eDNA Sampling in New Zealand Waterways – an Introduction

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SCIENCE + ENGAGEMENT

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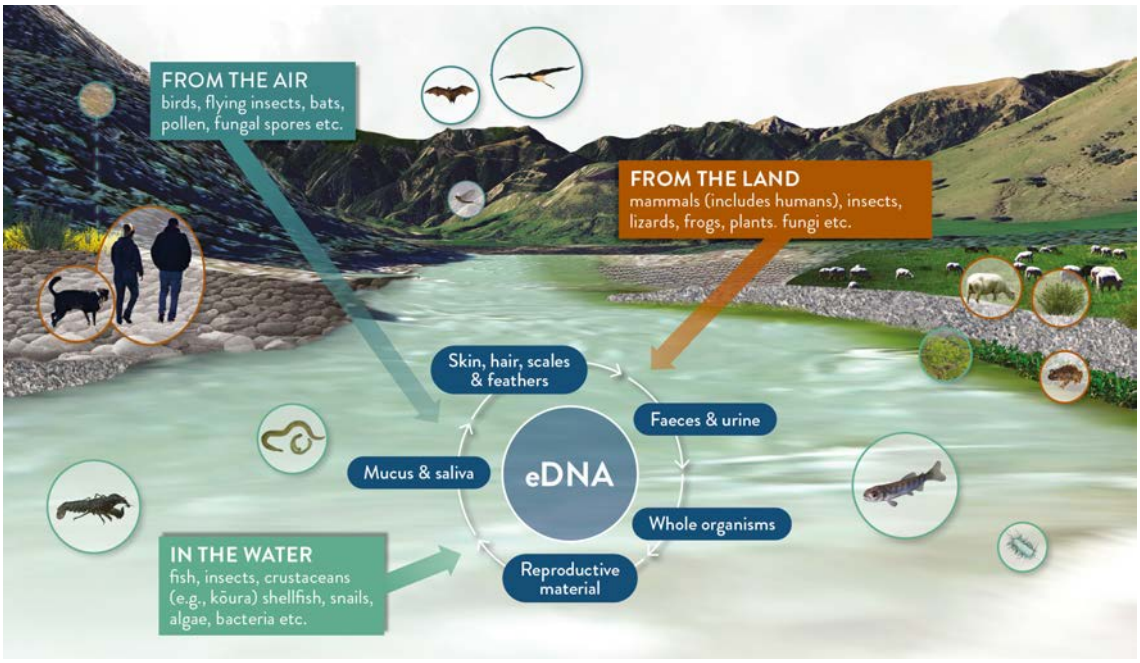
1 WHAT IS eDNA?

The genetic information for every living organism is held within a complex molecule called DNA (short for deoxyribonucleic acid), which acts like a blueprint for how the organism will look and function throughout its life. DNA is made up of chemicals – called bases – that are linked together like a chain to form long strands. The order of the chemical bases along a DNA strand creates a code for the expression of heritable traits in the organism. Depending on the species, a DNA strand can be hundreds of thousands to billions of bases long. Within these long strands of DNA, every species has short sequences of bases that are unique to that species or to a higher taxonomic group that species belongs to. These unique short sequences are called 'barcodes' and are a useful tool that scientists can use to identify a species just by looking at these sections of its DNA. A full copy of DNA is stored within nearly every cell in an organism, which means a single cell can be used to identify what species that organism belongs to. This is the case even if the cell is no longer attached to the organism, such as when it drops off the body (skin cells, hair cells, cells in fish scales, or slime from fish and invertebrates) or is released as reproductive material (gametes) or in waste products like faeces. The DNA that has been shed from an organism into the water, soil, or air where it lives is called environmental DNA, or eDNA.

2 HOW DOES eDNA HELP US UNDERSTAND OUR CATCHMENTS?

Our waterways act as reservoirs for genetic material, collecting and transporting eDNA that has been released by organisms that live in the water and on the land within the catchment.

Using specialised filters, eDNA can be extracted from water samples collected in aquatic environments. The eDNA strands are amplified and sequenced by specialist equipment in a laboratory setting, and then the sequences are compared to a huge reference database that contains the known barcodes for species found around New Zealand. If a section of the sequenced eDNA matches the barcode of a known species in the reference database, we can determine that species was present in that environment.



3 ADVANTAGES OF USING eDNA TO STUDY A CATCHMENT

Biodiversity is a measurement of the variety of species that live in a particular area. Field surveys have traditionally been used to measure biodiversity, involving a variety of methods to manually catch, identify, and count all of the organisms that can be found in an environment. While there will always be a place for these traditional surveys, eDNA has many advantages that make it a powerful tool to compliment biodiversity studies.

3.1 eDNA can measure biodiversity more efficiently than traditional survey methods

Some traditional surveys often use specialised equipment that require training and permits, and can be time-consuming and labour-intensive to undertake. They also require a strong working knowledge of local taxonomy, which takes time and experience to develop. Alternatively, collecting an eDNA water sample requires a few pieces of equipment that most people can use with little prior training, and it can be done more quickly and with less effort than traditional survey methods. While processing an eDNA sample does require specialised skills and equipment, this is work is outsourced to laboratories that are equipped to process samples at scale. Ongoing advancements are also helping to improve the affordability and accessibility of eDNA processing for community groups and citizen scientists.

3.2 eDNA can provide a more comprehensive measure of biodiversity than traditional survey methods

Traditional surveys rely on catching or observing all of the organisms in an environment so they can be identified and counted. Shy, rare, or well-camouflaged species may be overlooked, and mobile species may be missed if they move away from the area before their presence is recorded. Biodiversity can be further underestimated through the misidentification of similar-looking species or the omission of very small species. Particularly tiny organisms (such as larval fish and invertebrates, microalgae, and bacteria) are generally not included unless a specialised survey is undertaken to study them. A number of different traditional survey methods would need to be employed to capture the full range of species present in an environment. Alternatively, eDNA can provide an ecosystem-wide measure of biodiversity with a high level of accuracy using a single method. As all living organisms have genetic material, sampling eDNA can simultaneously detect the presence of organisms of all sizes and life stages, including species that are difficult to see and even those that have recently left the area.

3.3 eDNA is less invasive than traditional survey methods

Collecting data during a traditional survey may cause some disturbance to local habitats and/or organisms. The effects of these disturbances are usually minor, but can be more significant when fragile habitats or species are present. Collecting an eDNA sample simply requires the collection and filtration of a water sample, causing a minimal degree of disturbance, which may be a consideration if you are wanting to avoid disturbing areas with rare or fragile habitats/species.

3.4 eDNA can be used in a larger range of conditions than traditional survey methods

Regardless of specific methodologies, traditional surveys require the ability to access a sampling site to allow the species present to be collected and recorded. These surveys can also be labour-intensive and may require several people and multiple hours to complete. In some situations, traditional surveys may be impractical or impossible, such as in very small streams, in areas overgrown by thick vegetation, or when time and manpower is limited. As the access and effort needed to collect an eDNA water sample is minimal, it can be a good option to use in situations where traditional surveys would be difficult to undertake.

3.5 eDNA can sample a larger reach of the river than traditional survey methods

Traditional surveys provide a picture of the organisms that live within the sampled area of the river, which is usually limited to a small area due to the logistics of undertaking a hands-on survey. It is likely that eDNA can indicate what is living across a much larger area, with recent research indicating that eDNA from some freshwater fish species is detectable up to approximately 4 kilometres downstream of where the organism shed the genetic material. While the distance of eDNA detection does depend on factors, such as species and river flow, it is apparent that collecting water samples from a flowing river can provide an indication of the biodiversity across a longer reach of the river.

3.6 eDNA data can be improved over time as the reference database grows

Once an eDNA sample is collected and sequenced, the data can be stored electronically in perpetuity. In the initial processing, the eDNA sequences from the sample are compared to the barcodes that are known at the time of processing . If the sample eDNA sequence does not match a known species barcode, it may be assigned to a higher taxonomic level (such as family or genus) or remain unidentified. However, scientists are working hard to expand the database of species barcodes, and some of the currently unidentified sequences may be able to identified retroactively. The resolution of the data may improve if sequences that are currently attributed to higher taxonomic levels can be identified to species level in the future. The electronic storage of eDNA data allows for this possibility of data refinement over time.

4 LIMITATIONS OF USING eDNA TO STUDY YOUR CATCHMENT

Although it is a very powerful tool, eDNA won't tell you everything you may want to know about what is living in your catchment. It is important to understand the limitations of eDNA, and what the data will and won't tell you.

4.1 eDNA can only indicate the presence or absence of a species

One of the biggest constraints of eDNA is that it can only indicate the presence or absence of a species in an area, and does not provide any quantitative information. For example, the eDNA results won't tell you the size, age, or sex of the individual organism that the DNA came from, nor how many individuals within a given species are present in the environment. It also can't indicate whether the individual was stationary or migrating through the habitat, or even whether it was alive or dead. If you are interested in determining information beyond the presence or absence of species, then you will need to undertake hands-on surveys instead.

NOTE: A large amount of eDNA in a sample may not necessarily mean that there are many individuals of that species in the environment. There are a lot of factors which can determine the number of DNA sequences detected in a sample, such as the rate at which DNA is shed by an organism or how far away the sample is collected from the organism that shed the DNA. For example, a single longfin eel that is nearby your sampling site may shed a lot of DNA into the environment and produce a stronger signal than a larger population of redfin bullies residing a few kilometres upstream due to distance from the sampling site, individual size, and how they shed DNA. Spawning events can also cause artificially large spikes in eDNA, as genetic material is released into the water as part of the reproductive cycle. This increased presence of DNA in the water column can be particularly pronounced for species that mass spawn, with many individuals releasing reproductive material at the same time.

4.2 eDNA can only be detected in the environment for a short time

Depending on environmental conditions, eDNA is only detectable in the environment for a few hours to a few days before it begins to break down. Factors such as UV exposure, water temperature and pH, microbial activity, and the level of suspended fine sediment in the water column can impact the stability of the genetic material. In addition, eDNA detection rates often decline after periods of heavy rainfall due to the eDNA being diluted in a larger volume of water or flushed from the system altogether. These are not factors you should expect to control, so it is important to recognise the limitation of eDNA when interpreting your results.

4.3 eDNA can underestimate biodiversity

An eDNA sample will rarely identify all of the species present in an area, as the reference database does not have barcodes for every species in New Zealand. The short duration that DNA persists in the environment can also result in some species not being accounted for, particularly in the case of migratory species (if sampling is undertaken during a season when they are not in the area) or species that shed small amounts of DNA. The habitat preference or life history strategy of a species may also impact eDNA detection rates. For example, DNA shed by species that live on the riverbed may not be detected in samples collected from surface waters if the water column is stratified (such as in deep rivers, lakes, and tidal coastal habitats) and the eDNA cannot reach the main water flow in the waterbody. Consider where and how samples should be collected, and whether it is valuable to collect samples during multiple seasons in the year to make sure you are capturing a more complete picture of local biodiversity.

4.4 eDNA can overestimate biodiversity

Biodiversity can also be over-represented in an eDNA sample, particularly in areas with human influence. Sewage/stormwater discharges and runoff from land can release foreign DNA in a waterway. DNA can even be shed by dead organisms for a time, so activities like feeding the fish in your local river can release non-native DNA. A flowing river may carry eDNA from upstream areas, which could indicate the species is present in a larger area than is accurate. Similarly, eDNA from marine species is likely to be detected in water samples collected in estuaries and coastal areas during an incoming flood tide. It is important to keep these things in mind as you interpret your results.

4.5 eDNA samples can be contaminated during sampling

eDNA samples are very susceptible to contamination during sample collection, as anything that makes contact with the water could potentially shed additional eDNA into the water and be captured in your sample. Whilst some of these 'contaminant' results can be removed from the dataset post-collection if they are obvious (such as human DNA that may have come from the sampler), there is the potential for species to be recorded through sample contamination that would be impossible to determine post-collection. For example, the gear we use and the boots or waders we wear can be contaminated with DNA that was picked up from the locations where it was previously used. It is important to have a strong awareness and understanding of how to collect your eDNA samples to minimise contamination. Best practice guidelines include wearing disposable gloves when handling sample collection equipment, ensuring all gear is appropriately cleaned in advance, and always standing downstream of the area where you are collecting your eDNA samples. Further tips for minimising contamination are discussed below.

4.6 Errors can happen when eDNA samples are processed

While the processing of eDNA samples is generally accurate, it is not always perfect. Sometimes mistakes are made when the eDNA is sequenced in the lab, which could lead to a species that was present in the environment being missed (known as a false negative) or a species that wasn't present being identified (known as a false positive). Even if the eDNA is accurately sequenced, errors may also be made when assigning a species to the DNA sequence. If the sequenced eDNA exactly matches the barcode of a known species in the database, a strong positive identification can be made. If the eDNA sequence does not exactly match a barcode, it may be assigned to the closest matching sequence, which may not be the correct species, or it may remain unidentified. Scientists are working hard to expand the database of species barcodes and increase the accuracy of identifications, but it is a big job and errors are still possible.

4.7 eDNA sampling won't give you much first-hand experience in your catchment

Carrying out eDNA sampling is a simple process of collecting and filtering water samples that are then sent to a specialised laboratory to be processed. This means it will give you little first-hand experience seeing and identifying the organisms living in your waterways. To better understand the context around your eDNA results, you will need to have some familiarity with the local aquatic species and an understanding of the area of your catchment. You may find it valuable to undertake additional biodiversity surveys or organise guided explorations designed to strengthen the connection between your community and the catchment. If these are undertaken on the same day that eDNA samples are collected, it is important to collect the water samples first to minimise the potential for contamination.

5 COLLECTING eDNA SAMPLES FROM YOUR CATCHMENT

5.1 Why to collect eDNA samples

Before you collect eDNA samples, it is important to establish the purpose of your sampling and identify any questions you would like to answer with the resulting data. Clearly understanding your goals will help you select your sampling sites, decide the best time(s) to collect your samples, and determine which sample collection method is most appropriate.

A few examples of the questions eDNA can help you answer:

» What is the overall biodiversity of my catchment?

» What species are present in the areas where I don't have the capacity to undertake traditional survey methods?

» Where should I target traditional survey methods? (For instance, eDNA may help you determine where a species of interest is living, then you may undertake traditional surveys to get quantitative measurements like size ranges and population density.)

» Is a particular species of interest (such as a threatened, taonga, or pest species) present in my catchment?

» How does the biodiversity in my catchment compare to another catchment?

» Are there any fish migration barriers in my catchment?

» Does an existing source of contamination (such as runoff from contaminated land) have an effect on local biodiversity?

5.2 Where to collect eDNA samples

The purpose of your sampling should inform the specific area that you would like to target with your eDNA sampling. This may be a small area that will only require sampling at a single site, or it might cover a larger area of the catchment that requires multiple sites to fully investigate. Recent research indicates that eDNA for some key freshwater fish species can travel up to approximately 4 kilometres downstream before it is no longer detectable, and so you many consider spacing sampling sites several kilometres apart to effectively sample a longer reach of a river. You may also consider selecting sites several kilometres downstream from a known source of foreign DNA (such as sewage or stormwater discharge sites or popular swimming areas) to avoid contaminating your results, unless you are looking to investigate the impact of such contamination. If possible, choose sampling sites with fast-flowing well-mixed water and avoid collecting samples from stagnant pools.

5.3 When to collect eDNA samples

Collecting eDNA samples in the summer months is usually recommended, as samples collected in the summer generally capture a more complete measure of local biodiversity due to the behaviours of aquatic species. For example, the preferred time of year for detecting the presence of freshwater fish species in New Zealand is 1 December to 30 April, as this is when fish species are generally more active and therefore shed more DNA into the water column. The greater amount of eDNA in the water means there is a higher chance of your sample detecting the presence of that species. In many areas of the country this time of year is also usually less rainfall, which may increase the likelihood of detecting eDNA due to a lower chance of rain flushing or diluting the DNA or washing away the organisms present in

the stream. Note however, that some regions in New Zealand vary in terms of when there is typically more rainfall, whilst glacial or mountain-fed systems can experience more runoff during spring/summer snowmelt and lower/more stable flows during winter. It may be appropriate to collect eDNA samples outside of the summer months in some cases, and ultimately, your location and the purpose of your sampling should inform the timing your sample collection. It may also be relevant to sampling across multiple seasons to answer your identified questions. In any season, avoid sampling in an area that has received more than 10 mm of rainfall in the previous 2–3 days.

5.4 How to collect eDNA samples

There are two methods that are commonly used to collect eDNA samples. Which method is right for you will depend on the questions you are looking to answer, and the environmental conditions and ability to easily access your site(s). Each method requires some specialised equipment, which will need to be sourced before undertaking eDNA sampling.

1. **Active sampling:** This is also called syringe sampling, as a large plastic syringe is used to hand-filter a water sample through a specialised filter that collects the eDNA. The collection of a single samples takes approximately 5–10 minutes, then the filter is removed from the syringe and the sample is preserved and sent to a specialised laboratory for processing.
   - This is the most commonly used eDNA sampling method in New Zealand, as it is highly effective in most waterway conditions.
   - It is best to use these active sampling in areas with clear water and moderate flow, avoiding stagnant pools and backwater areas.
   - Use a caulking gun to assist in pushing water through the filter, as it is very difficult and tiring to do by hand.
   - Current recommendations include the collection of six replicate samples at one site, which means more time (up to one hour) on site, and more cost for the sample testing. Time and cost constraints need to be weighed against the desire to get as comprehensive a sample as possible.

2. **Passive sampling:** A specialised filter is placed into the current of a river or stream, then left for 24 hours so the eDNA can be filtered out and retained as the water flows through. Two visits to your site are required (one to deploy and one to collect your sample 24 hours later), and the filter must be secured so that it remains in place and submerged for the entire deployment. Once retrieved, the filter is preserved and sent to a specialised laboratory for processing.
   - This is a good option to use in areas with excess fine sediment or for sampling immediately after a rainfall event.
   - Passive sampling also has a high detection rate for terrestrial species in the catchment, and is commonly used by predator-free groups around New Zealand to identify areas where trapping is needed and investigate the effectiveness of existing pest eradication efforts.
   - If access to your site is problematic, passive sampling may not be ideal as you will need to access the site twice.
   - Current best practice recommendations include the collection of six replicate samples at one site. Although all six samples can be collected simultaneously with a passive sampler, there will be more cost for the sample testing. Cost constraints need to be weighed against the desire to get as comprehensive a sample as possible.

5.5 Tips for maximising eDNA detection

» Best practice recommendations include the collection of multiple eDNA samples (i.e., replicates) at each site/sampling event to increase the chances of detecting more of the species present in your area, and thus capturing a more accurate biodiversity measurement. A trial study undertaken by regional councils across New Zealand has shown that six is the optimal number of replicates to detect species presence.
A summary of this work can be found here:
https://s3.ap-southeast-2.amazonaws.com/wilderlab.docs/Resources/High_rep_trial_infosheet.pdf

» Preserve your sample as quickly as possible after collection to prevent the DNA from becoming degraded.

» It is a good idea to avoid collecting samples after a period of heavy rainfall, which may flush or dilute the eDNA, wash away the organisms in the stream, or stir up sediments into the water column that will quickly clog the eDNA filters. If the river flow is elevated or the water looks cloudy on the day you have chosen to collect eDNA samples, come back to sample on another day if possible.

» If migratory or seasonal species may be present in your catchment, you may choose to sample eDNA several times throughout the year. You may also choose to sample multiple sites to ensure you are capturing a more complete picture of biodiversity in the area.

5.6 Tips for minimising sample contamination

» Because eDNA detection is extremely sensitive, it is important to ensure your water sample will contain DNA material only from the environment you are interested in. It is important to wear disposable gloves whenever handling eDNA equipment to minimise the likelihood of introducing foreign DNA from your hands to the sample.

» Use new single-use equipment or sterilise equipment before sampling to avoid contaminating your samples. A 10% bleach solution can be used to remove traces of DNA from most equipment, or if it is made from materials that can be damaged by bleach (such as metal and neoprene), it is recommended to soak the equipment in hot soapy water and thoroughly rinse with clean water before use.

» If possible, avoid entering the water and collect your samples while standing on the land at the water's edge. This is particularly important if you are sampling in still waters, such as lakes or wetlands. If you must enter the water, face upstream while sampling and ensure you are collecting water from upstream of where you or anyone else is standing. Spend some time planning your entry into the water to make sure you always remain downstream of the site where you collect samples.

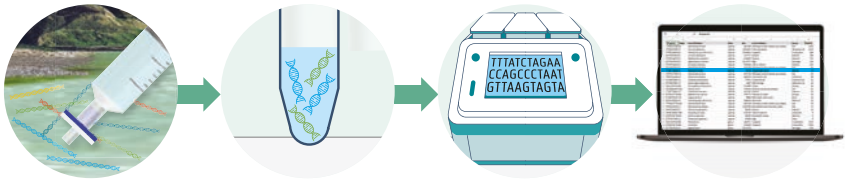
» If you will be collecting samples from multiple sites, start at the downstream-most site and work your way upstream. Unless it is relevant to the questions you are hoping to answer with your eDNA data, avoid sampling downstream of locations where there is likely to be contamination from foreign DNA (such as farm runoff, sewage and stormwater discharges, fish bait).

5.7 Non-reusable material

» Because eDNA detection is extremely sensitive, it is important the water sample is not contaminated with eDNA not from the area that you are wanting to sample. This means using disposable equipment for the collection of each sample, which results in a lot of non-recyclable material that has to be thrown out. If the option is available from your eDNA laboratory, ordering kits with sample replicates as one unit (as opposed to separate samples) can help to reduce the amount of disposable material generated.

6 PROCESSING eDNA SAMPLES

Your eDNA samples will need to be send to a specialist laboratory to be processed. These laboratories will have the equipment and expertise necessary to extract, amplify, and sequence the eDNA that was filtered out of your water sample. They will also be able to compare the sequenced DNA to a reference database of known barcodes to identify what species or taxonomic group the genetic material came from, and provide you information about the biodiversity of your catchment.



eDNA sampling through filter | DNA extraction & amplification | PCR analysis & sequencing | Species identification

7 eDNA DATA OWNERSHIP

Another thing you will need to consider is who will own and have access to the eDNA data you collect. This will depend on your group, where your sites are located, and how your work is being funded. If your work is receiving public funding or other assistance, data sharing may be expected or required and it is a good idea to establish the terms from the outset. If your sampling sites are on private land or sites of cultural significance, it is important to have a conversation with the land owner and/or mana whenua to establish who will own and/or access the data. DNA and associated eDNA data from native species are considered taonga according to te ao Māori. Any eDNA collection should be done in consultation with mana whenua in advance, allowing for time to consider tikanga and your proposed work.

8 eDNA – ONE TOOL AMONG MANY!

Finally, it is important to remember that eDNA is one tool that exists among a suite of available tools we can use to study a catchment. It can provide an initial indication of catchment biodiversity and help you answer many questions, but it won't tell you everything you need to know. In most cases, eDNA is best used alongside other traditional hands-on survey methods (such as visual assessments, electrofishing, kicknet sampling, spotlighting, or trapping/netting) to increase our understanding of your freshwater catchments.

9 WHO CAN PROVIDE eDNA TESTING IN NEW ZEALAND?

Wilderlab

Wilderlab (www.wilderlab.co.nz) is a commercial laboratory based in Wellington that specialises in eDNA testing. They were one of the first suppliers of eDNA test kits and sample processing services in New Zealand, and as of 2023 are the main suppliers for the general public. They have several types of user-friendly eDNA sampling kits available, which are outlined below. All Wilderlab kits include a preservative that is designed to be easily added to your sample in the field to prevent the degradation of the DNA in your samples during transportation and increase the accuracy of the resulting data.

- The eDNA syringe mini kit includes the main equipment you will need for active sampling. It is best practice to avoid drawing fine sediments and debris into the syringe as this can clog the filter and impact your ability to filter the amount of water needed for a sufficient sample. A standard 1.2 µm filter is included with each syringe kit, however a 5.0 µm filter is also available if you are sampling in an area with a high sediment load.

- The eDNA peg-mount passive sampler kit includes the main equipment you will need for passive sampling. It includes a stainless-steel peg for attachment to the riverbed. This sampler is best suited to smaller and shallower streams and wadable rivers to ensure the filter remains at the appropriate depth.
- The eDNA manifold-mounted passive sampler kit includes most of the equipment you will need for passive sampling, however mounting equipment is sold separately. This sampler can be used in wadable and non-wadable rivers, and be best suited for higher replicate sampling.

As of 2023, Wilderlab offers two levels of sample processing (i.e., eDNA packages). You will need to consider the range of species you are hoping to detect and your cost constraints to decide which package is right for you.

- The basic eDNA package will detect and identify DNA present in your sample from mammal, insect, crustacean, fish, bird, lizard, and frog species.
- The comprehensive freshwater eDNA package will detect same groups as the basic package, as well as freshwater mussels/kākahi, plants, algae, microorganisms, and the newly identified invasive golden clam. The comprehensive analysis also includes a new ecological health score (see: https://www.wilderlab.co.nz/tici).

Cawthron

The Nelson-based independent science organisation Cawthron Institute provides some commercial eDNA testing services (https://edna.cawthron.org.nz), with tests for a variety of topics including fish species detection, fish diseases and pathogens, harmful algae and cyanobacteria and their toxins, and for marine biosecurity.

Ampersand Technologies

Ampersand Technologies (www.ampersandtech.co.nz) is a start-up led by Massey University staff, focusing on agricultural, horticultural, and environmental applications of eDNA.

10 MORE INFORMATION

» www.epa.govt.nz/assets/Uploads/Documents/Wai-Tuwhera-o-te-Taiao/Designing-your-eDNA-project_final.pdf

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