ensure the capture of smaller eDNA particles. In Aotearoa-New Zealand, Wilderlab sample kits typically include a 1.2 µm, 30 mm cellulose acetate syringe filter, although this can be increased to 5 µm when sampling turbid waters.

Box 2. New Zealand Regional Council Active versus Passive Sampling Trial

Introduction

Following the high replicate validation trials, Wilderlab in collaboration with nine Regional Councils carried out further eDNA sampling trials aimed at comparing the use of passive filters with active syringe filters across various locations. The primary objectives of this trial were to assess differences in the number of detected species (species richness) between syringe-based and passive sampling, particularly for monitoring fish and macroinvertebrate populations. This investigation sought to determine the potential utility of passive eDNA sampling compared with syringe sampling.

Methods

As with the high replicate trials, sampling spanned a wide range of habitats and hydrological characteristics. At each site, six replicate Wilderlab eDNA syringe samples and six Wilderlab passive samples were collected. Passive samplers were deployed for 24 hours, while at the same site, at sample point adjacent to the passive sampler, syringe sampler replicates were collected. Samples were processed using Wilderlab's freshwater assay panel comprising 11 metabarcoding assays.

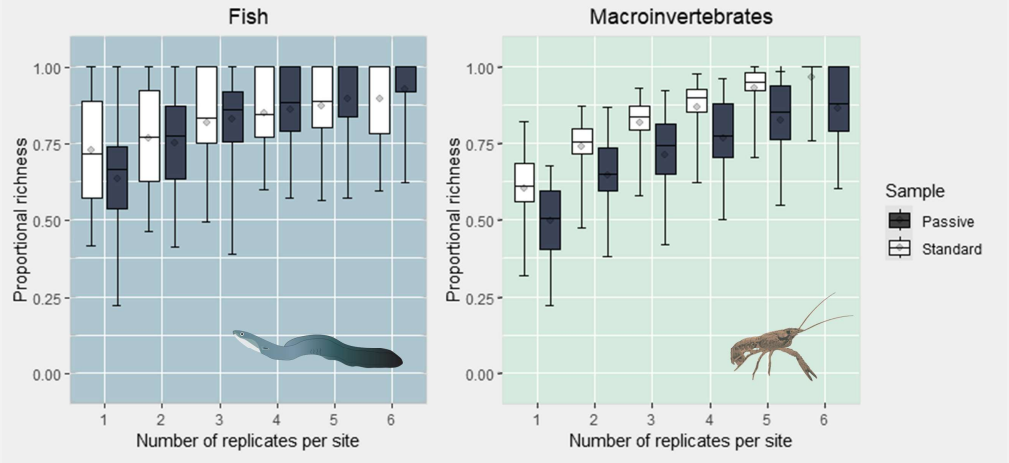


Figure 6-2: Species accumulation curves comparing cumulative proportion species richness in passive and standard sample types for fish (left) and macroinvertebrates (right)

Results

ANOVA revealed no significant differences in fish species richness between the standard and passive sampling methods ($p = 0.10$), although there does seem to be some bias towards underestimating compared to the standard. However, for macroinvertebrates, the standard sampling method significantly outperformed the passive method for detecting higher proportional richness across all replicates ($p < 0.002$). Although performance for detecting proportional richness was lower than expected and lower than that that of standard samplers, passive samplers detected a larger number of terrestrial species.

Table 6-1. Mean species/taxa proportional richness (%) and replication differences.

Group	Passive		Active	
	n = 38		n = 38	
	1 rep	6 reps	1 rep	6 reps
Fish	67.1	96.3	70.3	94.3
Macroinvertebrates	50.4	83.4	62.4	98.4

7 Wilderlab active (standard) syringe sampling[1]

Wilderlab's active samplers are a user-friendly manual filtration-based kit, which use a large barrel syringe to push water through a combination of filters (containing a 1.2 µm, 30 mm cellulose acetate syringe filter), capturing eDNA material from up to 1 L water.

Figure 7-1: Wilderlab syringe mini kit equipment, including one pair of gloves, one large and one small syringe and one filter. Image sourced from Wilderlab.co.nz.

7.1.1 Protocol

To perform active syringe sampling, a Wilderlab high-rep active sampler kit is required, which comes with one pair of gloves, two large barrel syringes, six preservative syringes, and six filters (Figure 7-1). Other useful items that are not provided in the kit include:

- A hand-held GPS
- A fine-tipped marker pen
- Safety glasses (the solution in the small preservative syringe is classified as an irritant).

It is important to **collect all replicates from the same spot for each sampling site**. At each site:

1. Wade out to collect the **sample in the thalweg**, which is the deepest or main flow of the stream. If the thalweg is too deep, sample as close to the thalweg as possible ensuring the site has flowing water that appears to be well mixed.
2. Open the Wilderlab sample bag, take the gloves out of the sample bag, put them on, and take out the large syringe. Draw up 50 ml of water from just below the surface of the water. Take care not to suck up any sediment from the bottom.

[1] Wilderlab Ltd products are used as examples in this context due to their widespread use in New Zealand. However, it is important to emphasise that their mention here does not constitute an endorsement of their products. There may be various alternative options available in the market for eDNA sampling equipment and related products that can be used.

3. Gently screw the filter on to the large syringe taking care not to overtighten, then push the plunger down to squeeze the water out through the filter. Avoid getting air bubbles in the filter as they can be difficult to push through.

4. Unscrew the filter from the large syringe and continue drawing and filtering **until 1 L of water has been filtered (20 syringefuls), or the filter is clogged**. If this happens, gently pulling back on the plunger may sometimes dislodge any particles trapped in the filter.

5. Unscrew the filter and draw 50 ml of air into the large syringe. Re-attach the filter and squeeze the air through the filter to remove excess water, while holding the syringe vertically with the filter pointing down.

6. Holding both the large syringe (with the filter still attached) and the small syringe (with black cap attached) in the same hand and in an upright orientation, transfer the black cap from the small syringe on to the outlet end of the filter.

7. Unscrew the filter (with the black cap now attached) from the large syringe and screw it on to the small syringe.

8. Push the plunger of the small syringe to inject the preservative into the filter. Shake well while holding the plunger down. Do not remove the syringe or cap from the filter. Do not worry if there are any air bubbles in the filter or if the plunger springs back – this is normal.

9. Place the filter with both the black cap and small syringe still attached into the sample bag.

10. **Repeat this process five times within the same location for a total of six replicates**, the standardised method for optimal detection (see Section 5.3, Box 1)

11. Seal the sample bag and record the sample details in the space provided. Ensure that the coordinates are entered in WGS84 decimal format (for example -41.30951, 174.82110 as displayed on Google Maps).

12. Submit samples for analysis using Wilderlab online submission portal before returning samples back to Wilderlab. This process ties your samples to your contact information using their UID (Unique Identifier) or Kit number.

Field methods for collecting active eDNA samples are summarised in Appendix A.

Figure 7-2: Active eDNA sampling using the Wilderlab barrel syringe and filter. Image sourced from Wilderlab.co.nz.

7.1.2 Banking sample replicates

Field validation trials indicated that six replicates of eDNA samples provides approximately 90% detection efficacy, or 90% of all species detected by eDNA (see Section 5.3 Box 1). For sampling results to be comparable between sites and across Aotearoa-New Zealand, a consistent and standardised approach is necessary. Should budget prevent six replicates from being undertaken at each sampling site, then collecting six samples but storing unprocessed replicates (using the provided preservative syringe and black cap for storage) should be considered. By banking replicates, this allows future completion of the recommended six replicates per site should budget allow or a more detailed investigation of taxa at a particular site be warranted. However, it is recommended that all six replicates are analysed at each survey site to allow a nationally consistent, standardised dataset to be developed in all regions.

Using Wilderlab Active Samplers

KEY REMINDERS

Rainfall Timing:	Avoid sampling after heavy rain, as it can flush eDNA out of the system and dilute target organism signals. Sampling should be performed when streams are at or **near base flow, not discoloured, and have received minimal rainfall in the past 2 - 3 days** (no more than 10 mm).
Contamination Prevention:	Prevent contamination by **wearing provided gloves** when handling the filter and standing downstream while taking your sample.
Replicate Consistency:	Collect all replicates from the same spot at each sampling site to maintain consistency. **Sample in the thalweg**, which is the deepest or main flow of the stream. If the thalweg is too deep, sample as close to the thalweg as possible ensuring the site has flowing water that appears to be well mixed.
Filter Care:	Avoid premature clogging the filter by refraining from sucking up large organic material or sediment.
Safety Measures:	Ensure safety by wearing nitrile gloves (provided in the kit) and safety glasses, as the small preservative syringe solution is an irritant.
Sample Preservation:	Prevent sample leakages by keeping the small preservative syringe and black cap attached to the filter when packing it.

8 Wilderlab passive sampling[2]

Wilderlab passive samplers are specially designed for capturing biodiversity assessments on a broader scale, making them well-suited for detecting various freshwater species, as well as terrestrial mammals, birds, and invertebrates. These samplers use manifold mounts, which are essential for high-replicate sampling, allowing for up to six replicates. They can be effectively deployed in both low and high order streams and rivers. Please note that passive manifold mount samplers are deployed for a 24-hour period and necessitate two site visits, one to set and one to collect the samples.

Figure 8-1: Wilderlab passive sampler kit equipment including two pairs of gloves, six filter pods, syringes and pottles and one manifold mount. Images sourced from https://wilderlab.co.nz.

8.1.1 Protocols

To perform passive sampling using manifold mount samplers, a two-site visit approach is necessary. The Wilderlab high-rep passive sampler kit includes two pairs of gloves, six filter pods, six preservative syringes, and six pottles (Figure 8-1). In addition, you will require a manifold mount, which is not included in the kit. Other useful items that aren't included in the kit include:

- A hand-held GPS.
- A fine-tipped marker pen.
- Safety glasses (the solution in the small preservative syringe is classified as an irritant).
- Hammer and waratah (for deploying the manifold mount).
- Flagging tape (to mark the site).

Ensure that this equipment including the waratah and manifold is decontaminated between sites and transported in a clean manner.

[2] Wilderlab Ltd products are used as examples in this context due to their widespread use in New Zealand. However, it is important to emphasise that their mention here does not constitute an endorsement of their products. There may be various alternative options available in the market for eDNA sampling equipment and related products that can be used.

Site Preparation:

1. Wade to the **deepest or main flow of the stream (i.e., the thalweg)**. If it is too deep, get as close as possible to ensure a well-mixed site.
2. Set up the eDNA manifold-mount following Wilderlab's instructions. Make sure the top of the manifold is about 5 cm below the water's surface. Be aware of flow variability (e.g., a hydropeaked river), and consider setting the manifold sampler lower to keep them submerged during lower flow. **Do not deploy the manifold on the receding limb of a flood,** where water levels are declining over time. This could expose the sampler and affect sample quality.
3. Choose a location and time when water levels are relatively stable or rising.
4. Wait 5 minutes to allow any stirred-up sediment to flow downstream. This helps to prevent cross-contamination from handling and any previous sampling events.
5. Open the Wilderlab passive sampling kit, put on gloves, and gently insert each eDNA filter pod into place. **Use six replicates, which is the standard method for optimal detection** (see Section 5.3, Box 1). Ensure the leaf guard faces upstream for a secure fit (Figure 8-2).

Sampling:

6. Record sample and site details, including location coordinates in WGS84 decimal format (e.g., -41.30951, 174.82110 as shown on Google Maps).
7. Leave the passive sampler with the six filter pods deployed for 24-hours.

Retrieval:

8. After 24-hours, return to the site and take out the additional pair of gloves from the Wilderlab sampling kit.
9. Retrieve one filter pod at a time. Hold the tag and pull on the easy-pull tag at the back of the filter pod to remove the sponge filter. Make sure the leaf guard points downward. Avoid touching the filter directly. Hold the tag and flick the filter downwards to remove excess water.
10. Take out the sample jar from the kit and place only the sponge filter inside.
11. Remove the small syringe containing the preservative, unscrew the cap, and pour all the preservative into the sample jar with the filter.
12. Seal the jar tightly and shake it well to ensure the preservative is evenly distributed throughout the filter.
13. **Repeat these steps for the remaining five filter pods in the manifold**. Place all sample jars in the sample bag and complete the sample information on the back of the bag.
14. Submit samples for analysis using Wilderlab online submission portal before returning samples back to Wilderlab. This process ties your samples to your contact information using their UID (Unique Identifier) or Kit number.

For detailed field methods on Wilderlab DNA passive samplers, refer to Appendix B.

Figure 8-2: Deployed Wilderlab passive sampler manifold attached to waratah taken within a stream. Sourced from https://wilderlab.co.nz.

Deploying Wilderlab Passive Samplers

KEY REMINDERS

Rainfall Timing:	Avoid sampling after heavy rain, as it can flush eDNA out of the system and dilute target organism signals.
Contamination prevention:	Prevent contamination by waiting for 5 minutes to allow any stirred-up sediment to flow downstream, wearing the provided gloves when handling the filter, and positioning yourself downstream of the passive sampler manifold.
Deployment Depth:	Deploy the sampler in or nearby the thalweg, at least 5 cm below the water surface to keep it submerged for 24-hours.
Safety Measures:	Ensure safety by wearing nitrile gloves (provided in the kit) and safety glasses, as the small preservative syringe solution is an irritant.
Site Marking:	Use flagging tape or GPS to mark the site for easy retrieval.
Sample Preservation:	When preserving and packaging your sample, remember to screw the sample jars on tight and shake well to ensure that the preservative gets well dispersed throughout the filters.

9 Results of eDNA sampling – what do they mean?

9.1 Positive detections - presence

Positive detections suggest the DNA of the species is present at the sampling location at the time of collection. However, these results cannot distinguish living or dead organisms, age/size, sex, or stationary versus migratory (due to eDNA transport in water).

- Repeated spatial and temporal sampling efforts can increase confidence in results, which is particularly important for estimating relative abundance from eDNA.
- Positive detections can inform where to direct traditional sampling methods.
- False positives can be avoided/minimised using field and laboratory controls.

9.2 Negative detections - absence

Negative detections (absence) occur when the organism is possibly too rare or below limits of detection with eDNA. However, negative detections do not always confirm that a species is absent. Here, the limitations of the technique must be considered with respect to environmental conditions/inhibitors, as well as sampling effort. There can, for instance, be several unidentified sequences that remain at the end of the eDNA results that can be a combination of organisms not yet described or not yet in a reference database. These sequences can continue to be updated with new taxon information as new reference sequences become available.

9.3 Wilderlab results[3]

Wilderlab results are provided in both an Excel (Figure 9-1) and online format.

Excel results

The Excel format contains three tabs consisting of:

Metadata

The sample submission form includes details related to the job and sample, along with supplementary fields, any relevant laboratory notes, and your account particulars.

Aggregated results

A simplified version of the eDNA results, excluding DNA sequence barcodes, and presenting a single entry for each distinct taxon (such as species or genus) detected in the samples.

Full results

The full eDNA results including DNA sequence barcodes and the assay codes that sequences were detected on, with one row for each unique eDNA sequence found in the samples.

[3] Wilderlab Ltd products are used as examples as they are currently the most widely deployed supplier in New Zealand. Other service providers may produce the results in a different format.

Online sample report

Online sample reports are accessible through the eDNA Explore Map and feature lists of identified species with search and filter functions, images of detected species and Biodiversity Wheels. These reports serve as dynamic records of your results and are routinely refreshed as additional sequence data becomes accessible.

Figure 9-1: Example of Wilderlab full eDNA results supplied in the excel spreadsheet.

9.4 eDNA positive criteria

The establishment of definitive criteria for eDNA positive detections is currently lacking. Instead, the strength of evidence for a species' presence relies on the frequency and consistency of positive eDNA samples collected at a specific location. This assessment takes into account various factors, including existing knowledge about the species' distribution, habitat, and behaviour, as well as information about the ecosystem's ecological characteristics and hydrodynamics. For instance, a single positive eDNA sample provides relatively weak support compared to the weight of evidence derived from multiple positive samples collected over an extended period, spanning multiple years (Jerde et al. 2011).

9.4.1 eDNA sequence counts

Sequence counts in eDNA analysis refer to the number of times a unique sequence or unique taxon was detected in each sample. Several factors influence these counts, including the proximity of organisms to the sampling point, the presence of dead or decaying organisms, environmental conditions that can accelerate or decelerate eDNA breakdown, and assay biases that might lead to preferential detection of specific groups of organisms.

Interpreting eDNA sequence counts and filtering data

The interpretation of eDNA sequence counts can vary depending on the specific research question or application. For example, the selection of specific filter parameters should align with the risk

tolerance profile of the user, as different applications may necessitate varying levels of stringency (e.g., biosecurity applications versus rare species). Based on current data, and due to the high sensitivity of eDNA testing, we propose the following guidelines:

- consider sequence counts >100 in at least 2 out of the 6 replicates as indicative of a "true detection".
- Counts falling below this threshold should be flagged as "tentative" or "trace detection" and further interpretation can be undertaken by an expert in the field of eDNA and the ecology of the relevant species.
- In cases where repeated sampling efforts yield consistently low sequence counts, the decision to conduct follow-up eDNA sampling or similar biological surveys (e.g., electric fishing) should be based on the significance of the taxon under consideration.
- Moreover, any reads of unidentified species should be excluded from the analysis.

Finding the right balance is essential, as overly stringent filters can increase the risk of false negatives, while excessively relaxed filters may lead to false discoveries. For instance, data demonstrating the relationship between eDNA results and fish abundance shows that a single detection with a sequence count of 128 was associated with the presence of giant kokopu (*Galaxias argenteus*), which was confirmed through electric fishing. The electric fishing data indicated a relative abundance of 2 individuals per 150 m or 0.42 per 100 m^2 (Appendix D). The threshold for a "true detection" is recommended based on current data but is subject to revision as more information becomes available.

9.4.2 Importing eDNA data to the New Zealand Freshwater Fish Database (NZFFDB)

Sharing fish (and other biological) records and maintaining an up-to-date national database for freshwater fish is critical for freshwater management. Importing eDNA survey data into the NZFFDB can be done via a user account. To contribute new records request an account by sending an email to fwdba@niwa.co.nz.

It is important to note that integrating eDNA data into databases requires rigorous quality checks to ensure accuracy and reliability of the records entered. Here are some checks and procedures that should be implemented prior to adding records to the NZFFDB:

1. **Verification of collection procedures**: ensuring eDNA samples collected follow standardised protocols, including verifying risks from decontamination and cleanliness of equipment (log potential contamination in example field sheet in Section 5), ensure optimal replication of samples have been taken and list replication number on the NZFFDB form.
2. **Cross-checking with known distributions**: compare eDNA results with the known geographical distribution of species. If a species is detected outside its known range, the result should be discussed with a Freshwater Fish expert for further verification.
3. **Reviewer Expertise**: Have eDNA records reviewed by experts familiar with both eDNA and the species detected.
4. **Thresholds for eDNA detection**: we recommend sequence counts >100 in 2 or more of the 6 replicates as a threshold for a "true detection". Anything below the threshold should be considered uncertain and require additional validation.

10 Prevalence index

A prevalence index has been proposed for categorising fish and large macroinvertebrate (e.g., kōura and kākahi) DNA reads from the six replicate multi-species (community) DNA analysis (Andy Hicks, MfE, Pers. Comm). Using data where DNA results can be identified to the species level, the index categorises DNA from trace levels to very high levels based on the level of detections across all species (Table 10-1). That is, for each replicate, the % of each species' DNA reads relative to the total DNA reads across all species is calculated. The median % of each species' reads across replicates with positive detections, relative to the number of the six replicates that the species was detected in provides the prevalence index for each species (Table 10-1).

Table 10-1: Prevalence index for DNA reads from six replicate multi-species analyses. For each species, match the median % of reads (calculated for each replicate as the % of that species' DNA reads relative to the total DNA reads across all species) to the number of replicates the species was successfully detected within.

	DNA Prevalence Index						
Median % across all 6 reps (excluding zeros)	**>20%**	Low	Moderate	Moderate	High	Very high	Very high
	10-20%	Low	Low	Moderate	High	High	Very high
	5-10%	Low	Low	Low	Moderate	High	High
	1-5%	Very low	Very low	Low	Low	Moderate	Moderate
	<1	Trace	Trace	Very low	Low	Low	Low
		1	**2**	**3**	**4**	**5**	**6**
		Number of reps					

The prevalence index was examined for four fish species (īnanga (*Galaxias maculatus*), banded kōkopu (*G. fasciatus*), kōaro (*G. brevipinnis*) and brown trout (*Salmo trutta*)) across 401 sites surveyed nationwide (

Figure 10-1 – Figure 10-6). Īnanga, banded kōkopu and kōaro were utilised as these species represent a gradient of penetration inland, from īnanga, a lowland species found at less than 400 m elevation, to kōaro, known to penetrate well inland (>300 km) and to high altitudes (>1100 m). Brown trout provide an example of a fish introduced to both lowland and high elevation waterways. For all four species, the distance inland and elevation where DNA was detected matched that seen from physical records held in the Aotearoa-New Zealand Freshwater Fish Database. The majority of moderate to very high reads for īnanga occurred at less than 125 m elevation and 50 km inland (

Figure 10-1). In contrast, moderate to very high reads for banded kōkopu and kōaro were found at higher elevations extending over 250 m (

Figure 10-2 &

Figure 10-3). In line with distributional data for brown trout, moderate to very high DNA detects span a broader range of low to high elevations and distances inland, particularly in the lower South Island (

Figure 10-4).

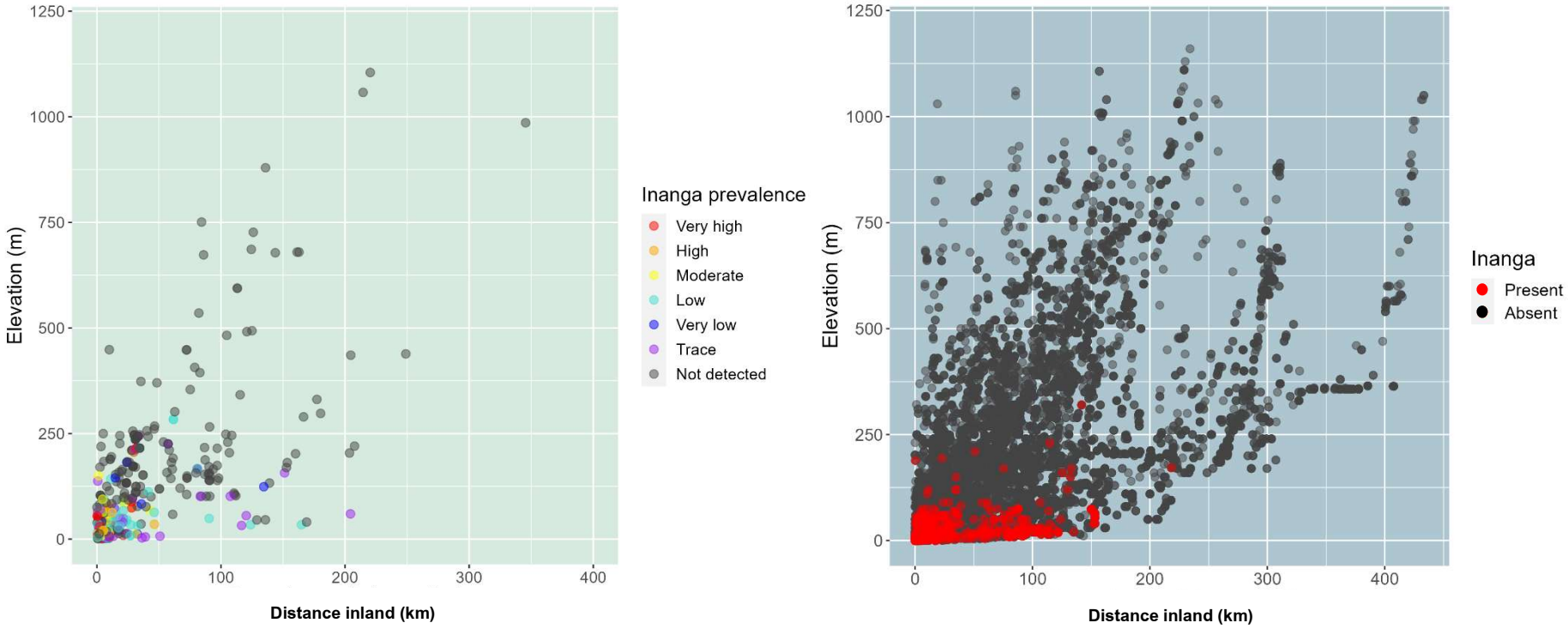


Figure 10-1: Prevalence of īnanga DNA reads (left) and īnanga presence as per Aotearoa-New Zealand Freshwater Fish Database (right) as a function of elevation (m) and distance inland (km).

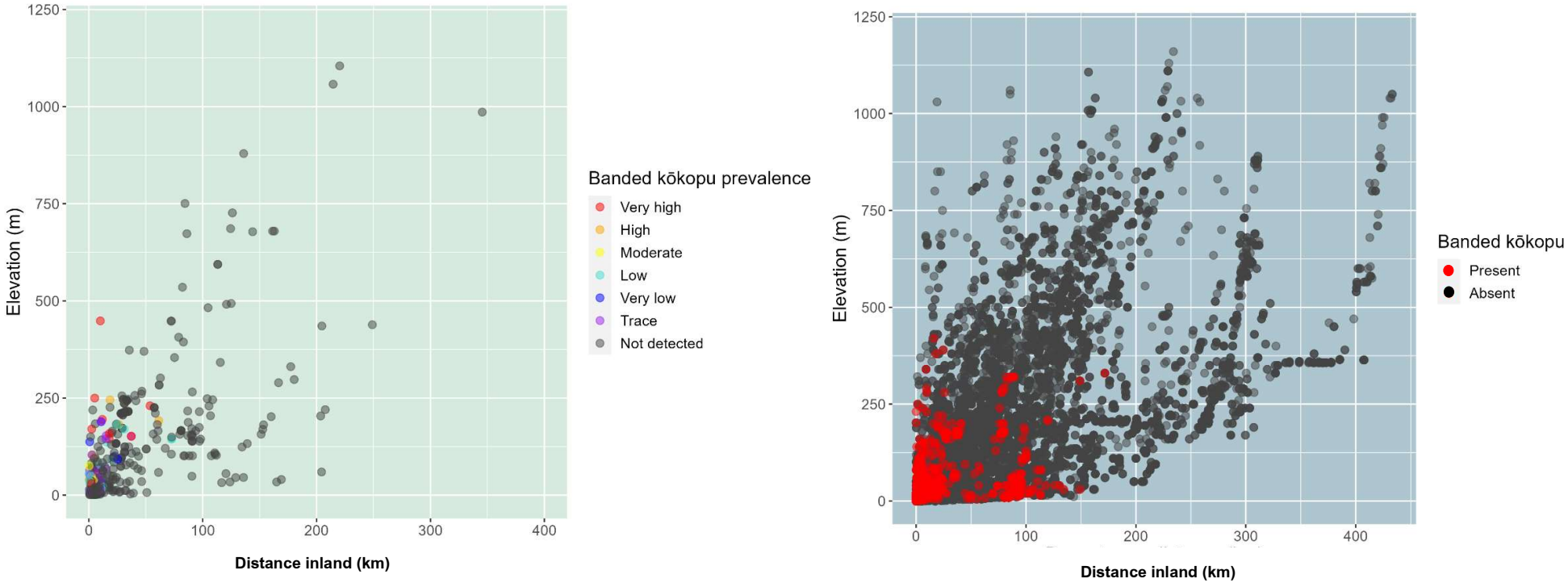


Figure 10-2: Prevalence of banded kōkopu DNA reads (left) and banded kōkopu presence as per Aotearoa-New Zealand Freshwater Fish Database (right) as a function of elevation (m) and distance inland (km).

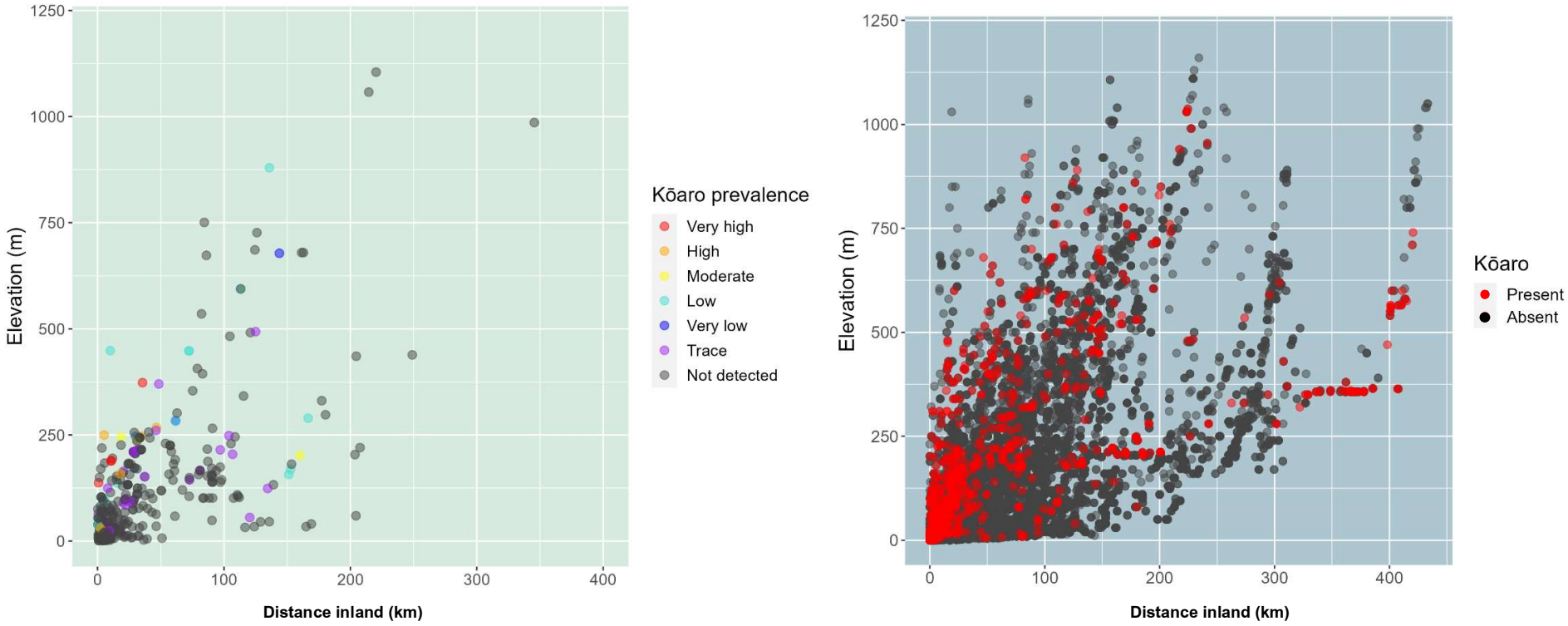


Figure 10-3: Prevalence of kōaro DNA reads (left) and kōaro presence as per Aotearoa-New Zealand Freshwater Fish Database (right) for as a function of elevation (m) and distance inland (km).

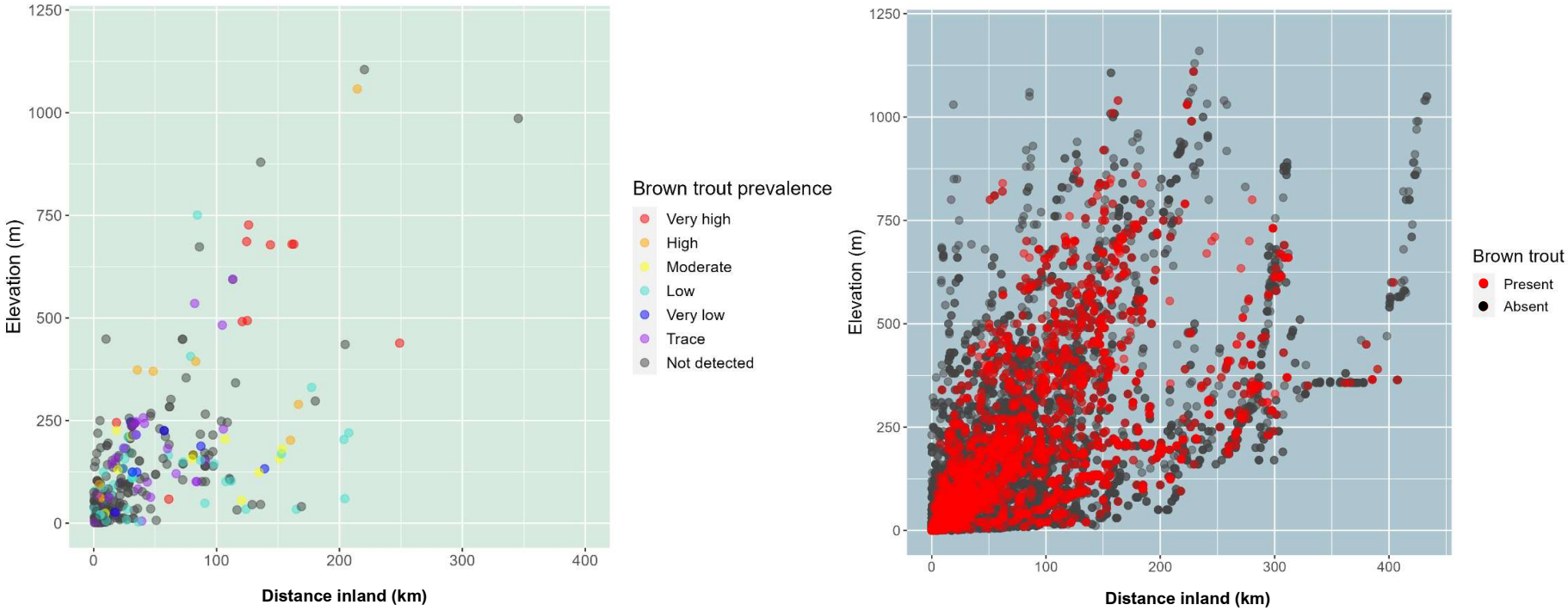


Figure 10-4: Prevalence of Brown trout DNA reads (left) and brown trout presence as per Aotearoa-New Zealand Freshwater Fish Database (right) as a function of elevation (m) and distance inland (km).

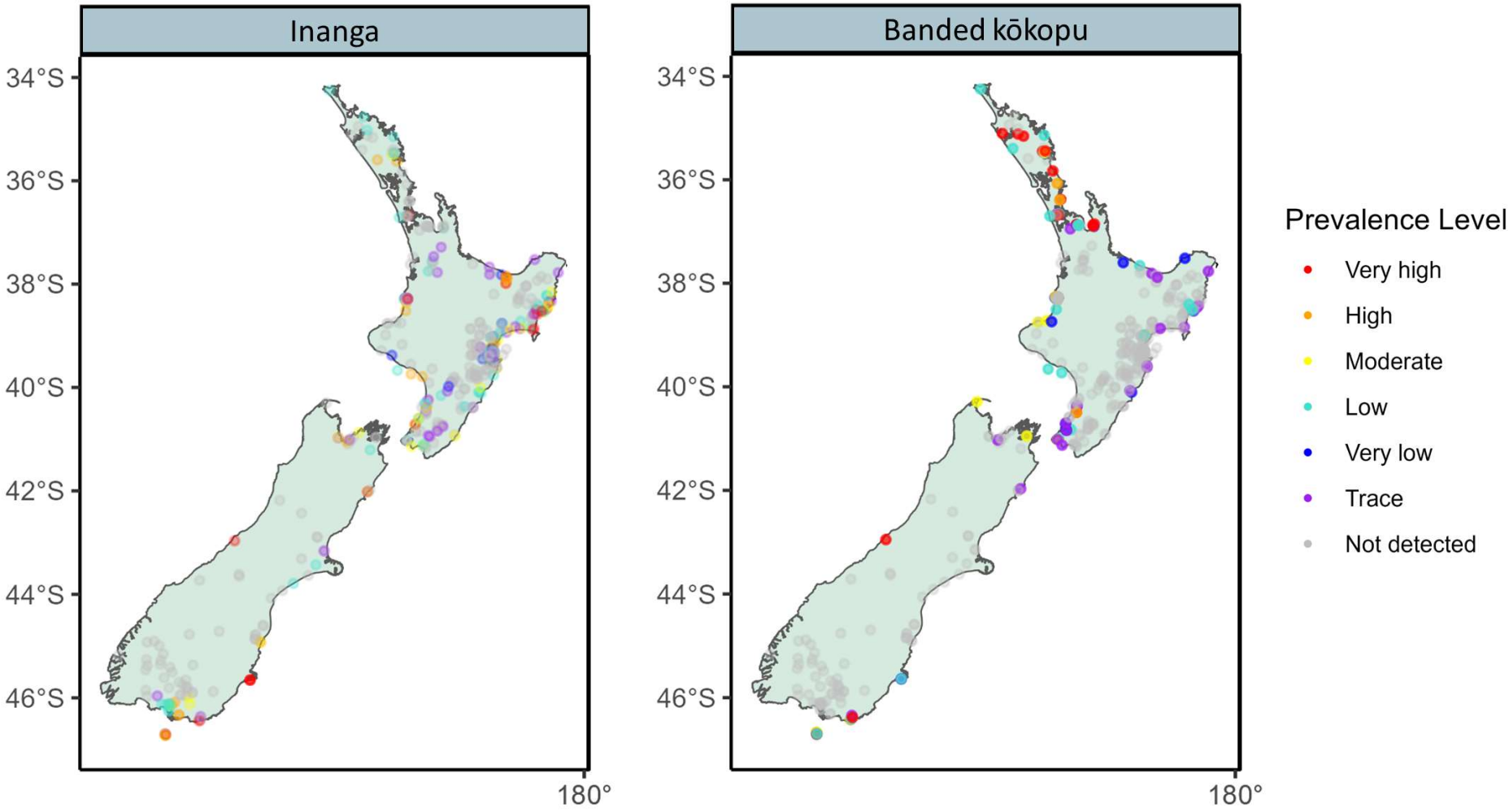


Figure 10-5: Distribution and prevalence of īnanga (left) and banded kōkopu (right) DNA reads across Aotearoa-New Zealand.

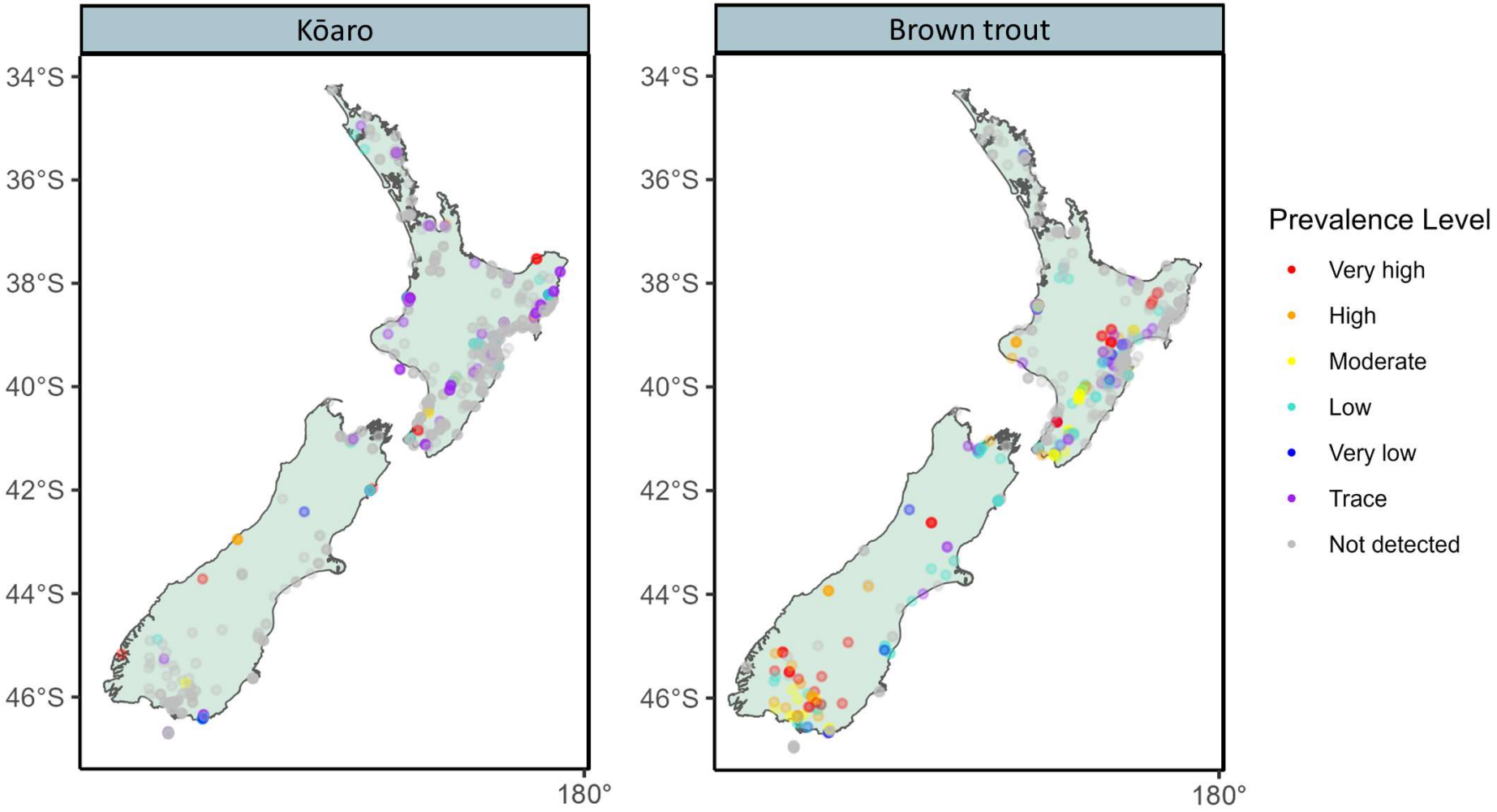


Figure 10-6: Distribution and prevalence of kōaro (left) and brown trout (right) DNA reads across Aotearoa-New Zealand.

Presently, the prevalence classes have not been validated with physical fish catch data. Consequently, while the prevalence index suggests that fish species are present and provides a scale for DNA reads, **these categories have not been correlated with fish abundance**. As covered in Section 3, the variability in production, degradation, transport, and detectability of DNA currently precludes quantification of species from water samples. However, with additional data collected, it may be possible to link the prevalence index to the relative abundance of species and help identify temporal changes in fish community structure. Rourke et al. (2021) reviewed 63 studies and found 90% identified positive relationships between eDNA concentrations and the abundance and/or biomass of target species, although the strength of these relationships were variable. Rourke et al. (2021) attributed this variation around the positive correlation to key biotic (taxon, life history, diet, metabolism, and behaviour) and abiotic (water flow, temperature, and capture method) factors. Further work understanding the influence of biotic and abiotic factors on eDNA detection will be beneficial in understanding how best to develop and apply a prevalence index of DNA reads to population abundance and/or biomass across different fish species.

11 eDNA versus conventional electric fishing

11.1 Conventional monitoring tools

The initial step in evaluating the suitability of emerging technologies like eDNA sampling is to compare their outcomes with those of traditional sampling methods (Lahoz-Monfort and Tingley 2018). Conventional tools for monitoring fish populations, such as capture-based techniques (e.g., electric fishing, netting, trapping; David et al. 2010), offer valuable insights into fish species and their populations, however, these methods also come with limitations (Table 11-1)

Table 11-1. Benefits and limitations of traditional fish capture methods (electric fishing, netting, trapping) compared with eDNA sampling.

Benefits Compared to eDNA sampling	Limitations compared to eDNA sampling
Precise data collection on abundance, size, sex, etc.	Labour-intensive.
Precise knowledge of location of species at the time of capture.	Restricted spatial and temporal coverage.
Focus on specific habitats or species (e.g., allowing researchers to target specific species or life stages.	Can exhibit selectivity concerning habitat and species.
Precise data collection on habitat associations at which species were caught.	Can be invasive (impractical for surveying small streams and endangered species).
Makes direct observations.	Relies on experienced taxonomists.

Biodiversity surveys conducted using eDNA can offer several advantages when compared to traditional methods. However, it is important to note that the choice between eDNA surveys and traditional electric fishing depends on research or management goals, site conditions, and the characteristics of the target species. Each method has strengths and weaknesses, and researchers/practitioners should select the most appropriate method based on their specific objectives and constraints (Table 11-2).

Table 11-2. Benefits and limitations of traditional fish capture methods (electric fishing, netting, trapping) compared with eDNA sampling.

Benefits compared to traditional methods	Limitations compared to traditional methods
Heightened detection sensitivity.	No ability to observe fish behaviour or collect data on demographic parameters.
Increased precision in identifying species.	Presence/absence data; reads do not directly correlate to abundance/biomass data.
Reduced taxonomic bias (e.g., can positively identify cryptic species).	Dependency on DNA databases and reference libraries.
Enhanced cost-effectiveness.	Risk of contamination in sample processing.

Benefits compared to traditional methods	Limitations compared to traditional methods
Broad-Scale Monitoring.	Inefficient for small-scale, fine-grained studies. Can be highly imprecise with respect to location.

11.2 Exploring fish detection and abundance through eDNA

eDNA sampling has demonstrated increased effectiveness in detecting species or communities when compared to traditional methods, as observed in studies such as Lugg et al. (2017). eDNA sampling is particularly well-suited for establishing fundamental data on species distributions and can be used for regular monitoring programs aimed at tracking changes in species distributions across time and space. However, as eDNA sampling integrates information across unspecified spatial and temporal scales, it constrains the ability to pinpoint locations and delineate species distributions at a fine scale.

When more detailed information is required about population dynamics or individual health, including aspects like reproductive output, juvenile recruitment, sex, size structure, and abundance, eDNA is not a suitable method, but can serve as a valuable tool in guiding and complementing the selection of sampling locations for traditional and more time-intensive capture methods. Notably, as technological advancements continue, eDNA methods may have the potential to provide insights into some aspects of populations and individuals, as evidenced in studies by Sigsgaard et al. (2016), Bylemans et al. (2017), David et al. (2021) and the comprehensive review of Rourke et al. (2021).

Using Waikato Regional Council eDNA and electric fishing data a generalised mixed effects model was conducted to examine the relationship between eDNA average count data (fish species only) based on six replicates with electric fishing abundance data (single pass over 150 m) across four Aotearoa-New Zealand streams (sampled by Waikato Regional Council). The fixed effect considered was fish density per 100 m^2 while random effects were chosen to be fish species nested within each site. A skew normal distribution was selected since the response was positively skewed. Model validation was performed using prior and posterior predictive checks, effective sample size, and R-hat diagnostics. The model was set up to account for the variation in replicates by including a measurement error component, which allowed the model to be more flexible when finding a line of best fit. Results indicated a positive association between average species sequence counts and number of fish per 100 m^2 (slope = 15.09; 95% CI [12.62, 17.54]; Figure 11-1).

No significant differences in species richness were observed when comparing the results of six replicate eDNA samplings with the electric fishing data obtained from the same streams (all adj $p > 0.49$). Species richness increased from an average of 6.4 ± 2.3 for species detected in one eDNA replicate to 9.8 ± 2.2 for six eDNA replicates, while electric fishing found 6.6 ± 3.0 species across sites (see Figure 11-2). Here, it is important to be aware that electric fishing solely captures the species present at a specific site, whereas eDNA sampling can also detect genetic material from species located at an unknown distance upstream. Consequently, there is a potential bias to detecting additional or rarer/elusive species.

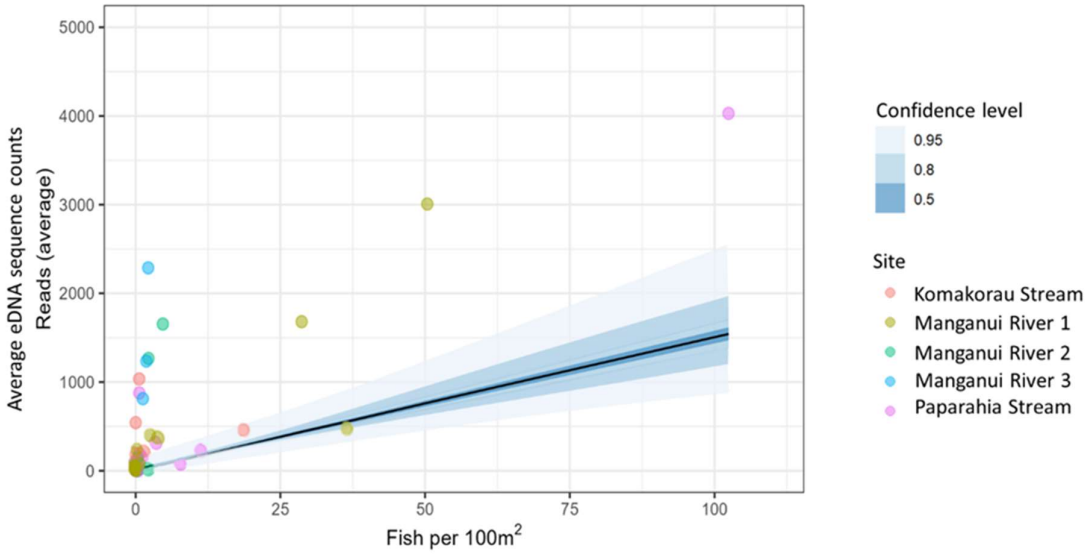


Figure 11-1. Relationship between average eDNA reads and fish species abundance (100 m^2). The black line indicates the model predicted mean, with credible interval levels indicated by 0.5, 0.8 and 0.95. Data points are coloured by site. **Dots represent species abundance within each site.**

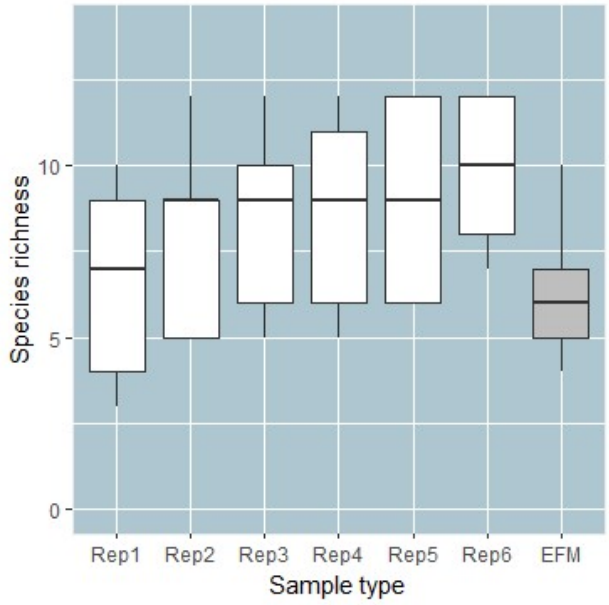


Figure 11-2: Species richness obtained via eDNA sampling (1 – 6 replications, white) and electric fishing (grey). While eDNA and electric fishing data were collected from the same site, eDNA sampling can detect genetic material from an unknown distance upstream, and, therefore, there is a potential bias to more species being detected.

12 Using eDNA to examine the effectiveness of fish passage barrier remediation

To examine the effectiveness of fish passage barrier remediation, Franklin et al. (2024) recommends a before-after-control-impact (BACI) survey, where the control reach is located downstream of a structure and the impact reach is upstream. A BACI approach to eDNA sampling would provide qualitative information on species richness and presence/absence of individual species upstream and downstream of an instream obstacle both before and after remediation. Presently, eDNA sampling is not quantitative as there is not enough information to translate DNA reads into an accurate measure of abundance or density. As eDNA reads do not correlate directly to fish abundance, it should be noted that if the instream structure is an impediment but passable intermittently, eDNA sampling may not conclusively determine a change in species abundance after remediation. In addition, eDNA sampling cannot determine if certain size classes of fish species were, or are, restricted by the instream structure (e.g., small juveniles) or the proportion of fish able to pass. However, for structures that likely form a severe impediment to fish passage, eDNA sampling could be useful for examining differences in species diversity downstream and upstream of the structure and changes in species diversity following management interventions. In particular, if weak swimming fish such as īnanga are absent above a structure before remediation, then eDNA could provide evidence for successful passage past the obstacle post-remediation (see Section 12.4).

In addition, eDNA sampling can help identify if undesirable fish species start penetrating past a purpose-built instream structure. In several parts of New Zealand, exclusion barriers have been constructed in waterways to protect native fish such as non-migratory galaxiids and mudfish from exotic or undesirable native predators (Franklin et al. 2024). Selective barriers have also been developed that enable the passage of native fish capable of climbing the wetted margins of instream obstacles but prevent undesirable exotic fish species from passing upstream. Here, targeted eDNA sampling could detect early breaches of purpose-built instream barriers by undesirable fish.

12.1 Survey method

Where possible a BACI approach should be undertaken as this is a robust method for assessing restoration success, controlling for spatial and temporal variation (Bunt et al. 2012, Mahlum et al. 2018). Mahlum et al. (2018) demonstrated that the natural temporal and spatial variability of fish movements often resulted in a higher risk of drawing false conclusions from the more commonly used BA (before-after) and CI (control-impact) survey methods when compared to the robust BACI design. However, the complexity of river landscapes can result in instream structures being located within a variety of locations with site-specific river conditions. Consequently, three approaches are recommended contingent upon structure location (Figure 12-1):

A. **Single stream BACI survey**. If the habitat below the structure is comparable to that above the structure and similar fish populations would be expected to occur both downstream and upstream of the obstacle, then a BACI survey should be undertaken (Figure 12-1). It is recommended that the control site is located 300 – 500 m below the instream structure to effectively sample DNA from fish resident within the waterway as well as those congregating below, or delayed by, the structure.

B. **Twin stream BACI survey**. If the instream structure is located close to the confluence of a stream or where the habitat changes significantly downstream of the structure and, therefore, a different fish community would be expected to be present upstream and downstream of the

structure, then locating the control site in a nearby/neighbouring stream is recommended (Figure 12-1). Here, the applicable control site should:

a. Be barrier free.

b. Be located at a comparable distance inland and elevation to where the instream structure being assessed is located.

c. Have the same fish community as the study stream.

C. **BA survey**. If selecting an appropriate control site within the survey stream or nearby waterway is deemed unfeasible then a BA survey using eDNA upstream of the structure before and after remediation is the third option (Figure 12-1). Here, a desktop based assessment is carried out to gather available knowledge on the fish species that are likely to be reaching the structure and requiring passage upstream. A suggested approach is:

1. **Examine fishing records from recent surveys and the Aotearoa-New Zealand Freshwater Fish Database (NZFFDB)**. Download all records held in the NZFFDB (https://nzffdms.niwa.co.nz/search). Outside of ArcGIS and other GIS platforms, the NZ species DB (downloadable from Jowett Consulting – NZ Species DB) is a useful tool for visualising records from the NZFFDB and examining stream gradients, elevation and distance inland. In general, records from the last 15 years will be the most reliable indicator of fish community presence.

2. **Engage with mana whenua to identify mahinga kai sites**. Discussions with mana whenua will identify if mahinga kai were traditionally gathered from the impacted stream and what fish species were historically present in that region.

3. **Examine the Freshwater Fish Probability of Capture models.** Leathwick et al. (2008) used the NZFFDB records and the River Environment Classification (REC1) to generate spatial predictions of freshwater fish probability of capture across Aotearoa-New Zealand. Crow et al. (2014) updated the model of Leathwick et al. (2008) using the REC2 and where data were available, completed separate probability of capture models for different fishing methods. The model of Crow et al. (2014) can be accessed through NZ River Maps. However, GIS layers are also available.

   The model of Crow et al. (2014) also includes a classification threshold based on Cohen's Kappa. For each species in the model, the Cohen's Kappa value represents the probability threshold above which a prediction value is considered more likely than not to indicate a species could be present. For any stream segment if the predicted probability of capture is higher than the Cohen's Kappa value plus one standard deviation then the species is likely to be present. Similarly, if the predicted probability of capture is lower that the Cohen's Kappa value minus one standard deviation then the species is most likely to be absent. Values within the range of the Kappa value plus or minus the standard deviation have a varying degree of confidence in both categories and should be assigned as indeterminate.

   A caveat of using the probability of occurrence models, however, is that they predict poorly for certain species that are underrepresented in the NZFFDB (e.g., lamprey). As such, these models should be viewed with caution and used only as an indicative guide to help identify stream segments more likely to contain target species by having higher probability values.

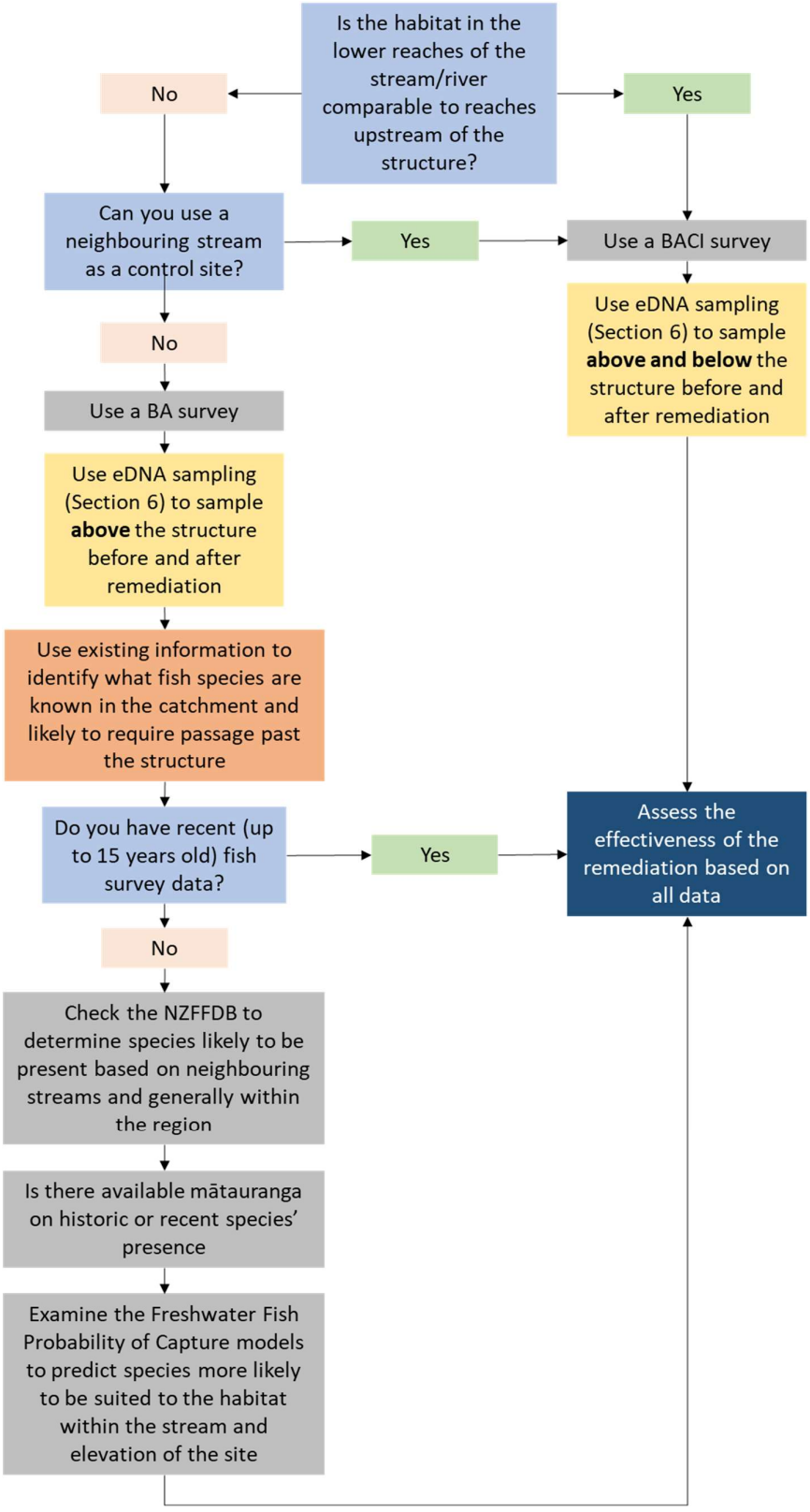


Figure 12-1: Decision tree to guide the most applicable eDNA survey method for a given site. Abbreviations: BACI, before-after-control-impact; BA, before-after.

12.2 Protocols

When undertaking eDNA sampling as part of a BACI or BA survey it is critical to ensure that data are collected in a consistent, standardised and reproducible way. This means that for both the control and impact reaches, and before and after remediation:

- Sampling is carried out when the target fish species are migrating and likely to have reached the site (based on the distance inland).
- Sampling upstream and downstream of the structure is carried out on the same day and the before and after surveys are carried out at the same time of year (i.e., within the recommended December to March timeframe).
- The same sites are used for each repeat survey.
- Sampling is carried out under similar conditions (e.g., similar flows).

Sampling protocols are outlined in Section 7 & 8. To maximise detection of the species present, it is recommended that for each of the before, after, control and impact samples:

- Utilise the 6 x 1 L replicate or 6 x passive sampler method.
- Samples should be taken at the thalweg of the stream or as close to the thalweg as possible.
- Samples upstream of the structure should be taken where the stream is unimpacted by the structure itself, i.e., upstream of any impoundment of the stream.

12.3 Frequency of sampling

Fish migrations are highly variable and can occur in pulses or triggered by specific environmental cues. Consequently, migration past the structure will also be highly variable and as such, eDNA sampling at one point in time may lead to false conclusions being drawn from once off or short-term sampling. To increase the likelihood of drawing valid conclusions from eDNA monitoring of fish passage remediation success, we recommend the following:

- One pre-remediation sampling at each site.
- Annual sampling at each site for three years post-remediation.

Based on target fish species, if the monitoring results indicate that the works have not improved fish passage past the structure, then further modifications will be necessary. Further monitoring should then continue annually for three consecutive years post each successive modification. Additional monitoring using other methods may also be required to evaluate the success of remediation against other performance standards (e.g., abundance or size based measures).

12.4 Indicator species

At each site, the target species for passage may influence when to use eDNA sampling for assessing the effectiveness of barrier remediation over traditional fishing techniques. For example, īnanga is a weak swimming fish and used as an indicator species for fish passage remediation as if īnanga can pass the obstacle, it is likely that all other species will also be able to navigate the impediment. Īnanga are essentially an annual species with adults migrating down to the estuary to spawn each

autumn and the majority dying after spawning. As such, over winter there should be little to no īnanga DNA present above instream structures that are located upstream of spawning grounds. Consequently, eDNA detection of īnanga upstream of instream structures during the recommended December to March sampling period would originate from upstream migrating juveniles successfully passing the structure that season. In this regard, where populations are present, īnanga may be an effective indicator species in eDNA monitoring of barrier remediation.

13 Summary of the challenges in eDNA sampling

This chapter summarises and further discusses the challenges (continuing from Section 2.3) that can arise during eDNA sampling.

13.1 Potential sources of false positives

eDNA sampling relies on inference because it is nearly impossible to directly observe organisms shedding DNA molecules or track the movement of specific DNA molecules in aquatic systems. Understanding and addressing potential sources of false positives are critical for the accurate interpretation of eDNA data in ecological studies and environmental monitoring.

Allochthonous eDNA

Allochthonous eDNA, originating from outside the sampling area, can lead to false positives. It may be transported and deposited through various mechanisms, such as the relocation of sediment containing eDNA, human deposition of material, (e.g., people discarding fish frames, fish-based fertiliser), faecal deposition by terrestrial animals or aquatic predators (Darling and Mahon 2011, Mahon et al. 2013)

Disturbances

Environmental disturbances can introduce DNA from species that are not present or have been extirpated, leading to false positives in eDNA detections (Turner et al. 2015). While it is anticipated that these signals from alternative sources may be weak or inconsistent over time, more comprehensive research is needed to understand their potential impact on the abundance and distribution of the focal species' eDNA.

Contamination

Contamination during sample collection, processing, or analysis may lead to false positives in eDNA data. Inadequate sample handling and storage practices can introduce foreign DNA into the samples, leading to false positives. Proper storage conditions, sampling and handling are essential to maintain sample integrity. Remembering that all equipment used for eDNA sampling must be kept clean - avoiding contact with surfaces in vehicles and boats that may be contaminated.

Cross-reactivity

Some eDNA assays may cross-react with DNA from closely related species, potentially causing false positives (Thomsen and Willerslev 2015).

Bioinformatics and data analysis errors

Errors in data analysis, including misinterpretation of results or incorrect filtering criteria, can contribute to false positives if not appropriately addressed during analysis.

13.2 Inference across space and time

Study design considerations

eDNA results may not provide immediate real-time information about species presence due to various factors, including site characteristics, eDNA transport, and the time required for sample processing and laboratory analysis. To account for variability in eDNA concentration and distribution, it is essential to incorporate these factors into eDNA study designs. For example, collecting water samples from multiple locations within a site and pooling them for analysis can provide a more

representative assessment. Alternatively, targeting specific habitat components known to be relevant to the species of interest can enhance detection accuracy. In flowing water systems, the movement of water can result in eDNA concentrations that may not correlate with local species presence over significant distances (Pilliod et al. 2013).

eDNA degradation

eDNA degrades over time when exposed to the environment. Degradation rates can vary but generally limit the detectability of eDNA in water to periods ranging from 1 day to 8 weeks (Dejean et al. 2011). A time lag between species presence and eDNA sampling can lead to false conclusions of species absence due to degradation. This can limit the scope of eDNA studies, with only small segments of genetic material remaining, particularly in warm conditions (Strickler et al. 2015, Goldberg et al. 2018, Harrison et al. 2019, Moushomi et al. 2019, Murakami et al. 2019).

13.3 Inferring presence versus viable populations

Challenges for rare and declining species

eDNA detection provides valuable insights into monitoring invasive species and established populations, offering early signals of their presence (Smart et al. 2015). However, it presents unique challenges when it comes to rare or declining species. For such species, eDNA detection offers only partial information, as it may not distinguish individuals in "sink" habitats from those in reproducing, stable populations, and it might miss critical details like sex, body condition, and directional hybridization. Overreliance on eDNA for monitoring rare species, when direct observation is possible, could inadvertently conceal actual declines and hinder conservation efforts. To address these challenges, eDNA surveys can be integrated into an occupancy modelling framework to detect landscape-level population trends (MacKenzie et al. 2013).

eDNA versus species abundance

Although research has demonstrated correlations between eDNA quantities and organismal abundances in controlled experiments and some field sites, this relationship can be influenced by outliers (Spear et al. 2015). Biotic factors (such as taxon, life history, diet, metabolism, and behaviour) and abiotic factors (including water flow, temperature, and capture method) have been identified as significant influencers on the positive correlation between eDNA reads and population abundance (Rourke et al. 2021). Nevertheless, further research is necessary to gain a more comprehensive understanding of the relationship between abundance and eDNA detection reads.

14 Conclusions

The use of eDNA methods for species detection may offer some advantages over traditional sampling techniques. It minimises the impact on sensitive species, enhances the effectiveness of surveys, and presents an opportunity to detect rare and elusive species. Additionally, eDNA sampling can introduce efficiency gains and cost reductions, particularly through lesser reliance on expert staff and consultation time. This can aid in increasing our understanding of freshwater species distribution at a broad scale, particularly in regions with limited or non-existent fish community records.

These guidelines for eDNA monitoring in lotic systems are the result of collaboration and expertise from the Environmental DNA Working Group, a consortium of freshwater experts from regional councils, Ministry for the Environment, Department of Conservation, NIWA, Waikato University and Wilderlab. The aim of this manual was to establish a standardised national approach to eDNA monitoring in lotic environments, ensuring uniformity in effort and methodologies across similar applications. While these guidelines primarily focus on fish and macroinvertebrate sampling, reflecting the availability of comprehensive reference libraries and validated field trials, they remain adaptable and responsive to the continuous advancements in eDNA methods and applications.

Although eDNA has been shown to be successful for biodiversity monitoring in lotic systems, there are still a number of challenges specific to stream and river systems that must be overcome to achieve accurate, standardised tools that can be routinely and reproducibly implemented. Key guidance points in this manual include optimal eDNA field sampling and capture methods (including passive and syringe-based filtration techniques), interpreting eDNA sampling results and the concept of the eDNA Prevalence Index, proposed for assessing the presence and prevalence of DNA from detected species. Furthermore, it explores use of eDNA monitoring in comparison to conventional electric fishing and its practical application in evaluating fish passage barrier remediation efforts.

These guidelines highlight the potential of eDNA monitoring, a rapidly evolving field expected to become a key part of biodiversity and ecosystem health assessments. As the science of eDNA continues to advance, both in sampling methods and analyses, these guidelines will evolve to ensure they remain at the forefront of eDNA applications in Aotearoa-New Zealand.

15 Acknowledgements

The collaborative effort in creating this eDNA guidelines for lotic environments involved the contributions of numerous individuals and organisations, reflecting the collective knowledge and expertise provided through the eDNA working group.

The development of these guidelines would not have been possible without several key contributors; Shaun Wilkinson and Amy Gault from Wilderlab Ltd, whose insights, advice, and the utilisation of their findings, reports, images, and data were invaluable to this project. Josh Smith at Waikato Regional Council, along with Bruno David (formerly of Waikato Regional Council), for generously sharing eDNA and electric fishing data, as well as an early draft of an eDNA manuscript. Andy Hicks from the Ministry for the Environment, whose support and input improved the project and Thomas Moore for his valuable guidance in data analysis.

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We hope that this guide will prove a useful resource for users as they either begin or continue to integrate these eDNA into the suite of tools used for biomonitoring.

16 Glossary of abbreviations and terms

Amplification	The process of creating copies of a particular region of DNA (the amplicon), usually through a PCR reaction using primers and enzymes such as polymerases. Non-target amplification refers to the unintended amplification of DNA from taxa that the primers were not designed to amplify.
Barcoding	The taxonomic identification of a species by sequencing specific genetic markers, such as COI (Cytochrome c oxidase subunit I) or *rbcL* (Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit), typically from a single specimen.
Bulk sample	A mixed community sample of organisms or their tissues (macroinvertebrates) such as would be collected in a net or trap.
DNA (Deoxyribonucleic Acid)	A molecule that carries genetic information in living organisms and viruses. It consists of a double helix structure made of four nucleotides (A, C, G, T) and encodes instructions for an organism's development and functioning.
eDNA/Environmental DNA	Genetic material (e.g., DNA fragments) shed by organisms into their environment, such as a stream or river. eDNA can be used for species detection, identification, and monitoring by analysing the genetic material present in environmental samples.
Filter	Membrane filter for the capture of eDNA constructed out of a wide range of synthetic materials with specific pore sizes.
Genetic marker	A specific DNA region or sequence that is used to identify or differentiate organisms at the genetic level. Genetic markers are often unique to particular species or taxa.
High-throughput Sequencing	DNA sequencing technology that produces millions of DNA sequence reads in parallel. Enables thousands of different organisms from a mixture of species to be sequenced at once, to obtain community data from a single analysis (i.e. metabarcoding).
Lentic	Still water, i.e lakes and ponds.
Lotic	Flowing water, i.e streams and rivers.
Metabarcoding	The taxonomic identification of multiple species derived from a mixed sample. This involves PCR (Polymerase Chain Reaction) amplification and high-throughput sequencing of genetic markers to detect various species simultaneously.

Negative control	A negative control is used to check for potential contamination. A negative site control refers to a sample collected from a field site where the target taxon is known to be absent. A negative filtration control is a sample where DNA-free water is filtered alongside the eDNA samples to check that DNA is not transferred between samples. Negative laboratory controls consist of DNA-free samples processed alongside the test samples at each stage of the process to check for (cross-)contamination. In the context of DNA extraction, a negative control should not contain a DNA template and in the context of PCR, a negative control should not give amplicons.
PCR (Polymerase Chain Reaction)	A laboratory technique used to amplify and replicate a specific DNA segment, making it possible to detect and analyse even trace amounts of genetic material.
PCR (Polymerase Chain Reaction) inhibition	Where PCR is hindered or suppressed due to the presence of substances or factors that interfere with the amplification process (e.g., contaminants, inefficient DNA extraction).
Replicates	Multiple, independent samples collected from the same site or environment to increase data reliability and account for variation.

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Appendix A Environmental effects on eDNA

We gathered environmental data (hourly averages), comprising variables such as discharge ($m^3 s^{-1}$), temperature (°C), and turbidity (NTU), from regional councils across New Zealand. Our aim was to explore the associations between passive sampler eDNA-detected richness of fish and macroinvertebrates, as well as total eDNA counts (fish and macroinvertebrates). We found no significant relationship between discharge ($m^3 s^{-1}$) and fish (β = -0.0003, SE = 0.0016, t = -1.75, p = 0.09) and macroinvertebrate eDNA richness (β = -0.0283, SE = 0.016, t = -1.8, p = 0.08, Figure A-1), or between discharge and total eDNA sequence counts (β = -0.0001, SE = 0.0001, t = -1.91, p = 0.07, Figure A-2).

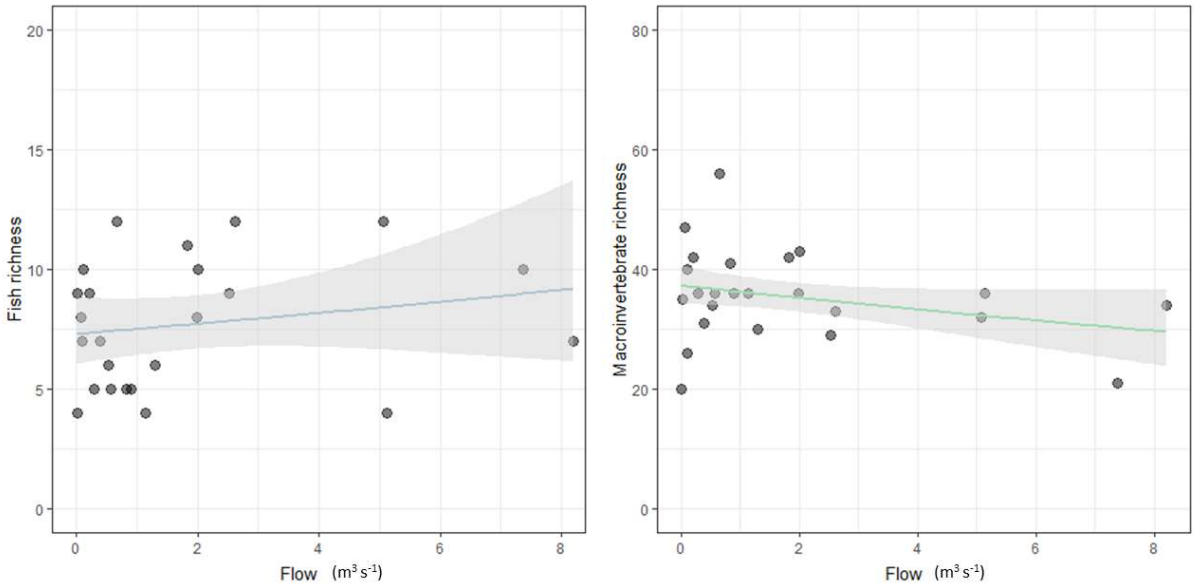


Figure A-1: Relationship between discharge/flow ($m^3 s^{-1}$) and richness of fish (left) and macroinvertebrates (right) detected by passive eDNA samplers. Grey shaded area indicates 95% confidence intervals.

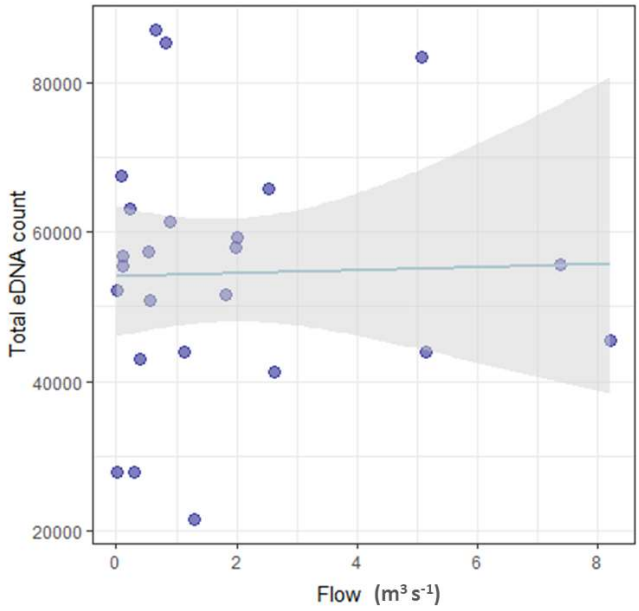


Figure A-2: Relationship between discharge/flow ($m^3 s^{-1}$) and total eDNA count of fish and macroinvertebrates detected by passive eDNA samplers. Grey shaded area indicates 95% confidence intervals.

Similarly, when testing for effects of water temperature, no significant relationships were found between water temperature (°C) and richness in macroinvertebrates (β = -0.00295, SE = 0.00035, t = -0.85, p = 0.39) and fish (β = -0.0001, SE = 0.0001, t = -1.29, p = 0.21, Figure A-3), or between temperature and total eDNA count (β = -0.00069, SE = 0.00136, t = -0.51, p = 0.62 from the passive samplers (Figure A-4).

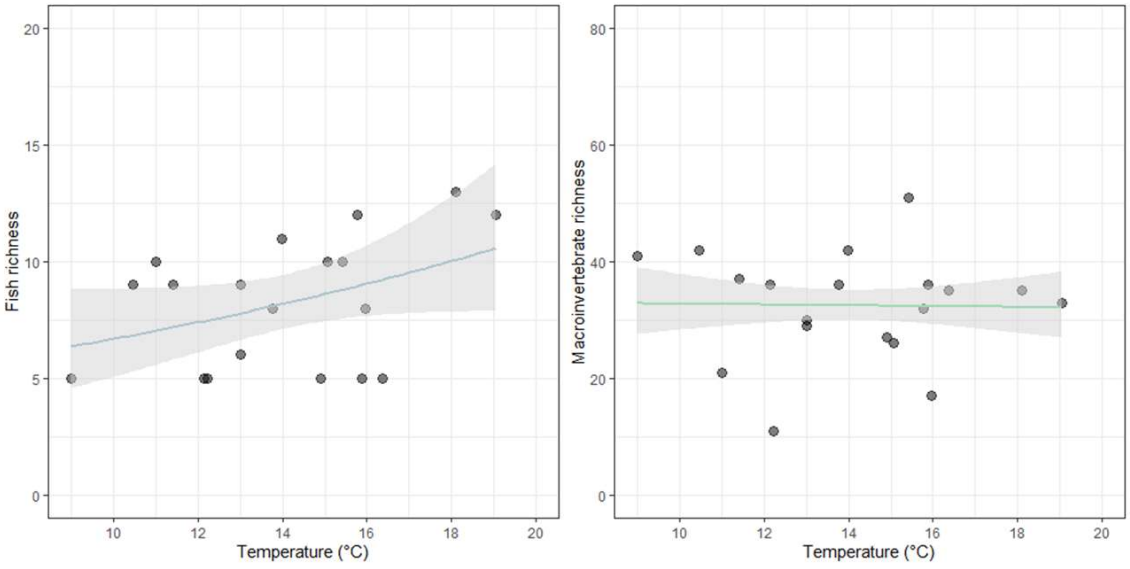


Figure A-3: Relationship between temperature (°C) and richness of fish (left) and macroinvertebrates (right) detected by passive eDNA samplers. Grey shaded area indicates 95% confidence intervals.

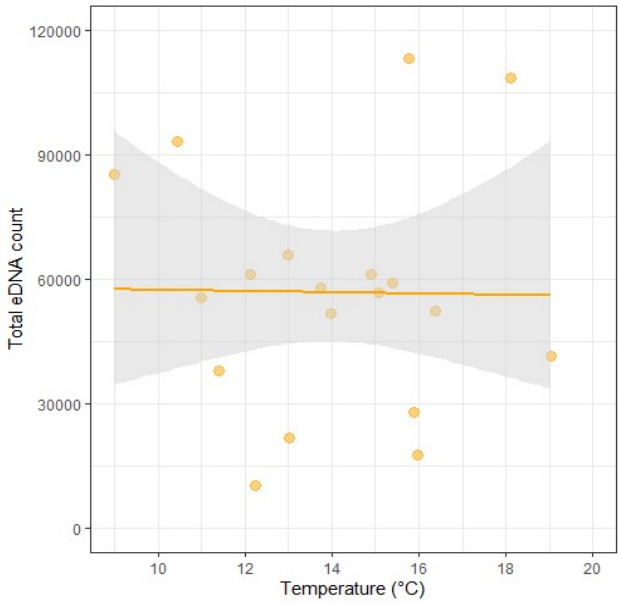


Figure A-4: Relationship between temperature (°C) and total eDNA count of fish and macroinvertebrates. Grey shaded area indicates 95% confidence intervals.

As for flow and temperature, no significant relationships were observed between turbidity and fish richness (β = 0.001138 SE = 0.0011, t = 05, p = 0.28), macroinvertebrate richness (β = -0.00364 SE = 0.0021, t = 1.79, p = 0.17, Figure A-5), and the total count of eDNA from the passive samplers, although this relationship marginally significant (β = -0.0001 SE = 0.0001, t = 2.22, p = 0.08, Figure A-6). It is important to note that the sample size used in this analysis was low as data were only available for nine sites.

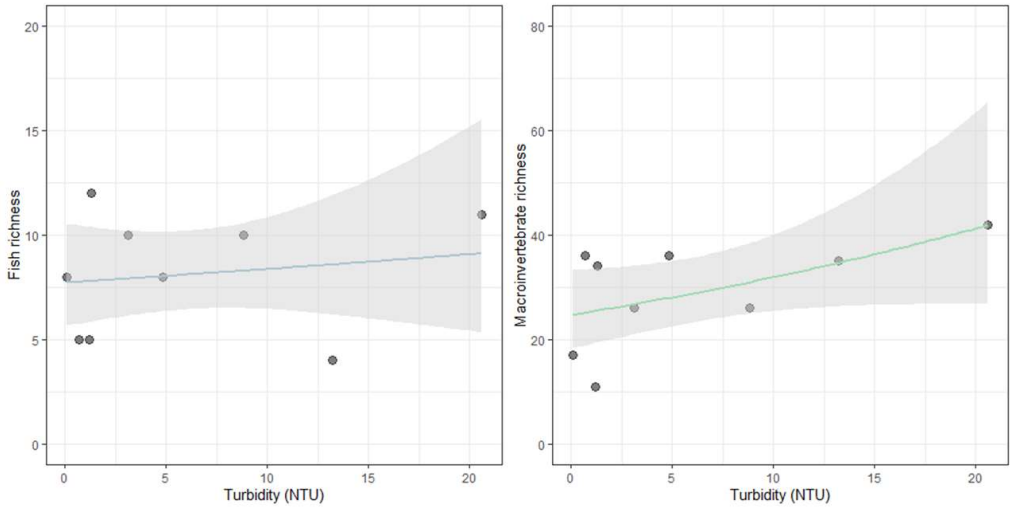


Figure A-5: Relationship between turbidity (NTU) and proportion richness of fish (left) and macroinvertebrates (right) detected by passive eDNA samplers. Grey shaded area indicates 95% confidence intervals.

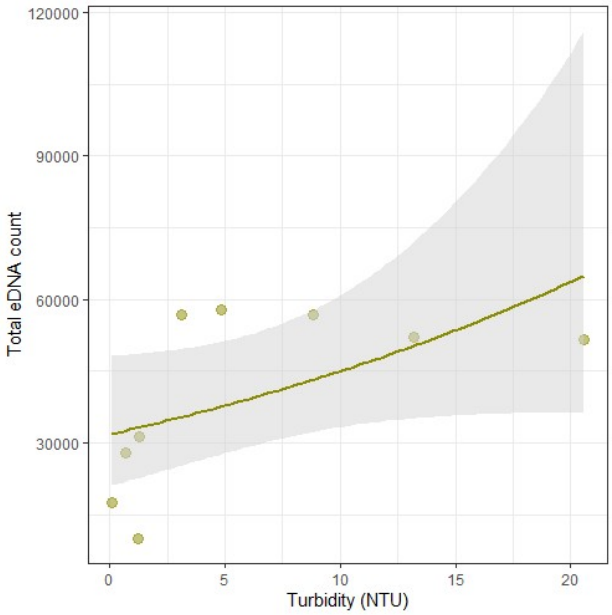


Figure A-6: Relationship between turbidity (NTU) and total eDNA count of fish and macroinvertebrates detected by passive eDNA samplers. Grey shaded area indicates 95% confidence intervals.

Appendix B Wilderlab active sampling protocols

eDNA Mini Kit Instructions

WILDERLAB

If you would like more information including instructional videos, please scan the code to visit wilderlab.co.nz/directions

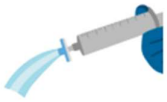
1

Take the gloves out of the sample bag, put them on, and take out the large syringe. Draw up 50 ml of water from just below the surface of the water. Take care not to suck up any sediment from the bottom.

2

Gently screw the filter on to the large syringe taking care not to overtighten, then push the plunger down to squeeze the water out through the filter. Avoid getting air bubbles in the filter as they can be difficult to push through.

3

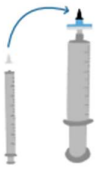


Unscrew the filter from the large syringe and continue drawing and filtering until 1L of water has been filtered (20 syringefuls), or the filter is clogged. If this happens, gently pulling back on the plunger may sometimes dislodge any particles trapped in the filter.

4

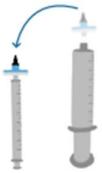
Unscrew the filter and draw 50 ml of air into the large syringe. Re-attach the filter and squeeze the air through the filter to remove excess water, while holding the syringe vertically with the filter pointing down.

5



Holding both the large syringe (with the filter still attached) and the small syringe (with black cap attached) in the same hand and in an upright orientation, transfer the black cap from the small syringe on to the outlet end of the filter.

6



Unscrew the filter (with the black cap now attached) from the large syringe and screw it on to the small syringe.

7

Push the plunger of the small syringe to inject the preservative into the filter. Shake well while holding the plunger down. Do not remove the syringe or cap from the filter. Don't worry if there are any air bubbles in the filter or if the plunger springs back – this is normal.

8

Place the filter with both the black cap and small syringe still attached into the sample bag.

9

Seal the sample bag and record the sample details in the space provided. Ensure that the coordinates are entered in WGS84 decimal format (for example -41.30951, 174.82110 as displayed on Google Maps).

Submit your samples online at wilderlab.co.nz/submit-samples

Print and sign the chain of custody (CoC) form that has been emailed to you after sample submission. Include this in the parcel containing your samples (no refrigeration necessary).

Send the samples by standard courier to:

Wilderlab NZ Ltd
Level 2, 129 Park Road
Miramar
Wellington 6022

Small packages can be sent by post to:

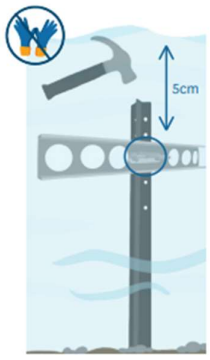
Wilderlab NZ Ltd
PO Box 15059
Miramar
Wellington 6243

Appendix C Wilderlab passive sampling protocols

eDNA Manifold-mount Instructions

WILDERLAB

Manifold set up:



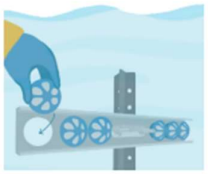
Hammer your waratah into the stream bed, in an area of moderate to high flow.

Attach the passive sampler manifold to the waratah using the provided U-bolt and nuts with the edged section of the manifold facing upstream. Ensure the top of the manifold is approximately 5cm below the stream surface.

Leave the manifold for approx. 5 minutes prior to inserting the filter pods to reduce the risk of cross contamination between sampling events and sites.

Deploying the passive sampler:

1



Put on a pair of gloves from the kit, and carefully push each filter pod (up to 6 can be used) into place with the leaf guard facing upstream. Ensure a tight and secure fit.

2

Record the location co-ordinates in WGS84 decimal format (for example -41.30951, 174.82110 as displayed on Google Maps).

Leave the sampler deployed for 24 hours.

Retrieving the passive sampler:

1

Put on the remaining pair of gloves. If you have more than one filter pod, work with one at a time: gently remove a filter pod from the manifold by pushing on the edges of the pod from the back.

2

Pointing the leaf guard to the ground, pull on the easy-pull tag protruding from the back of the filter pod to remove the sponge filter. This motion squeezes out excess water from the sponge filter.

3

Avoid touching the filter directly. Holding the tag, flick the filter in a downward motion to rid the filter of even more excess water.

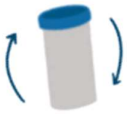
4

Take the sample jar out of the kit and place only the sponge filter in the sample jar.

5

Take out the small syringe containing preservative and unscrew the cap. Dispense all preservative into the sample jar containing the filter.

6



Screw the jar lid on tightly, then shake the sample jar well to ensure distribution of the preservative throughout the filter.

7

Repeat these steps for all filter pods in your manifold. Place all sample jars in the sample bag, and complete the sample information on the back of the bag.

If you would like more information including instructional videos, please scan the code to visit wilderlab.co.nz/directions

Submit your samples online at wilderlab.co.nz/submit-samples

Print and sign the chain of custody (CoC) form that has been emailed to you after sample submission. Include this in the parcel containing your samples (no refrigeration necessary).

Send the samples by standard courier to:

Wilderlab NZ Ltd
Level 2, 129 Park Road
Miramar
Wellington 6022

Small packages can be sent by post to:

Wilderlab NZ Ltd
PO Box 15059
Miramar
Wellington 6243

Appendix D Wilderlab data interpretation guide

Version 1.2.6 – August 2022

Interpreting eDNA results

WILDERLAB

Your results will be provided in both an Excel and online format

1 Excel spreadsheet - Made up of three tabs:

Metadata

The job and sample information included on the sample submission form, including additional fields such as a passcode to access your online sample reports, any laboratory notes, and your account information.

Aggregated

A simplified version of the results without DNA sequence barcodes and with one row for each unique taxon (i.e. species, genus, etc) found in the samples.

Full

The full eDNA results including DNA sequence barcodes and the assay codes which sequences were detected on, with one row for each unique eDNA sequence found in the samples.

Example of full results

① Sequence	② Target	ScientificName	Rank	TaxID	CommonName	Group	③ 518902
ATCCTTGTTT	TP	Nasturtium officinale	species	65948	Watercress	Plants	2366
TCTTGCAGC	CI	Orthonychiurus folsomi	species	2581074	Springtail	Springtails	1701
TTAGCCCTA	RV	Homo sapiens	species	9606	Human	Mammals	138
TTTACTCTA	WV	Lumbriculus variegatus	species	61662	Blackworm; California blackworm	Worms	447
TTTATCCGC	CI	Deleatidium lilli	species	1968926	NZ mayfly	Insects	761
TCTTTCAGC	CI	Coloburiscus humeralis	species	241031	NZ spinygilled mayfly	Insects	545
TTTATTTTTA	WV	Chaetogaster diaphanus	species	212246	Oligochaete worm	Worms	118
TTTATCTTAT	WV	Aulodrilus pluriseta	species	76585	Aquatic oligochaete worm	Worms	71
TTTAGACAC	WV	Galaxias brevipinnis	species	66447	Koaro	Fish	121

④

① **Sequence:** This is a short stretch of DNA which can be used to identify different taxa, often referred to as 'sequence' or 'barcode'.

② **Target:** This is the code for the assay which the sequence was detected on.

③ **UID:** This header is your samples' unique identifying number and can be easily matched to your personally assigned sample names by referring to your Metadata sheet.

④ **Sequence count:** This is the number of times a unique sequence (Full sheet) or unique taxa (Aggregated sheet) was detected in each sample taken.

2 Online sample report

Each eDNA sample recieves an online sample report which can be viewed via the eDNA Explore Map. All sample reports are passcode protected (included on your Metadata tab), unless you specified to make them publicly accessible on the sample submission form.

These reports are a more interactive and visual way to explore, communicate and share your eDNA results. It includes lists of detected species with searching and filtering capabilities, detected species images, Biodiversity Wheels and ecological health insights.

These reports are also a living copy of your results, which are regularly updated as new sequence information is made available. For example, a sequence identified as 'Insect' today, could be refined to *Nesameletus*/Swimming mayfly tomorrow!

We are always trying to find new ways to help you visualise and interpret your eDNA results, so the format and contents of these reports are ever evolving.

1

Version 1.2.6 – August 2022

Understanding sequence counts

There are several factors which influence eDNA sequence counts including the distance of organisms from the sampling point, the presence of dead/decaying organisms, environmental factors which can speed up or slow down eDNA breakdown, and assay biases, which can lead to preferential detection of certain groups of organisms. Because of this, these counts can only loosely be used to predict the abundance/biomass of organisms in the local environment.

Interpretation of sequence counts can vary depending on the research question you are looking to ask of the dataset.

Due to the high sensitivity of eDNA testing, a low sequence count in few replicate samples should be considered a tentative detection. Depending on the importance of the taxa, follow up eDNA sampling or similar biological survey should be used to determine presence or absence.

Unexpected detections or non-detections

Occasionally, some organisms that you had expected to find are not detected in your eDNA sample. This can occur if certain taxa are out of scope of our assays, missing from the reference database, or if insufficient replicates are taken. **For most applications, we recommend taking 6 replicate samples** at each sampling site to maximise the detection rate and reduce false negatives. There are usually several unidentified sequences which remain at the end of your sample results which are a combination of organisms not yet described or not yet in a reference database. These sequences can continue to be updated with new taxon information as new reference sequences become available, so the resolution of your data can continue to improve over time.

On the other hand, some organisms which you may not have expected to find are detected in your eDNA sample. Due to the sensitivity of eDNA testing, field gear, vehicles, run-off, and animal faeces, are all potential pathways for DNA to be introduced into your sampling area. This is when local knowledge and context of the site is particularly important and can be used alongside your understanding of sequence counts to reduce misinterpretation and false positives.

It is very helpful for us if you let us know if you notice any taxa that might be missing (e.g. found in high abundance near the sampling site), as we can often use this information to improve our assay panel and reference database for the future.

Broader ecological insights

We have been hard at work optimising our new riverine ecological health index, the taxon-independent community index (TICI), and it is now available for any river/stream sample processed with our comprehensive assay panel.

The TICI is similar to the New Zealand's macroinvertebrate community index (MCI), except the tolerance values have been assigned to DNA sequences from across the tree of life, instead of just invertebrate taxa. Read more about the TICI here.

TICI score	TICI value
< 80	Very Poor
80 – 90	Poor
90 – 100	Average
100 – 110	Good
110 – 120	Excellent
> 120	Pristine

To help interpret the TICI score we include:

TICI nseqs: The number of individual indicator sequences included in the TICI score.

TICI reliability: The reliability/robustness of the score as determined by the number of composite tolerance values.

TICI quantile: This indicates the position of your score amongst 52 well known river sites around the country (taken as part of a national eDNA trial carried out by Aotearoa's regional councils 2021/2022).

TICI rating: Qualitative value which aligns with the MCIhb scoring system.

TICI dial: On your online sample report, you can find a visual dial to report your TICI score.

2

Appendix E Sequence counts versus fish abundance

Site	Scientific name	Common name	Group	rep1	rep2	rep3	rep4	rep5	rep6	av_reads	SD	hits	fish_150m	fish_100m	fish_prese	edna_pres
Paparahia Stream	Gobiomorphus huttoni	Redfin bully	Fish	5092	4359	3738	2578	3327	5079	4028.8	915.0824	6	487	102.42	1	1
Paparahia Stream	Galaxias fasciatus	Kokōpu,Banded kokopu	Fish	1423	1093	954	364	731	700	877.5	333.295	6	3	0.63	1	1
Paparahia Stream	Anguilla australis	Tuna; hao; aopori; hikum	Fish	190	422	294	8	348	592	309.0	182.2846	6	17	3.57	1	1
Paparahia Stream	Anguilla dieffenbachii	Tuna; kūwharuwharu; re	Fish	124	239	251	238	182	268	217.0	49.25444	6	54	11.36	1	1
Paparahia Stream	Galaxias maculatus	Īnanga,Inanga	Fish	366	690	0	33	0	0	181.5	262.5171	3	4	0.84	1	1
Paparahia Stream	Cheimarrichthys fosteri	Panoko; pānokonoko; pā	Fish	156	0	0	640	10	30	139.3	230.3813	4	6	1.26	1	1
Paparahia Stream	Geotria australis	Piharau; kanakana,Pouch	Fish	0	314	0	126	0	0	73.3	117.0508	2	4	0.84	1	1
Paparahia Stream	Galaxias argenteus	Giant kokopu	Fish	128	0	0	0	0	0	21.3	47.70278	1	2	0.42	1	1
Paparahia Stream	Galaxias brevipinnis	Kōaro; maehe,Koaro	Fish	0	17	0	0	30	80	21.2	28.57981	3	0	0	0	1
Paparahia Stream	Galaxias argenteus/postv	Kokōpu,Giant or shortjaw	Fish	23	0	0	0	0	16	6.5	9.411872	2	0	0	0	1
Paparahia Stream	Retropinna retropinna	Ngaore; paraki; pōrohe,C	Fish	5	0	29	0	0	0	5.7	10.5935	2	0	0	0	1
Paparahia Stream	Gobiomorphus cotidianus	Titikura,Common/Cran/C	Fish	52	44	52	46	211	33	73.0	62.043	6	37	7.78	1	1
Paparahia Stream	Gobiomorphus hubbsi (Te	Bluegill bully	Fish	0	0	0	0	0	0	0.0	0	0	2	0.42	1	0
Manganui River_Big	Gobiomorphus huttoni	Redfin bully	Fish	1824	722	2590	700	2059	2029	1654.0	705.7367	6	34	4.69	1	1
Manganui River_Big	Anguilla dieffenbachii	Tuna; kūwharuwharu; re	Fish	530	1063	1627	1752	886	1740	1266.3	468.7745	6	16	2.21	1	1
Manganui River_Big	Anguilla australis	Tuna; hao; aopori; hikum	Fish	0	255	218	66	0	268	134.5	115.6053	4	4	0.55	1	1
Manganui River_Big	Galaxias brevipinnis	Kōaro; maehe,Koaro	Fish	92	0	0	386	0	66	90.7	136.9266	3	0	0	0	1
Manganui River_Big	Galaxias postvectis	Shortjaw kokopu	Fish	67	90	0	0	0	98	42.5	43.50383	3	0	0	0	1
Manganui River_Big	Galaxias argenteus/postv	Kokōpu,Giant or shortjaw	Fish	0	0	167	0	0	0	27.8	62.23723	1	0	0	0	1
Manganui River_Big	Oncorhynchus mykiss	Taraute;tarauta; hāmana	Fish	0	0	0	0	154	0	25.7	57.39241	1	0	0	0	1
Manganui River_Big	Geotria australis	Piharau; kanakana,Pouch	Fish	0	0	0	0	57	8	10.8	20.85199	2	16	2.21	1	1
Manganui River_Big	Salmo Trutta	Brown trout		0	0	0	0	0	0	0.0	0	0	1	0.14	1	0
Manganui River_Small	Galaxias brevipinnis	Kōaro; maehe,Koaro	Fish	532	190	613	1263	1229	1047	812.3	395.2635	6	4	1.24	1	1
Manganui River_Small	Anguilla dieffenbachii	Tuna; kūwharuwharu; re	Fish	811	2671	1876	3090	2718	2558	2287.3	752.6579	6	7	2.16	1	1
Manganui River_Small	Gobiomorphus huttoni	Redfin bully	Fish	1643	540	772	2622	243	1598	1236.3	806.5822	6	6	1.85	1	1
Manganui River_Small	Salmo trutta	Taraute; tarauta,Brown t	Fish	0	0	0	0	250	0	41.7	93.1695	1	0	0	0	1
Manganui River_Small	Galaxias argenteus/postv	Kokōpu,Giant or shortjaw	Fish	0	62	0	163	0	0	37.5	60.51928	2	0	0	0	1
Manganui River_Small	Galaxias postvectis	Shortjaw kokopu	Fish	0	192	0	0	0	0	32.0	71.55418	1	0	0	0	1
Manganui River_Small	Anguilla australis	Tuna; hao; aopori; hikum	Fish	0	0	0	0	0	126	21.0	46.95743	1	2	0.617	1	1
Komakorau	Cyprinus rubrofuscus	Toretore,Koi carp	Fish	1009	1244	741	1418	1181	617	1035.0	280.9276	6	2	0.61	1	1
Komakorau	Gambusia affinis	Mosquitofish	Fish	187	881	331	348	1077	427	541.8	322.1161	6	0	0	0	1
Komakorau	Anguilla australis	Tuna; hao; aopori; hikum	Fish	387	123	362	705	651	463	448.5	193.3147	6	61	18.74	1	1
Komakorau	Anguilla dieffenbachii	Tuna; kūwharuwharu; re	Fish	44	273	204	285	220	213	206.5	78.76283	6	5	1.53	1	1
Komakorau	Retropinna retropinna	Ngaore; paraki; pōrohe,C	Fish	719	14	37	27	68	247	185.3	251.2626	6	0	0	0	1
Komakorau	Galaxias argenteus	Giant kokopu	Fish	180	0	137	221	0	0	89.7	92.88822	3	0	0	0	1
Komakorau	Galaxias argenteus/postv	Kokōpu,Giant or shortjaw	Fish	89	0	76	19	46	16	41.0	32.51666	5	0	0	0	1
Komakorau	Anguilla reinhardtii	Speckled longfin eel	Fish	126	0	0	0	0	0	21.0	46.95743	1	0	0	0	1
Komakorau	Galaxias maculatus	Īnanga,Inanga	Fish	9	0	104	0	0	11	20.7	37.53961	3	2	0.61	1	1
Komakorau	Scardinius erythrophthalr	Rudd	Fish	0	0	0	0	0	27	4.5	10.06231	1	0	0	0	1
Manganui River_410_8	Anguilla dieffenbachii	Tuna; kūwharuwharu; re	Fish	3075	2888	2960	3252	3511	2359	3007.5	354.7153	6	420	50.38	1	1
Manganui River_410_8	Gobiomorphus huttoni	Redfin bully	Fish	1288	1516	1607	2649	1688	1334	1680.3	455.4539	6	239	28.67	1	1
Manganui River_410_8	Anguilla australis	Tuna; hao; aopori; hikum	Fish	529	407	528	311	480	519	462.3	79.83664	6	305	36.59	1	1
Manganui River_410_8	Galaxias maculatus	Īnanga,Inanga	Fish	237	523	303	704	271	307	390.8	167.4857	6	22	2.64	1	1
Manganui River_410_8	Cheimarrichthys fosteri	Panoko; pānokonoko; pā	Fish	232	194	191	183	577	799	362.7	239.3036	6	33	3.95	1	1
Manganui River_410_8	Oncorhynchus mykiss	Taraute;tarauta; hāmana	Fish	20	212	248	247	297	343	227.8	101.8715	6	3	0.35	1	1
Manganui River_410_8	Geotria australis	Piharau; kanakana,Pouch	Fish	93	35	100	0	177	0	67.5	63.06808	4	7	0.84	1	1
Manganui River_410_8	Gobiomorphus gobioides	Tītarakura; tīpokopoko,G	Fish	0	0	0	0	147	0	24.5	54.78367	1	0	0	0	1
Manganui River_410_8	Gobiomorphus cotidianus	Tīpokopoko; toitoi,Comm	Fish	0	0	0	238	0	0	39.7	88.69736	1	0	0	0	1
Manganui River_410_8	Salmo trutta	Taraute; tarauta,Brown t	Fish	0	74	6	65	74	0	36.5	34.68789	4	0	0	0	1
Manganui River_410_8	Gobiomorphus hubbsi	Bluegilled bully	Fish	0	0	35	0	0	0	5.8	13.04373	1	0	0	0	1
Manganui River_410_8	Galaxias brevipinnis	Kōaro; maehe,Koaro	Fish	0	39	0	0	0	0	6.5	14.53444	1	0	0	0	1