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Guide for the Kaipātiki Project EcoHub: kākahi eDNA sampling in the Coatesville area

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Guide for the Kaipātiki Project EcoHub: kākahi eDNA sampling in the Coatesville area

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Prepared for Kaipātiki Project EcoHub

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Contents

1. Introduction

Cawthron Institute (Konstanze Steiner) and Environmental Impact Assessment Ltd (Brett Stansfield) were commissioned by the brokerage Access to Experts (A2E) to provide scientific expertise to the Kaipātiki Project EcoHub (Neil Henderson). Our objective was to support Neil Henderson in his goals to detect kākahi (freshwater mussel, *Echyridella menziesii*) in the Coatesville area using environmental DNA (eDNA).

This report serves as a short guide to the work undertaken, including the development of a sampling strategy, protocols and online training to facilitate eDNA sampling, fieldwork and water filtration; lists of necessary equipment, health and safety measures, and how to avoid contamination and biosecurity risks; and a step-by-step induction on how to carry out the tasks.

2. Sampling plan and strategy

Following a short literature review and discussion between Konstanze Steiner and Neil Henderson, we developed a sampling plan. This plan was then fine-tuned following a site visit with Brett Stansfield and subsequent site visits to the area.

2.1 Literature review

We carried out an initial literature review to collect information regarding eDNA sampling of freshwater mussels in lotic systems. This included screening about 20 publications covering areas such as dispersal distance of eDNA in lotic systems, other factors influencing eDNA detection, the recommended number of replicates for confident detection, and sampling strategies. While an extensive literature review is beyond the scope of this project, a simplified list of the most important information is presented below.

- Several publications found that eDNA derived from freshwater mussels has been detected up to 9 km downstream from the population (Deiner and Altermatt 2014; Wacker et al. 2019; Preece et al. 2021; Stoeckle et al. 2021), with the highest eDNA signal generally not in close proximity to the population but 100 m or even 2 km (Whitehead 2023) downstream from the source. However, some studies also recorded highest eDNA concentrations in close proximity to the population and no eDNA detection further downstream (i.e. 500 m, 1,000 m; Stoeckle et al. 2016).
- Detectability of eDNA was influenced by several factors, such as water temperature (high temperatures degrade eDNA), turbidity (inversely correlated to positive detection rate; Pilliod et al. 2014; Stoeckle et al. 2021), or rate of water flow and velocity (higher flows decrease eDNA concentration; Shogren et al. 2017; Shogren et al. 2019; Gasparini et al. 2020; Curtis et al. 2021). The habitat also had an influence, with eDNA more likely to be captured in riffles compared to pools or runs, based on the hypothesis that settling of eDNA might be prevented in riffle habitats due to their high velocity and turbulence (Preece et al. 2021). Other factors mentioned were nutrient concentration (Shogren et al. 2019) and stream geomorphology (Fremier et al. 2019). Recommended or suggested variables to collect alongside eDNA sampling in addition to those already mentioned are listed in Harrison et al. (2019) and include waterbody width and depth, salinity, pH, microbial growth and substrate type.
- Seasonality was shown to influence eDNA concentrations; however, findings are ambiguous. While warmer temperatures are associated with eDNA breakdown (Strickler et al. 2015), other studies found higher eDNA concentrations in warmer seasons due to increased organismal activity, metabolism and reproduction (De Souza et al. 2016; Wacker et al. 2019; Buxton et al. 2021; Chucholl et al. 2021; Curtis et al. 2021).
- Six replicates were previously recommended in Aotearoa New Zealand for syringe-based eDNA sampling for subsequent metabarcoding analysis. In the reviewed literature, 1–4 replicates of 0.5–2 L water samples were taken for subsequent targeted PCR analysis (quantitative or digital PCR, as planned in this study). The most commonly used number of replicates was 3–4 (Shogren et al. 2017; Wacker et al. 2019; Gasparini et al. 2020; Curtis et al. 2021; Preece et al. 2021; Whitehead 2023).

- Other recommended sampling strategies included sampling from downstream to upstream to avoid disturbing upstream sites that will be subsequently sampled, the inclusion of controls, and downstream collection of known and expected populations.

2.2 Suggested options for sampling strategies

Two different sampling strategies were explored, based on the desired outcome and accounting for the analysis of 32 samples (later increased to 62 samples) by the University of Waikato allowed by the budget. If run in triplicate, and considering positive and negative controls, approximately 19 sites / conditions can be sampled. The two sampling strategy options are described below:

1. **Identify 1–3 small populations of kākahi and 1–3 large populations of kākahi, and take samples at three distances from the source population.**
   Goal: Create an experiment with enough statistical power to gain insight into the relationship between the eDNA signal strength and population size and distance to the source population.
   Advantages: Use this information to generate more in-depth knowledge for future eDNA monitoring, e.g. providing an indication of how close the kākahi population is to where the eDNA signal was detected.
   Disadvantages: The relationship between eDNA signal strength and population size (or distance) is still not fully understood. Since many factors contribute to eDNA signal strength, a clear correlation cannot always be found. This might be possible to some extent in more stagnant waterbodies, but probably not in streams and rivers. For a reasonably reliable result, it is necessary to identify sites that contain kākahi populations but where populations are not present upstream or downstream of the site for the length of the chosen distances. The large transport distance of eDNA in rivers and streams means that it may be challenging to find such sites. Furthermore, it means that a positive eDNA signal may not provide much information to help with locating kākahi populations as they could be many kilometres upstream.
2. **Carry out Coatesville-wide screening of kākahi: select suitable and accessible sites in the Coatesville area.**
   Goal: Use eDNA detection to screen the downstream ends of tributaries for the possible presence of upstream kākahi populations. This will be used to identify and narrow down target sites for traditional monitoring (e.g. a positive eDNA signal means kākahi are somewhere in that given tributary).
   Advantages: Using all samples for this sampling strategy means a wide area can be screened and results can be provided quickly.
   Disadvantages: The low stream flow in this area could mean that eDNA is not transported far enough to reach the end of a tributary, potentially resulting in negative eDNA detections even if kākahi are present in that tributary.

2.3 Stream catchment walk to identify sampling sites

Brett Stansfield and Neil Henderson carried out a site visit to the Māhoenui Stream in the Coatesville area on 12 May 2024 (Figures 1 and 2; 1758648E, 5443476N). They found that stream banks were very steep, the stream was well shaded (90%) and the water flow was almost stagnant (water flow < 0.1 m/s). The water depth was between 0.06 m and 0.3 m, and the substrate was dominated by gravel and wood.

Figure 1. Māhoenui Stream during the site visit on 12 May 2024.

Neil Henderson carried out additional stream surveys along the Māhoenui Stream on 15 May 2024 (Figure 2, Appendix 4) to identify locations that are easy to access and have a greater water flow, which would be beneficial for eDNA detection at greater distances from potential kākahi populations. Study sites identified from these surveys are outlined in the final sampling strategy.

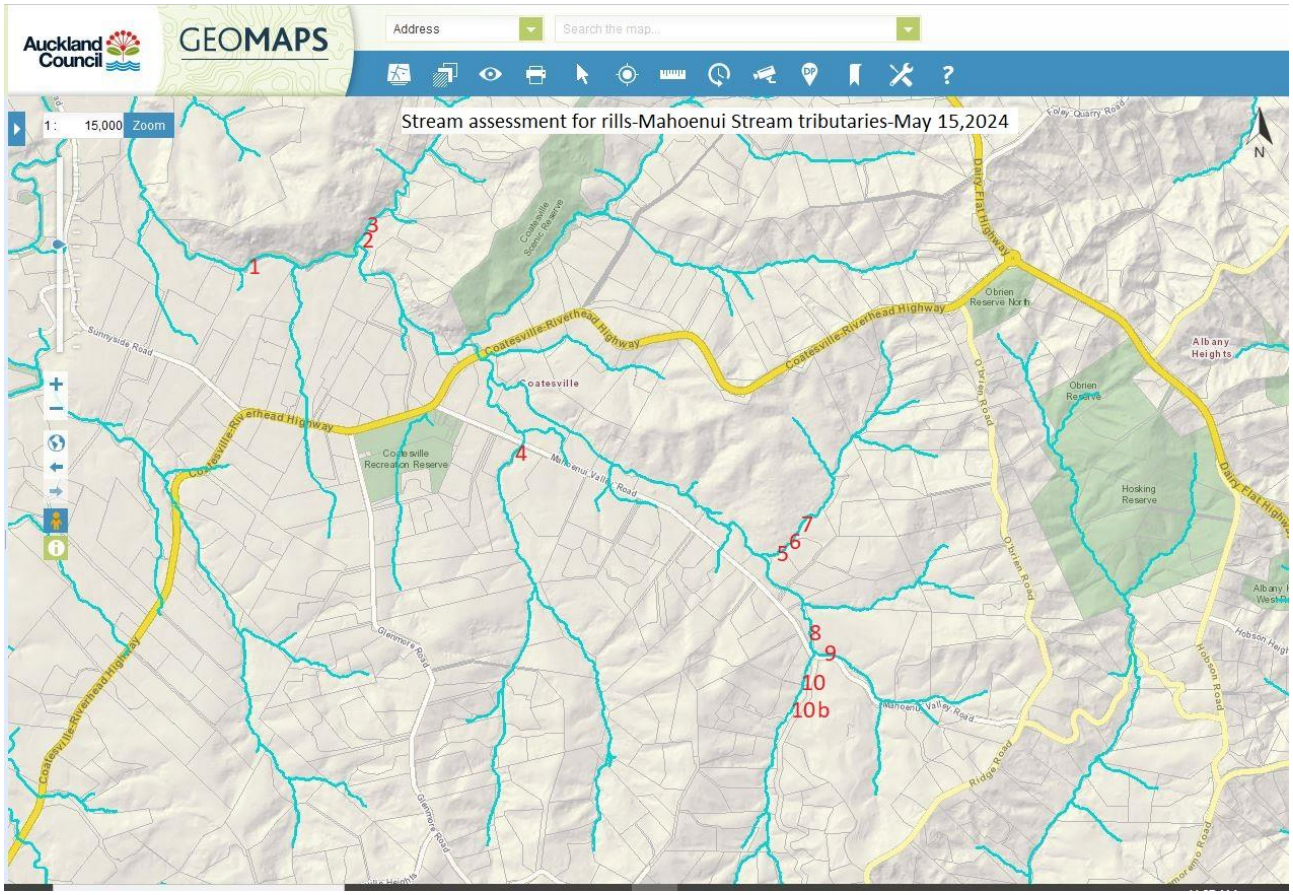


Figure 2. Locations along the Māhoenui Stream assessed by Neil Henderson and Brett Stansfield on 12 May 2024 (location 1) and 15 May 2024 (locations 2–10b) as potential sampling locations.

2.4 Final sampling strategy

The large transport distances of eDNA in lotic systems described in the literature made the initial option of testing the relationship between signal strength and population size and distance unfeasible. This approach could give only somewhat reliable results, and only if multiple sites with different-sized kākahi populations and without other populations upstream or downstream within the chosen distances could be found. This would be an interesting sampling approach for a future project if these sites can be identified.

In agreement with Neil Henderson, we decided to focus on the second sampling option, namely using eDNA to screen the downstream ends of tributaries for the presence of upstream kākahi populations in order to narrow down target sites for traditional monitoring. Our advice was to sample at the end of tributaries, before they join a stream. This was because the tributaries of the targeted streams (Māhoenui and Rangitōpuni) are usually between 150 m and 700 m long, and a positive eDNA signal would mean that only that specific tributary (upstream from its junction with the stream) would need to be searched for kākahi using traditional methods.

In addition, we discussed the following points for consideration during the sampling strategy:

- Include a positive control site (a site where kākahi are known to be present; sample to be taken immediately next to kākahi and up to 1 m downstream from the population) and a negative control (which can be tap water).
- Use three sample replicates, as this is the number most often reported in the literature for eDNA sampling and subsequent targeted eDNA analysis (droplet digital PCR, as opposed to metabarcoding community analysis, which would require more replicates).
- Avoid sampling after heavy rainfall, as this can dilute the eDNA and potentially disturb the organisms. If possible, we advise allowing a few days for organisms to return to their usual activities and for the stream to return to its pre-rainfall state.
- If possible, sample in riffles and avoid pools or runs.
- Consider seasonality, bearing in mind that a positive detection might be more likely in warmer months. This is a trade-off between degradation of eDNA due to warmer temperatures and, potentially, ultraviolet light, and more shedding of eDNA due to increased organism activity. Studies have often found that kākahi eDNA concentrations are higher in warmer months. This might also be due to increased reproduction and glochidia release, which usually peaks in February but can take place between November and March in the North Island. While this is an advantage in that higher eDNA signals will be picked up, it might produce noise in detecting adult organisms. The best time of year for sampling depends on the priority of the study goals. If the main goal is to determine the presence / absence of kākahi populations in a given tributary, high kākahi activity and high eDNA concentrations might be an advantage. If the goal is to measure relationships between eDNA signal strength and distance from populations and population sizes, periods of glochidia release should be avoided. However, it should also be noted that sampling in the winter months might be a necessity in the future and therefore the sampling strategy needs to be tested under these conditions (a future study taking samples from the same site in summer could be a great comparison to identify differences).

Given the low water flow observed during the first site visit, we predicted that eDNA might not be transported as far as described in the literature. We therefore decided to use a staggered approach, whereby for multiple tributary branches, one site (proposed sample Site 1 in Figure 3) will be sampled close to the junction of the main tributary with the stream in the first sampling round. If eDNA cannot be detected from these samples, a second site further upstream will be sampled (proposed sample Site 2 in Figure 3) in a second sampling round. Suggested sample sites are shown in Figure 3 and coordinates are provided in Table 1 (note that results from the first sampling round will inform the exact location of the second sampling round).

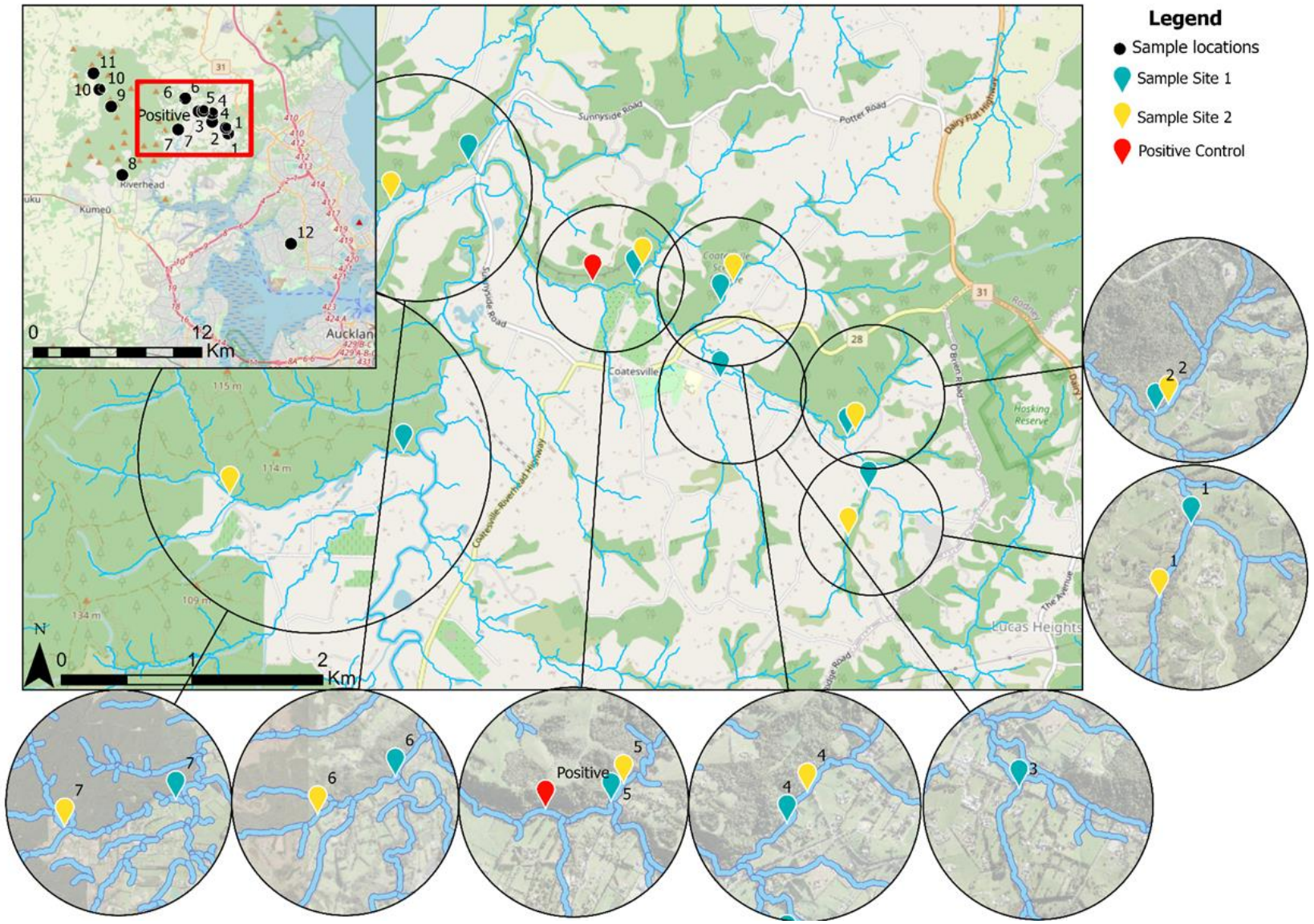


Figure 3. Final sample locations for kākahi eDNA stream sampling in the Coatesville area (locations 1–7).

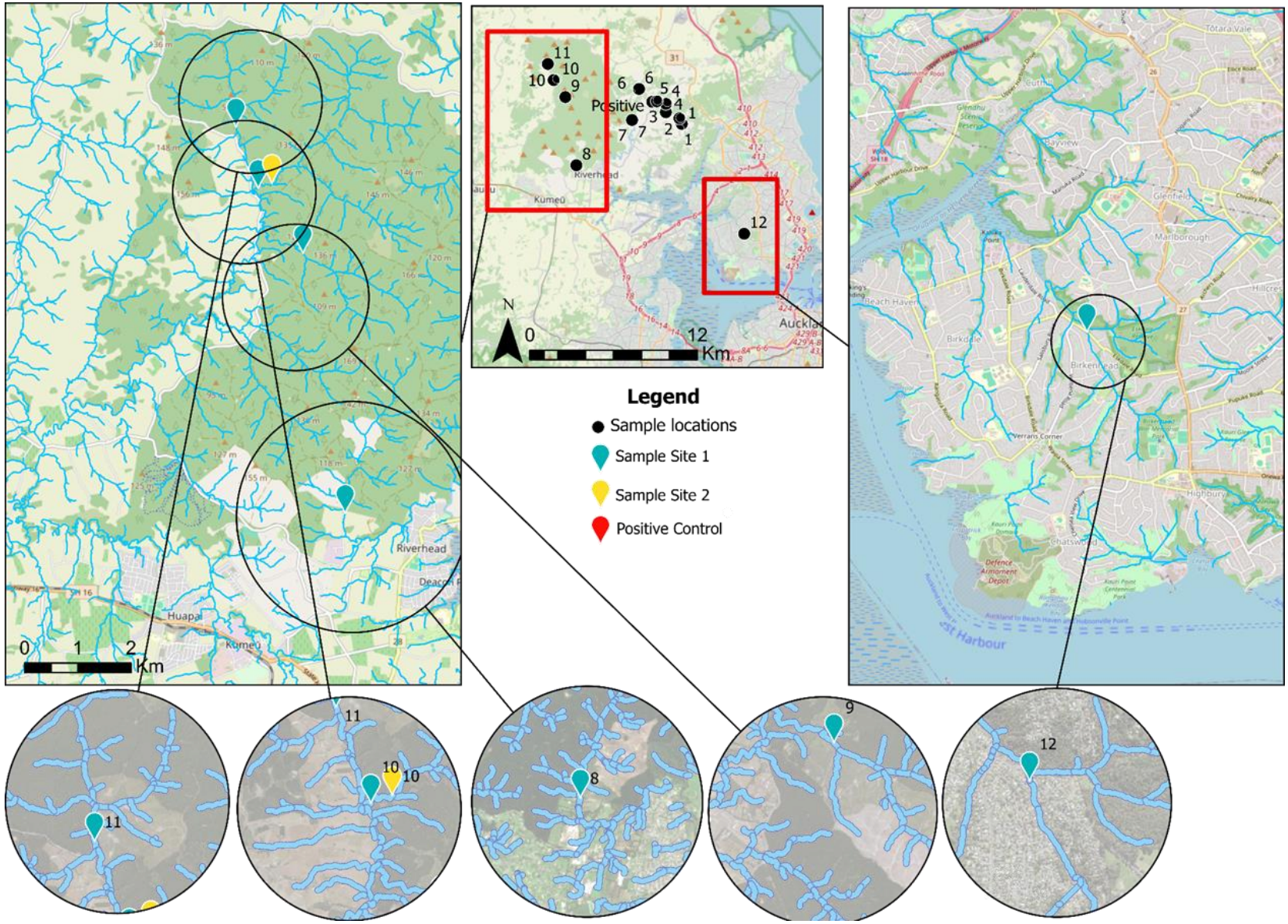


Figure 3 (cont.). Final sample locations for kākahi eDNA stream sampling in the Coatesville area (locations 8–12).

Table 1. Geocoordinates for sampling sites.

Location	Site	Latitude	Longitude	Sample round
Positive control	0	S36.71100	E174.63571	1, 2
1-Māhoenui 1	1	S36.72498	E174.65967	1
	2	S36.728199	E174.657998	2
2-Māhoenui 2	1	S36.7213	E174.65778	1
	2	S36.72095	E174.65846	2
3-Māhoenui 3	1	S36.71754	E174.64679	1
4–Coatesville Riverhead	1	S36.71223	E174.64669	1
	2	S36.710827	E174.647773	2
5-Hathaway Grove	1	S36.71063	E174.63930	1
	2	S36.70982	E174.63997	2
6-Robinson Road	1	S36.70287	E174.62490	1
	2	S36.705571	E174.618297	2
7–Croft Lane	1	S36.72297	E174.61974	1
	2	S36.7261	E174.6049	2
8-Forestry Road	1	S36.75282	E174.57566	1
9-Barlow Road	1	S36.70891	E174.56587	1
10-Campbell Road	1	S36.69803	E174.55624	1
	2	S36.697158	E174.559108	2
11-Ararimu Road	1	S36.68769	E174.55134	1
12-Eskdale Stream	1	S36.7952	E174.71136	1

3. Development of a protocol for eDNA sampling and water filtration

We developed a protocol for the Kaipātiki Project for sampling eDNA in the field, transporting and storing samples, and subsequent water filtration (Appendix 1). This protocol formed the basis for the online training we provided (Section 4.1). Furthermore, we provided equipment lists (Appendix 1), including items that will be supplied by Cawthron and those that need to be purchased (labelled as 'not provided' in the equipment lists), and a data collection sheet (Appendix 2).

4. Training for eDNA collection and water filtration

4.1 Online training

Online training for Neil Henderson, Brett Stansfield and volunteers helping with the project was carried out by Konstanze Steiner on 12 June 2024 via Teams. The training covered the following areas:

- An extensive description of what to look out for when collecting eDNA.
- How to use the water filtration station.
- Sample storage and transport.

The meeting was recorded, with the recording provided to Neil Henderson and Brett Stansfield.

4.2 Onsite training

Onsite training took place mostly in the field, and some observers attended training in the laboratory for filtrate processing and preparation of water samples.

Fieldwork training

This included:

- Identifying the site to be sampled.
- Understanding health and safety requirements in relation to working next to a stream.
- Wearing gloves during water sampling and changing these for each sample site.
- Taking samples without entering the water, if possible.
- Taking water samples from surface water.
- Taking water samples prior to taking additional measurements.
- Taking samples further upstream if the water has been disturbed beforehand, to avoid collecting sediment.
- Understanding how and why to take additional measurements, as described in Section 5.

Laboratory training

This included:

- Understanding why triplicate control water samples are used throughout the day – i.e. to check there is no cross-contamination of water samples (if eDNA is present in a control sample, then this would indicate that cross-contamination has occurred during the course of the day).
- Understanding the importance of maintaining a sterile work environment, including regular sterilisation of equipment, to ensure no cross-contamination between filtrate preparations.
- Recording water volumes filtered for each site.

5. Development of stream walk and training

During the stream walk, training was provided on why different variables are measured and how each measuring instrument works. Participants were instructed in how to populate a prepared form with measurements for each site.

Site selection

Attendees discussed why the particular sampling locations were chosen. For example, when undertaking stream gauging it is best to ensure that there are no obstacles (e.g. rocks, plants, weed, islands) directly upstream as these can divert flow, potentially rendering measurements invalid. In addition, a straight reach of stream is selected as water velocity at stream bends can be highly variable. This is because friction is generally greater on the inside of a bend compared to the outside, leading to higher velocities at the outside.

Substrate, water depth and water velocity

The importance of substrate, water depth and water velocity readings were discussed. Aquatic species all have environmental preferences, and so their presence or absence at a particular location could be due to these factors. Participants were shown how to measure wetted width and depth at set distances from the true left bank using a tape measure, and how to calculate cross-sectional areas from these measurements. They were then shown how to calculate volumetric flow (L/s) by multiplying cross-sectional area by average flow velocity for each transect, and how to assess percentage flow at each end of the transect (used to account for the smaller cross-sectional area at the sides of a stream). Finally, attendees were shown how to measure stream water flow using a YSI FP111 Flow Probe (for most sites, there was a measurable flow using this probe). The substrate was determined by measuring the dimensions of the stone immediately adjacent to the transect point. The second-longest dimension determines categorisation into silt, gravel, cobble, boulders or bedrock (see Appendix 3 for detailed differentiation of substrate).

Water temperature

As with substrate, water depth and water velocity, water temperature can affect which species live in a stream. Some aquatic fauna are highly temperature sensitive while others are less so, and therefore this variable can govern the biological community of a stream. Trainees were shown how to measure water temperature using a YSI Pro Plus meter.

Turbidity

The turbidity of a stream determines how well light is absorbed within the water column. High turbidity readings tell us that ultraviolet light may not penetrate the water very well, and conversely, low turbidity enables good penetration of ultraviolet light within the water column. In turn, this can affect the concentration of eDNA in a water sample because ultraviolet light denatures DNA. The turbidity reading is also useful when water samples are analysed in the laboratory as high turbidity may indicate that filter

papers could become clogged when only a small volume of water is being filtered. This provides an indication of whether any samples will take longer to filter. Trainees were shown how to measure water turbidity using a Hach turbidity meter.

pH

Water pH can have a large effect on the solubility of some contaminants, in turn influencing the aquatic life of a stream. pH has also been shown to have an influence on eDNA denaturation. Trainees were shown how to measure water pH using a YSI Pro Plus meter.

6. Appendices

Appendix 1. Protocols for eDNA sampling and water filtration

A1.1 eDNA field sampling protocol – sample collection and storage

Equipment

- Waders (not provided).
- Sterile gloves.
- Three sterile 2 L sample bottles per site (not provided).
- Chilly bin(s) (enough to transport all bottles back to the lab) and ice / pre-frozen ice packs (not provided).
- Marker pen.
- Data collection sheet (Appendix 2).
- Rubbish bag (not provided).

Step-by-step process

1. *Collecting samples*
   a) At each sample site, and while wearing a fresh pair of sterile gloves, rinse the three sterile 2 L sample bottles three times downstream from the site, then fill them with surface water. After sampling, dispose of the gloves in the rubbish bag.
   b) Store the bottles in a cooled chilly bin until processing (see 'Water filtration protocols' below).
   c) Take geocoordinates for each replicate, as well as the following measurements, if possible: water temperature, pH, flow velocity and / or discharge, turbidity, waterbody width and depth. If possible, also take note of the habitat (e.g. riffle, run or pool). (Note that instruments and instructions for this are not provided in this protocol.)
   d) Fill in the data collection sheet and, using a marker pen, label the bottles according to the following labelling system:

L1-S1-R1 (for Location1, Site 1, Replicate 1)

The filtration should be performed as soon as possible after sample collection (maximum 24 hours after collection). Make sure that samples are kept chilled / in shade / refrigerated until they are filtered.

A1.2 Water filtration protocol

Read the 'Health and safety protocol', 'Cross-contamination and quality control protocol' and 'Biosecurity and sample disposal protocol' below before proceeding with this protocol.

Equipment

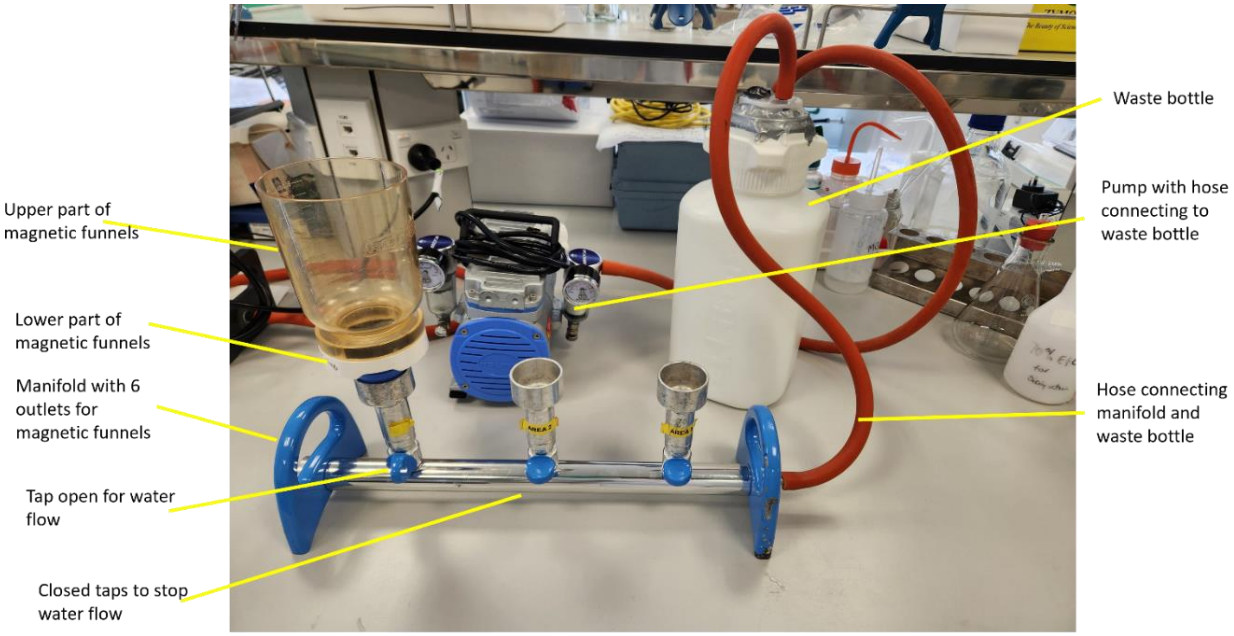
- The water filtration will ideally take place in a laboratory, but anywhere with a table and access to a freezer will suffice.
- Sterile nitrile gloves.
- Chemicals (not provided):
  - 50 mL Falcon 100% ethanol.
  - 500 mL of Janola or equivalent bleach with 42 g/L of sodium hypochlorite and 4.0% w/v of available chlorine.
- 1–2 containers (approximately 5–10 L volume) for sterilisation in between samples (not provided).
- Spray bottle or squirt bottle for cleaning and disinfecting (not provided).
- Filtration unit:
  - Threefold manifold.
  - Three magnetic filter funnels (consisting of top and bottom sections).
  - Wastewater bottle.
  - Electric pump.
  - Two lengths (~20 cm) of rubber tubing.
- Tap water.
- Paper towels (not provided).
- Metal tweezers.
- Metal scissors.
- Lighter (not provided).
- One box of 100 glass microfibre Whatman filters, grade GF/C (1.2 µm pore size, 47 mm diameter).
- Rack for 1.7 mL tubes.
- Two 1.7–2 mL sterile tubes per sample, plus three extra for controls (each sample is cut in half, so double the number of tubes is needed).
- Fine-tip marker pen.
- Box to store 1.7 mL tubes.
- Safety glasses and protective clothing (not provided).

Step-by-step process

Wear sterile nitrile gloves throughout the whole process.

1. *Cleaning / sterilising equipment*
    a) Set up a bleach bath: in a container / bucket, mix 500 ml Janola with 4.5 L tap water for a 10% bleach solution.
    b) Pour about 100 ml of the bleach solution into a spray bottle and clean working surfaces (i.e. laboratory bench).
    c) If no sink is nearby for rinsing with tap water, fill a second container / bucket with tap water. Ideally, use fresh water for each round of sterilisation.
    d) Soak magnetic filter funnels (top and bottom part), tweezers and scissors in the bleach bath for 20 minutes.
    e) Rinse sterilised soaked equipment thoroughly with tap water or wash in the water bucket and put on paper towels to dry.
    f) Flame the tweezers and scissors with ethanol (see 'Health and safety protocol' below for instructions on how to do this safely).

2. *Assembling the filtration unit*



    a) Screw the lid onto the wastewater bottle.
    b) Connect the manifold outlet to the small nozzle of the wastewater bottle using one length of rubber tubing.
    c) Connect the pump to the big nozzle of the wastewater bottle using the second length of rubber tubing.
    d) Make sure the third opening of the manifold is closed to create a vacuum.

e) Close all outlets of the filtration unit by turning each tap to the right.

f) To test that the system is set up correctly, add some water in one outlet, open the tap (turn it up) and switch on the pump. The water should flow into the wastewater bottle. Close the tap again.

g) Firmly press the lower part of the filtration funnel into the manifold outlets.

h) Using the sterile tweezers, add one GF/C filter, centred on the black circle of the lower unit of the filter unit.

3. *Labelling the 1.7–2 mL tubes and box*

a) Place the tubes in the rack, close the lid and label with the sample ID (two for each sample, differentiated with an 'A' and a 'B').

b) Label the box with your project name, date of sampling and date of filtering.

4. *Filtering the samples*

a) Gently swirl the 2 L sample bottle (collected from streams as part of the eDNA field sampling protocol) to ensure homogeneity of the contents.

b) Add 500 ml of the water sample onto the filter (use the measurement on the filter funnel) and open the tap (with the pump still running). Repeat this step until the whole sample has been filtered.

***Note**: If the sample water is very murky / turbid, the filter will clog quickly. To avoid this, you can reduce the filtration volume (although at least 1 L is recommended). Make sure you record the volume filtered.*

c) Process the other samples in the same way using the remaining filter funnels. Make sure you always add the same sample to the same funnel.

d) Keep an eye on the wastewater bottle as you will need to empty it at least once during the process. If the bottle overflows, the pump will be damaged. The fastest way to stop the water flowing is as follows:

   i) Turn off all taps below the filter funnels, then switch off the pump.

   ii) Take the rubber tubing off the wastewater bottle, unscrew the lid and discard the contents.

   iii) Reattach the tubing and lid to the empty wastewater bottle and proceed again (i.e. turn the pump on and open the taps).

e) At the end of the filtration process, when no water is visible on the filter, keep pumping for approximately 10 sec to dry the filter slightly.

f) Remove the upper part of the filtration funnel. Carefully cut the filter in half with sterile scissors, holding the filter at its edge with sterile tweezers. Fold the half filter using tweezers without disturbing the filtered area and place in two separate 1.7 mL tubes (labelled with the sample ID and 'A' and 'B' for differentiation).

g) Store the tubes on ice/ in the fridge until all samples have been processed, then store the whole box at -20 °C.

h) Before proceeding with the next filter, wipe off and carefully flame with ethanol, keeping the scissors open at all times (see 'Health and safety protocol' below for instructions on how to do this safely).

The filter funnels (top and bottom) need to be sterilised between samples. After one round of filtration (e.g. of three samples for a threefold manifold), repeat ALL steps – including step 1 (cleaning and sterilisation)– before proceeding with the next set of samples.

A1.3 Health and safety protocol

This protocol covers the required health and safety considerations when handling bleach and ethanol. Wear nitrile gloves at all times, safety glasses and protective non-flammable clothes.

- 100% ethanol is used to sterilise tweezers and scissors in this protocol. It poses a moderate risk as it is highly flammable.
  - Place the tube containing ethanol securely into a rack, cup or similar.
  - When sterilising equipment, place only the tip of the tool in the ethanol and then hold it in a flame. Ensure that there is no ethanol on the tool further up towards your hand.
  - When holding the tool in the flame, keep it away from any flammable material such as paper or tissues.
  - While flaming, hold the tool as straight as possible (i.e. not pointing down, as this will direct the flame towards your hand, and not pointing up, as this will cause flammable ethanol to flow towards your hand).
  - Place the tools on a rack or similar to cool down, making sure the sterilised tip is not touching anything.
- Bleach is used to sterilise equipment before starting and in between samples to avoid cross-contamination. It can be corrosive and cause serious eye damage. It will also damage clothing if spilled.
  - Wear nitrile gloves, safety glasses and protective non-flammable clothes.

Other hazards to consider include using an electrical pump near water. Make sure all electrical equipment is kept dry and separate from the filtering station.

A1.4 Cross-contamination and quality control protocol

To avoid cross-contamination of samples, wear gloves throughout the whole procedure and change them between working with samples from different sites. In addition, follow the disinfection and neutralisation protocols described above.

As a quality control, filter three 2 L quantities of tap water in addition to the samples and label these 'FC-R1', 'FC-R2' and 'FC-R3'.

A1.5 Biosecurity and sample disposal protocol

Sterilisation baths and samples can be flushed down the sink with plenty of water. If there are any biosecurity concerns, e.g. where invasive species may be present in your water samples, add bleach to the wastewater (one part Janola to nine parts wastewater) and wait for 1 hr before disposing of it in the sink.

A1.6 Troubleshooting

- Filter clogs before the whole sample is filtered. It is common for the filtration process to slow down as the filter absorbs particles from the water sample. If no progress is observed after a given time (e.g. 30 min), try filtering a reduced volume of water (although note that a minimum of 1 L is recommended). Always record the volume filtered as any changes will be calculated in the final results.
- Filter clogs very rapidly. In some cases this may be due to bigger particles or glochidia in the water. In this case, a prefiltration at 200 µM might be necessary.

Appendix 2. Data collection sheet

Label	Location#	Site #	Replicate	Lat (DMS)	Long (DMS)	Stream width (m)	Stream depth (cm)	temperature (°C)	pH	turbidity (NTU)	velocity m/s)	flow rate (L/s)	Volume filtered (ml)
L1-S1-R1 L1-S1-R2 L1-S1-R3	1 - Mahoenui 1	1	1 2 3										
L2-S1-R1 L2-S1-R2 L2-S1-R3	2 - Mahoenui 2	1	1 2 3										
L3-S1-R1 L3-S1-R2 L3-S1-R3	3 - Mahoenui 3	1	1 2 3										
L4-S1-R1 L4-S1-R2 L4-S1-R3	4–Coatesville Riverhead	1	1 2 3										
L5-S1-R1 L5-S1-R2 L5-S1-R3	5-Hathaway Grove	1	1 2 3										
L6-S1-R1 L6-S1-R2 L6-S1-R3	6-Robinson Road	1	1 2 3										
L7-S1-R1 L7-S1-R2 L7-S1-R3	7–Croft Lane	1	1 2 3										
L8-S1-R1 L8-S1-R2 L8-S1-R3	8-Forestry Road	1	1 2 3										
L9-S1-R1 L9-S1-R2 L9-S1-R3	9-Barlow Road	1	1 2 3										
L10-S1-R1 L10-S1-R2 L10-S1-R3	10-Campbell Road	1	1 2 3										
L11-S1-R1 L11-S1-R2 L11-S1-R3	11-Ararimu Road	1	1 2 3										
L12-S1-R1 L12-S1-R2 L12-S1-R3	xx (Positive Control)	1	1 2 3										
FC-R1 FC-R2 FC-R3	Tap Water	Filter Control	1 2 3										2000 2000 2000

Appendix 3. Physical habitat sheet

This sheet gives an example of the physical variables to measure at a habitat.

Wetted width [a]	3.1 m
Velocity [b]	<0.1 m/s
Shade [c]	90%

WELB [d]	Depth (m)	Substrate [e]
0.3	0.16	Silt
0.6	0.22	Medium gravel
0.9	0.25	Medium gravel
1.2	0.26	Large gravel
1.5	0.2	Small to medium gravel
1.8	0.23	Small to medium gravel
2.1	0.29	Small cobble
2.4	0.225	Wood 2.5 cm diameter
2.7	0.205	Wood 9 cm diameter
3	0.06	Wood 3 cm diameter

(a) Wetted width is the width of the stream that is wet.

(b) Velocity is estimated by timing how long it takes for a leaf to travel 1 m. In this example it took a leaf 10 sec to travel 1 m, so the velocity is 1/10 = 0.1 m/s.

(c) Shade is assessed at mid-stream as an estimate of how much shade occurs over a 180-degree horizon.

(d) WELB = water's edge left bank. Approximately 10 measurements are made across the stream-width transect from the WELB. So, if the wetted width is 3 m, you would undertake 10 equally spaced measurements at 0.3 m intervals. In the example above, the first observation was at 0.3 m, at which the depth was 0.16 m and the substrate was silt.

(e) For assessing substrate size, record the second-longest dimension measured, e.g. at WELB 1.2 m, the two longest dimensions of the gravel may have been 0.4 m and 0.26 m, and so you record the 0.26 m measurement. Substrate size categories are: silt =< 2 mm, small gravel = 2–8 mm, small to medium gravel = 8–16 mm, medium gravel = 16–32 mm, large gravel = 32–64 mm, small cobble= 34–128 mm, large cobble = 128–256 mm, boulders = > 256 mm, bedrock = where the base of the stream is a firm rock type that is too large to measure. For wood, record the diameter of the second-longest piece, e.g. at WELB 2.7, the wood may have been 75 cm long but its diameter is recorded, not its length.

Appendix 4. Photos from the 15 May 2024 Māhoenui Stream assessment

These photos were taken by Neil Henderson at each of the locations along Māhoenui Stream assessed on 15 May 2024. The locations are shown on the map in Figure 2.

Figure A4.1. Location 2.

Figure A4.2. Location 3.

Figure A4.3. Location 4.

Figure A4.4. Location 5.

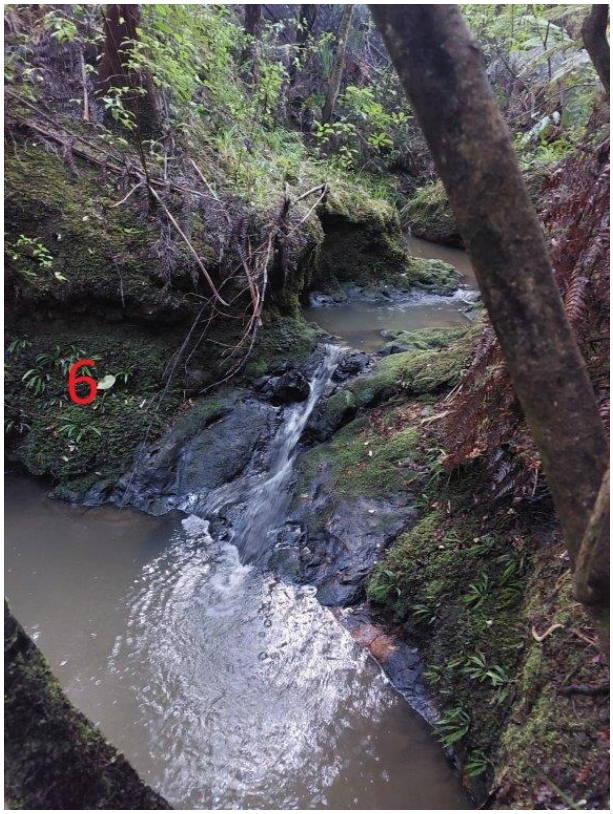


Figure A4.5. Location 6.

Figure A4.6. Location 7.

Figure A4.7. Location 8.

Figure A4.8. Location 9.

Figure A4.9 Location 10.

Figure A4.10. Location 10b.

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