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Regional guidelines for ecological assessments of freshwater environments - standardised protocol for adult freshwater mussel monitoring in wadeable streams

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Abstract

Freshwater mussel populations within New Zealand are understudied and little is known about their habitat preferences, population structure and general distribution, particularly in rivers compared to lake populations. This knowledge gap was identified by Waikato Regional Council and a standardised protocol for monitoring freshwater mussels in wadeable streams and rivers was developed for the two species which are present in the Waikato, *Echyridella menziesii* and *Echyridella aucklandica*. This protocol focuses on three aspects: 1. Assessing instream habitat preferences of mussels (where to look); 2. Producing standardised population density estimates (how many there are); and 3. Determining the size structure of populations (is the population healthy or ageing?). The aim is that this protocol can be used by other government authorities, private consultancies, consent holders, local community groups and other stakeholders to assess whether freshwater mussels are present and if so in what densities. To date, freshwater mussel monitoring in New Zealand has been mostly ad hoc and has not conformed to a consistent set of standards. The standardised monitoring methods outlined in the present document will generate data which is comparable across regions and at a national scale, and the usefulness of mussel monitoring data will thus be maximized. Due to the variety of potential monitoring goals we have provided a decision tree and three different options for freshwater mussel surveys: Protocol One - presence/absence, Protocol Two – population size structure (and presence/absence) and Protocol Three - population density and habitat associations. These options are outlined in section 3 of this document.

1 Introduction

Freshwater mussels (also known as Kākahi / Kāeo / *Echyridella* spp.) are endemic to New Zealand and are considered keystone species in freshwater ecosystems. Kākahi filter-feed at an average rate of 1 litre per mussel per hour (Walker et al. 2001), can bioturbate sediments (Collier et al. 2016) and are a culturally important taonga species to Maori. Due to the ability of Kākahi to clear sediment and algae from the water column, they are receiving increased attention from waterway managers. Kākahi populations are believed to be undergoing widespread declines, particularly in shallow lakes and streams, throughout New Zealand due to multiple, severe environmental stressors. There are two species found in the North Island of New Zealand: *Echyridella menziesii* which is distributed across all North and South Island regions and *Echyridella aucklandica* which is known to occupy areas throughout Northland, Auckland, Waikato, and in isolated locations around Whanganui, and in Lake Wairarapa, and Lake Hauroko of the South Island (Marshall et al. 2014). Further, a third species, *Echyridella onekaka*, is only found in the Northwest of the South Island (Marshall et al. 2014). The New Zealand Department of Conservation currently classifies *E. aucklandica* as Threatened - Nationally Vulnerable, and *E. menziesii* as At Risk -Declining (Grainger et al. 2014).

Drivers of freshwater mussel declines in New Zealand likely include predation by exotic species, water quality changes, contaminants, habitat loss, physical removal, excessive sedimentation and declines in dispersal vectors (e.g., declines in host fish populations (see below), or barriers which prevent host fish from moving into key habitats). As well as declines in abundance and distribution, many remaining populations show size distributions which are heavily skewed towards large adults, indicating that only limited recruitment is occurring, and populations are ageing as a result (James 1985; Rainforth 2008; McEwan 2012). Due to these declines, research efforts across New Zealand have increased in order to better understand these species. Records show that *E. menziesii* are present over a larger range within the Waikato region, whereas *E. aucklandica* has been recorded from a much smaller number of locations (Marshall et al. 2014).

Freshwater mussels have a complex life cycle that includes an obligate parasitic larva. During summer the males are thought to exude sperm into the water column and the females then draw this in through their inhalant siphon (Phillips 2007). Their eggs are then fertilized and brooded internally until they develop into larvae known as glochidia (McEwan 2012). Glochidia are then expelled into the water column where they attach onto fish, where they lives as parasites for a few weeks until they detach and transform into juvenile mussels and thereafter are found in the substratum (Clearwater et al. 2014, Figure 1). This is believed to generally occur over summer and with the use of intermediate fish hosts. This mechanism is generally believed to aid dispersal, and may be particularly important in streams, where there is a risk of being swept downstream (McDowall 2011). It was previously believed that kōaro (*Galaxias brevipinnis*) were the main fish host for glochidia (McDowall 2002), but glochidia have been reported on common and giant bully (*Gobiomorphus cotidianus* and *Gobiomorphus gobioides* respectively), long and shortfin eel (*Anguilla dieffenbachia* and *Anguilla australis* respectively) and rainbow trout (*Oncorhynchus mykiss*) (Clearwater et al. 2014 and references therein). Recent laboratory trials with upland bully (*Gobiomorphus breviceps*) have shown that they can act as hosts to glochidia, though īnanga (*Galaxias maculatus*) seem to be poor hosts (Bob Brown, Manaaki Whenua Landcare Research Pers. Comm. 1/11/2016). Redfin bullies (*Gobiomorphus huttoni*) have also been correlated with *E. menziesii* presence in predictive models (Death et al. 2017), and glochidia were found on common bullies in Lake Taupo (Clearwater et al., NIWA, unpublished study, 2018). Once glochidia are detached from their fish hosts, little is known about the next part of the life-cycle until they are found as adults. Adult *E. menziesii* have generally been found in a wide range of habitats from soft-bottomed lakes to fast-flowing streams with hard substrates, whereas little is known about the distribution and habitat preferences of *E. aucklandica* (Phillips 2007).

Population declines and skewed size distributions could be related to concurrent declines in host fishes (McDowall 2002), as without host fishes, mussels are not able to complete their lifecycles and/or disperse to suitable habitats. Freshwater mussels are free-living and usually semi-bury themselves in the substrate. They can also be quite mobile and are able to move around streambeds using their muscular foot. Juvenile mussels are rarely found and surveys tend to show populations dominated by larger mussels (James 1985, Roper & Hickey 1984), but this may be because they are living deep in the sediments or associated with in-stream macrophytes. It is thought that juveniles are likely to feed primarily on organic matter in the sediment (Nichols et al. 2005) rather than filter feeding from the water as the adults do (Roper & Hickey 1995). Collier et al (2016) suggested adult *E. menziesii* could also be using organic material in lakebed sediments as a food source. The differences in habitat and biology of juvenile mussels compared to their larger counterparts could mean that existing survey techniques may underestimate the abundance of juvenile mussels.

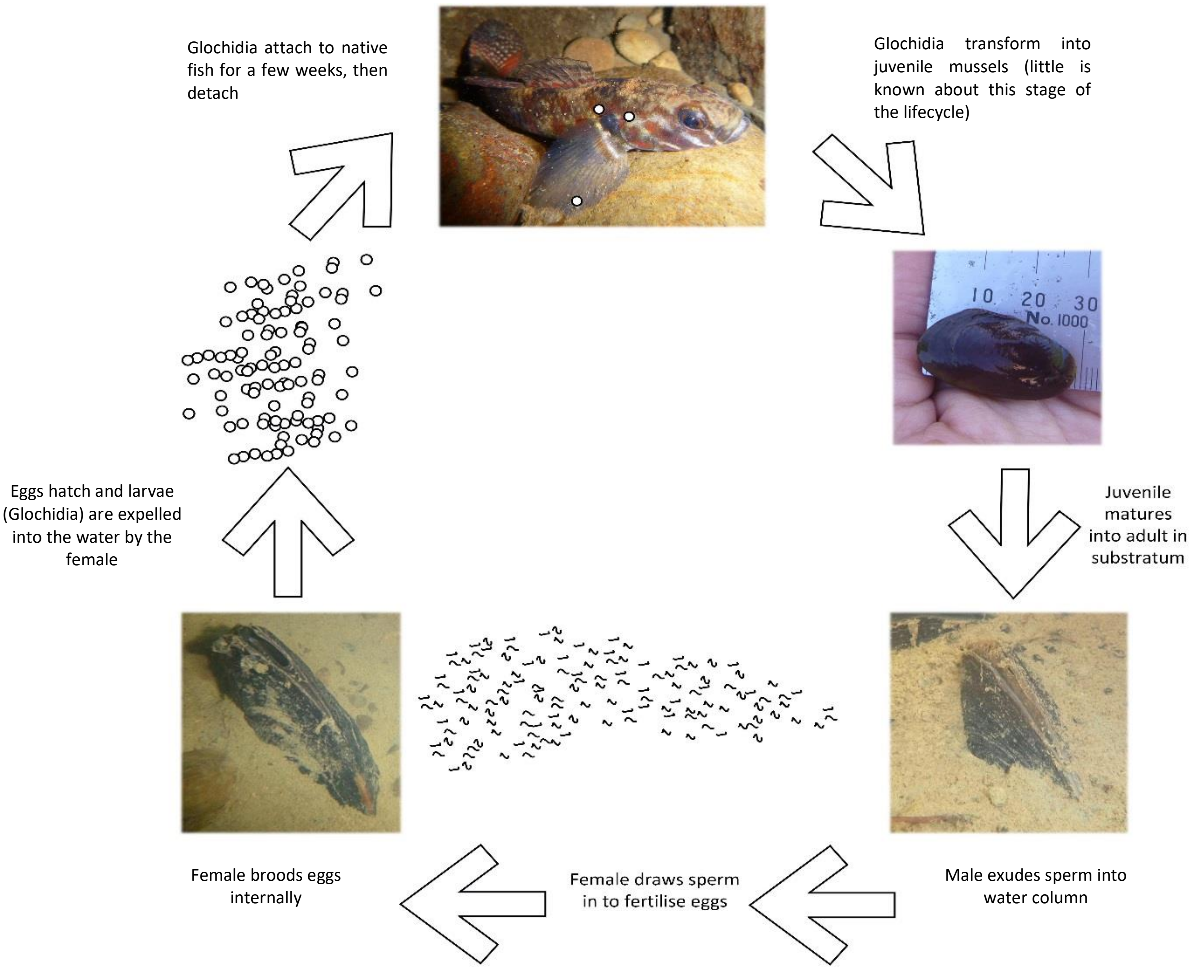

Figure 1: The lifecycle of the freshwater mussel (modified from McEwan, 2015).

There has been little research on the distribution and abundance of *E. menziesii* and *E. aucklandica* within streams and rivers as most research has focused on lake habitats. In addition, the more common *E. menziesii* has been the focus of more research, due to its commonalty as opposed to the rarer *E. aucklandica*. Freshwater mussels are not currently included in standard State of the Environment (SoE) monitoring protocols. Councils are receiving increasing pressure to incorporate iconic and taonga species such as freshwater mussels into SoE monitoring, so there is a pressing need for a consistent national protocol to be developed.

At least two councils have started monitoring programmes for mussels but are using different methods. Wellington Regional Council has developed a monitoring protocol, however, only targeted for use in lake systems (McEwan 2015), whereas Horizons Regional Council has undertaken several surveys to establish presence and population size within their regional boundary. There is therefore a need to calibrate these methods and establish national protocols to ensure data collected in different regions can be used for making national comparisons. Because of this knowledge gap, Waikato Regional Council decided an understanding of the state of freshwater mussel populations in the region was required (for both species) and developed a freshwater mussel survey protocol to provide consistent quantification of mussel populations across sites. It is intended that this protocol will act as an established framework to guide other groups undertaking similar research and so results will be comparable between regions.

The objective was to develop a reach scale survey design that would: 1. Effectively detect both freshwater mussel species present in the Waikato region; 2. Determine mussel abundance in a repeatable and standardised way; and 3. Gather much needed ecological information regarding size distributions, population health and habitat preferences.

2 Freshwater mussel survey protocol development

2.1 Discovery phase

In 2012 to 2013 a discovery phase was initiated to investigate potential freshwater mussel monitoring methods. The objectives as described above, were to undertake freshwater mussel surveys in a standardised way that could be repeated, and collect enough information to enable inferences to be made on the habitat, health and size mussel populations. Due to the lack of information on riverine mussel populations in New Zealand, increasing the number of measured parameters allows additional information to be collected, such as the size of the mussels, the health of the populations and their preferred habitat (where to find them). See Appendix 1 for details.

In order to learn more about freshwater mussel habitat needs, the collection of environmental data was included in the protocol. Site information that was collected during the discovery phase included physical stream measurements (widths, depth, velocity, area searched), water quality measurements, in-stream characteristics (i.e. substrate, macrophyte cover, large wood), stream catchment and reach information (i.e. land use, shade, dead mussel shells on bank) (**Table 1**). A Search Efficiency Score (SEC) for each site was also recorded which related to the clarity and ease in which the reach could be effectively searched for mussels.

Additional habitat information was collected at the individual mussel level (**Table 2**). Based on findings from the initial discovery phase trial (Appendix 1), it was decided, that a timed search was the best method to ascertain presence or absence. If mussels were found to be present, a 50-m reach would then be searched visually with a viewer and when mussels were detected a limited tactile search through the sediment would also be undertaken. Gaining a better understanding of population size structure and condition could identify whether the population was healthy in terms of recruitment as well as abundance. Mussel dimensions (length, width, depth, axis a, b and c respectively, see diagram in Appendix 6) and shell erosion were also recorded for each mussel. A maximum number of 50 individuals of each species were measured, but all mussels present were counted and habitat variables recorded.

Table 1: Parameters measured within the survey reach during the mussel surveys

Stream associated parameters	Explanation	Measures included (if applicable)
Channel dimensions (m)	Size and shape of the stream channel	Wetted width, Channel width, Thalweg depth (Deepest point)
Area unsearched (m^2)	The total area of the stream reach that wasn't included in the survey i.e. $2m^2$	
Water measurements	Measures of water quality	Temperature, Dissolved oxygen, Conductivity
Visual clarity	How clear the stream water column is	Clear, slightly turbid, highly turbid, stained
Water analysis	Samples taken to a laboratory to analyse water quality parameters	Water hardness, turbidity, dissolved metals (See Appendix 8 for more details)
Substrate composition (%)	What the bottom of the streambed is comprised of	Bedrock, boulder, cobble, large gravel, small gravel, sand, silt and clay
Embeddedness (%)	How much fine sediment covers the bottom substrate	
Aquatic vegetation cover (%)	Estimates the total cover of aquatic plants (Macrophytes) within the stream reach	
Fish species observed	Species of fish seen during mussel surveys	
Surrounding land use	Dominant land use surrounding reach	Indigenous forest, exotic forest, exotic scrub, pasture, urban, parkland
Organic matter cover (%)	Amount of organic matter within stream reach	Large wood, coarse detritus, fine detritus
Shade	Amount of shade due to canopy cover, covering the stream reach	Open, Partly shaded, Significantly shaded
Habitat Assessment field data sheet (HAFDS, Collier & Kelly 2007)	Regional specific habitat score for the stream reach based on multiple parameters such as sediment, substrate, riparian vegetation etc.	1-200 (N.B, Either hard or soft bottomed assessment based on what type of substrate makes up >50% of reach)
Search efficiency score	How efficient the mussel searching was (1-poor to 5-very good)	1-5
Searching time	How long it takes from the start searching point to survey and measure all mussels found within the 50m reach	
Number of dead shells	How many dead mussel shells are found within the stream reach	Number on stream banks, number in stream channel

Table 2: Parameters measured that are associated with individual mussels

Parameters associated with finding mussels	Explanation	Measures included
Mussel dimensions (mm)	Measures all dimensions of the mussel shell	Length, width, depth
Shell erosion	How worn the shell is	0%, 1-25%, 25-50%, 75-100%
Mussel location 1	Where the mussel was located when it was found	True left, true right, middle
Mussel location 2	Where the mussel was located when it was found	Outside bend, inside bend, straight
Mussel location 3 (associated habitat)	What habitat the mussel was associated with when it was found	Bank toe, undercut, rootmat, substrate, wood, sand bar, macryophytes
Flow type	What type of flow the mussel was found in	Run, riffle, pool, backwater
Substrate	What substrate the mussel was found in	Bedrock, boulder, cobble, large gravel, small gravel, sand, silt and clay
Search method	The type of search used to find the mussel	Visual, hand search
Mussel found	How the mussel was found within the stream	Emergent, buried

2.2 Selecting a monitoring site

This method covers mussel sampling of wadeable streams and rivers. We define wadeable stream sites as those where more than half of the sampling reach can be safely accessed by survey personnel. Site selection is dependent on the objectives of the study in question, however careful consideration needs to be given to the characteristics of potential stream sites. Stream size and depth, habitat present, shade, visual clarity and access are all important factors to consider when selecting a site for mussel surveys. We have summarized this process into a decision tree diagram (**Figure 2**). Depending on the reason for surveying, the site selection decision tree may improve chances for finding mussels. Whether the stream is soft or hard bottomed will also need to be recorded. If the stream bed is comprised of sand/silt or clay which is equal to or greater than 50% of the stream reach it is soft bottomed, whereas less than 50% is classed as hard bottomed (Collier & Kelly 2005).

Sampling time and frequency will depend of the objectives and timeframe of the study. It is recommended that mussel surveys are undertaken between November-May as this is the season when flows will usually be lowest and clarity should be good for viewing. Also, we have demonstrated that autumn was the most effective season for detecting mussels (see Appendix 1). Team size for searching depends on the size of the waterway (i.e., width), for a large site three to four people is generally advisable, as three can do the searching and one the recording, while as few as two people can efficiently search smaller streams. The time taken for undertaking a sample reach will vary based on the size of the stream and by the number of mussels that are found. To ensure surveys can be completed in an efficient and timely manner the number of people required should be considered.

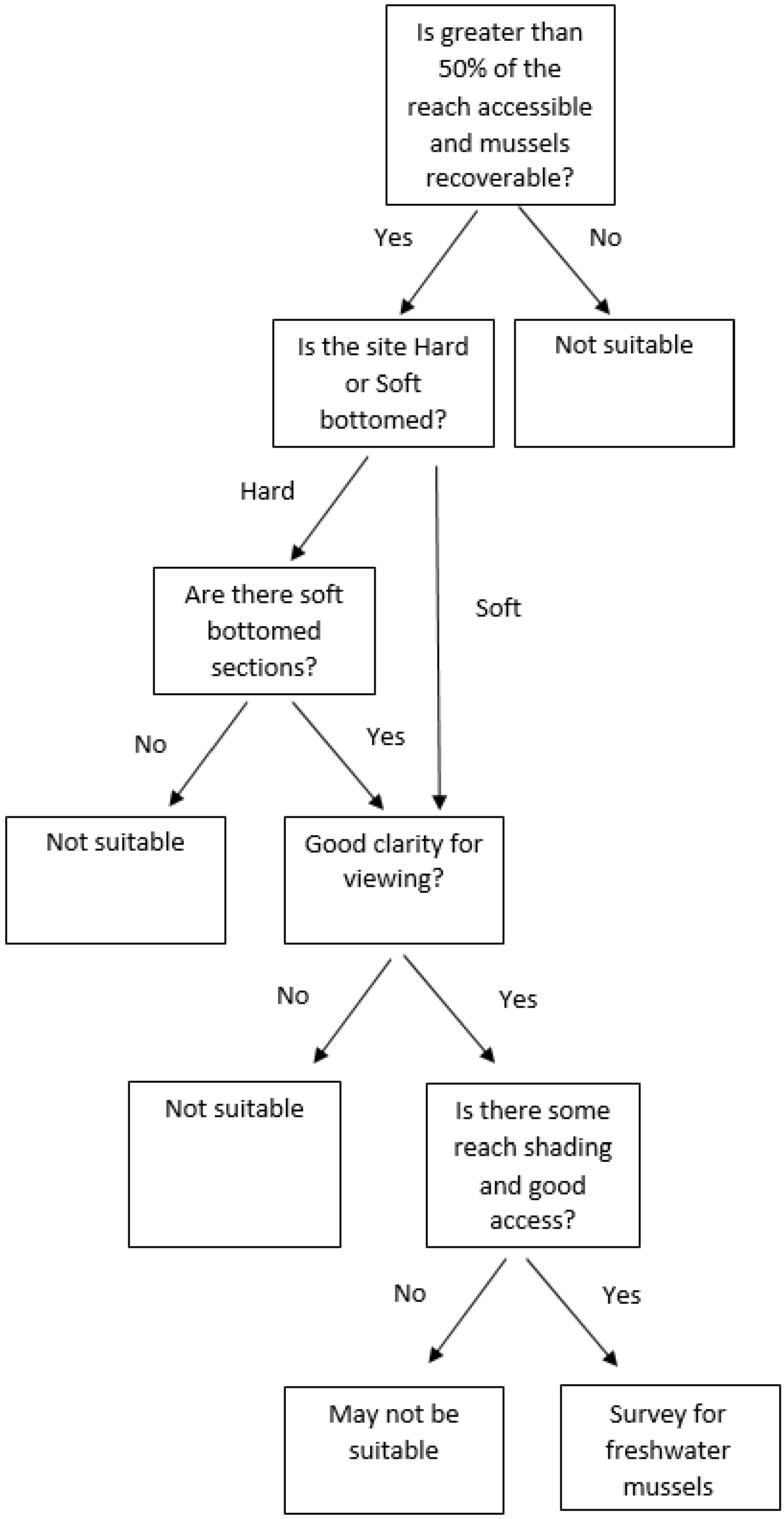

Figure 2: Decision tree diagram for mussel survey site selection.

2.3 Selecting a monitoring protocol

The purpose of this protocol is to describe the procedures used by Waikato Regional Council when undertaking surveys for freshwater mussels in wadeable streams, and to enable other groups conducting similar studies, such as community groups, regional councils or consultants to employ a consistent methodology. The intention is that method standardisation will lead to comparable data across regions and at a national scale. The output of this method is an estimate of mussel density per square metre and population size structure that will be comparable across the region and through time.

We propose three protocols for undertaking mussel surveys to cover the variety of situations that may be encountered in wadeable rivers and streams. Protocol 1 is designed to be a quick method to identify whether mussels are present or absent and if present, what species is there. This protocol may be a good choice for community groups which include mixed ages and abilities (see McEwan 2012, Wairarapa Moana 2015, 2016). Protocol 2 is the method we recommend for most situations. It requires a greater effort than Protocol 1, but provides valuable information on mussel species presence or absence, population size structure and provides a standardised, repeatable, density estimate. Protocol 2 is recommended as a minimum requirement to provide baseline information for State of Environment monitoring and potentially consent monitoring, however, in situations where a greater level of understanding is required Protocol 3 should be employed. Protocol 3 provides more detailed information about instream habitat associations (**Figure 3**, **Table 3**). Protocol 3 is seen as more suitable for consent applications and assessments as it helps identify instream habitats of importance.

The State of the Environment monitoring report by Catlin et al. (in prep) can be read in conjunction with this method guideline, to further inform decision-making about what protocol to use and what measurements could be left out and why. Based on the results obtained from undertaking the surveys (Catlin et al. in prep) that show mussel habitat preferences, we recommend that the majority of searching time when undertaking an initial 30 minute search should focus on bank habitat in runs and pools, and primarily silty substrates.

A decision tree is included in these guidelines to help select the best protocol depending on the aims of the survey. Three different protocols are provided for freshwater mussel surveys including: Protocol One for presence/absence, Protocol Two to provide size structure and population density, and; Protocol Three to provide size structure, population density and habitat associations. Different data sheets are required for each protocol. Irrespective of the protocol selected, we encourage completion of a Field Assessment Form (FAF). The field assessment form (FAF, as presented in Collier & Kelly 2005 which is specific for the Waikato region; Appendix 3), includes evaluation of reach canopy cover, fencing, and dominant land use. Protocol One only requires completion of a FAF (Collier & Kelly 2005) and the Freshwater Mussel Survey Form (Appendix 2) as it only examines presence/absence. Protocol Two includes data sheets to record species and mussel measurements (e.g., length, width and depth), while Protocol Three includes the latter plus habitat associations and search methods (Appendix 2).

Table 3: Guidelines for selecting a monitoring protocol

Protocol	Explanation	Suitable for
Protocol 1: Presence/Absence	Used for ascertaining the presence of freshwater mussel species	Target groups after a quick, easy survey to get baseline data
Protocol 2: Species density and size structure	Used for looking at species densities within a study reach and that populations size range i.e. ageing or not	SoE monitoring, consent monitoring. Uses medium effort to gain valuable data
Protocol 3: Presence, density, size structure and habitat	Uses the previous two protocols plus looks at individual mussels habitat preferences within a stream reach	Situations where a greater level of information is required, i.e. AEE, research projects

2.4 Deciding which variables to include

If a data intensive survey is required (Protocol 3), there are a number of habitat parameters which can be included if appropriate. These parameters were investigated during the discovery phase, and include:

Table 4: Mussel parameters that can be included in more intensive surveys (i.e. Protocol 3)

Parameter	Explanation	Reasons for inclusion
The number of dead shells	Count of the number of dead shell found within the survey reach. i.e., due to natural causes, rat or bird damage or flooding events.	May indicate predation pressure (i.e., if shells are bitten or otherwise characteristically damaged), a disease event, or deaths due to stranding after flooding. A community group may be interested in this if they are planning a trapping operation.
Shell thickening	Records whether shell thickening or layering is present on a mussel shell	Mussels with thickened shells are found alongside mussels without thickened shells therefore thickening may provide information about mussel health. Shell thickening in *E. menziesii* is sometimes caused by infestation with a commensal chironomid (Forsyth & McCallum 1978).
Shell erosion	Percentage of the mussel shell that has eroded	Rather than being an indicator of poor health alone erosion is also probably influenced by water chemistry, and physical abrasion (Rainforth 2008; and references therein), which in turn is likely related to substrate type and how much the mussels move around in the substrate.
Flow type	The type of flow the mussel is associated with is recorded. i.e., run, riffle, pool, backwater	The majority of mussels we found were in runs and not riffles (pools couldn't always be searched effectively) (Catlin et al. in prep). However, recording flow type could increase on information around mussel habitat preferences and whether they prefer riffles or other flow types in different streams. Otherwise, it could be removed from the individual mussel level and implemented at the reach scale e.g. percentage of pool/run/riffle in sampling reach.
Mussel location 1	The location of the mussel is recorded i.e., true right, true left bank or straight	The majority of mussels were found along banks (Catlin et al. in prep), which can be useful knowledge directing assessments for AEEs and consent applications in streams and rivers. It will also build upon the knowledge we have now and show whether mussels can prefer multiple locations within a stream.
Mussel location 2	The location of the mussel is recorded i.e., outside bend, inside bend, middle	Mussels were generally found in straight runs (Catlin et al. in prep), but greater knowledge is required on whether this is a true finding or region specific.
Mussel location 3	Records what type of habitat the mussel was associated with when it was found	Across our surveys there was a lot of variation in mussel location 3. More surveys are needed to include this and to establish habitat preferences for freshwater mussels.

Substrate	Record what substrate type the mussel is found in	Enables mussel habitat preferences to be recognised and compared across sites, regions and nationally. Substrate dominance could give insight on better sites to survey as well.
Mussel emergence	Whether the mussel is found visually or by hand search	Recording mussel emergence can give insight into whether juvenile mussels are getting found by hand search rather than visually in the substratum as the majority of adults do. Also enables detection of large mussel beds if the majority are buried.
Mussel search	Whether the mussel is emergent or buried when it is detected is recorded	We did find that three quarters of the mussels were emergent, however we do believe that hand searching for mussels is particularly important as the majority of juveniles (10-35mm) are found this way rather than visually. If specifically looking for juveniles, recording search method for each mussel may be useful to understand how they were detected (Catlin et al. in prep).

Mussel length can vary according to environmental conditions (Downing & Downing 1993) but it can also provide an indication of life stage, age and overall size and is the most useful shell measurement to understand mussel population structure. Thus, if time is limited for undertaking mussel surveys, measuring width and depth (mm) of the mussels could be omitted as these measurements can vary, dependent on factors affecting growth such as habitat, in-stream water quality and mussel health (Forsyth & McCallum 1978). In conclusion, if the aim of the survey is just to understand/record population size structure, all the remaining mussel-specific observations could be removed.

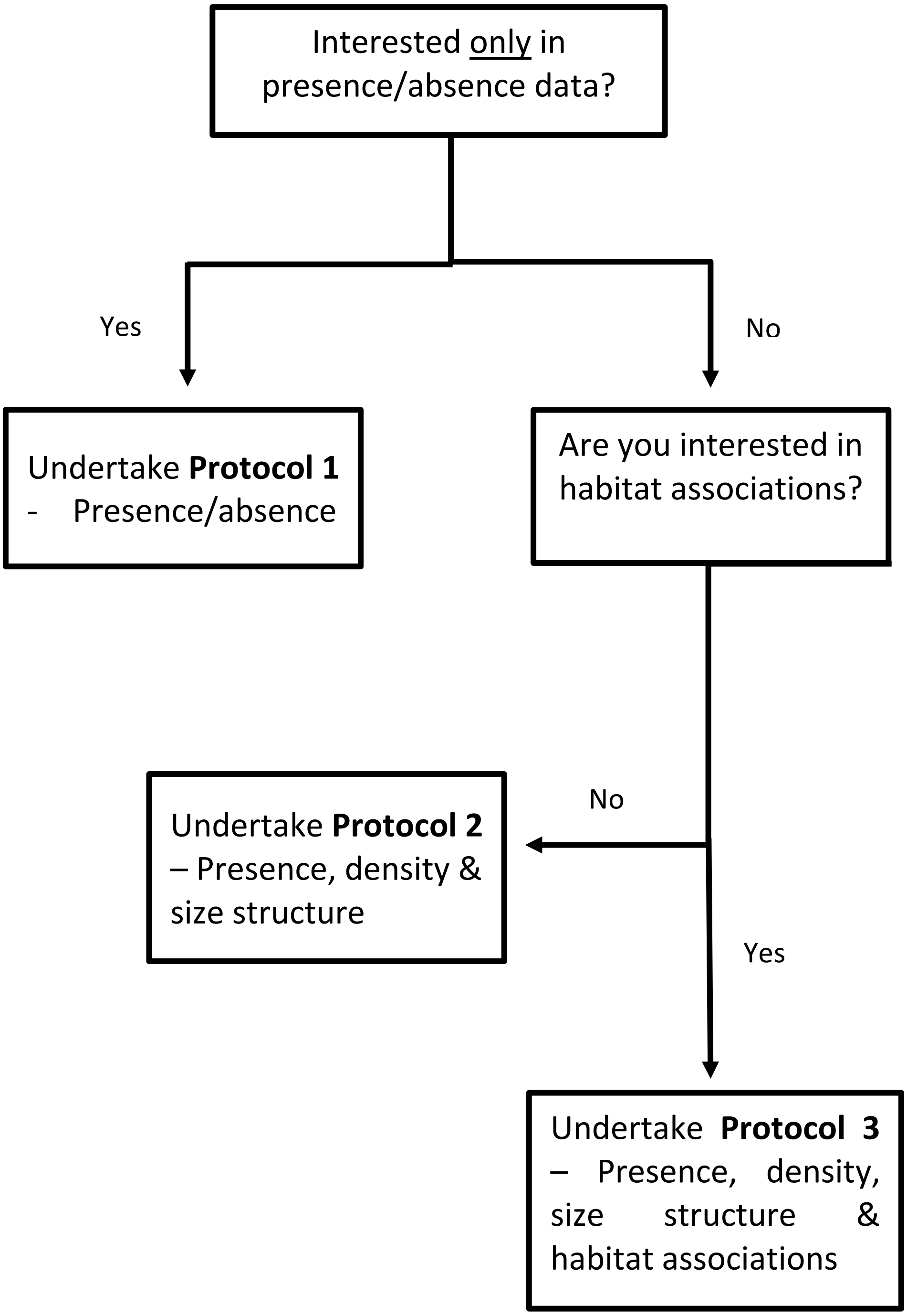

Figure 3: Decision tree for selection of freshwater mussel survey protocol.

3 Protocol One – Presence/ Absence

Once a site is selected (see **Figure 2**), begin by undertaking a 30 minute visual survey with underwater viewers (N.B., if two people are present 2 x 15 = 30 minutes) targeting likely habitats (i.e., along banks/undercuts/macrophytes/shaded areas/logs) to establish presence or absence. A full equipment list for monitoring can be found in Appendix 5.

If no mussels are found, take a GPS reading of the survey start and finish locations, take photos of the site and record that no mussels were present.

If mussels are present the site must be surveyed more thoroughly. Survey steps are outlined below.

1. Fill out a region-specific standard Field Assessment Form (FAF) for the site (canopy cover, fencing DO, conductivity etc.) – (N.B., region specific; Collier & Kelly (2005) for the Waikato).
2. Set up a 50 m reach from the point the first mussel was found. Take a GPS reading at start location, take photos of the site, and record the start time of searching.
3. Start an intensive search of the entire stream width looking for the presence of the other species.
4. Identify the species present (using the photo guide and comments in Appendix 7).
5. Once the 50m reach has been surveyed or both species of mussel have been found, then stop searching and record the finish time.

6. Add any relevant comments to the comments section.
7. Give the site a 'search efficiency' score (1= poor, 5 = good) taking into account visual clarity, depth and aquatic plant cover.
8. Finally fill out the Habitat Assessment Field Data Sheet (HAFDS) (N.B., slightly different forms need to be used for hard and soft bottomed streams; region specific, see Collier & Kelly 2005 for Waikato, or nationally used, Rapid Habitat Assessment see RHA, Clapcott 2015. Appendix 3).
9. Decontaminate all gear using check, clean, dry protocols (https://www.mpi.govt.nz/travel-and-recreation/outdoor-activities/check-clean-dry/) and a suitable disinfectant to prevent the unwanted spread of bacteria, viruses fungi and other infections i.e. Trigene

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4 Protocol Two – Species density and size structure

Once a site is selected (see **Figure 2**), begin by undertaking a 30 minute visual survey with underwater viewers (N.B., if two people are present 2 x 15 = 30 minutes) targeting likely habitats (i.e., along banks/undercuts/macrophytes/shaded areas/logs) to establish presence or absence. A full equipment list for monitoring can be found in Appendix 5.

If no mussels are found, take a GPS reading of survey start and finish locations, take photos of the site and record that no mussels were present. Then take water quality measurements of temperature (˚C), dissolved oxygen (DO%, DO mg L^{-1}) and specific conductivity (µS/ cm^{-1}). Lastly, Field Assessment Forms (FAF, region specific, Collier & Kelly (2005)) are to be filled out before moving onto the next site.

If mussels are present the site should be surveyed more thoroughly. Survey steps are outlined below.

1. Fill out a region-specific standard Field Assessment Form (FAF) for the site (canopy cover, fencing DO, conductivity etc.) – (N.B., region specific; Collier & Kelly (2005) for the Waikato).
2. Set up a 50 m reach, broken down into 5 x 10m subreaches from the point the first mussel was found. Take a GPS reading at start photos, take photos of the site and record the start time of searching.
3. At the start point and at the end of each subreach, record wetted width, thalweg depth and any areas that were unsearched due to depth etc.
4. Start an intensive search of the entire stream width slowly working your way upstream. Each time a mussel is found visually, remove it from the substrate and undertake a tactile (hand) search of the substrate one hand width around that mussel to detect any buried mussels nearby (also check the base and stalks of macrophytes for small juveniles with your hands). Record whether juvenile mussels were found by either "hand search" or "visual" if interested in juveniles, as little is known about usual location or habitat preferences in streams and rivers.
5. Identify the species present (using the photo guide and descriptions in Appendix 7).
6. Measure the length, width, depth of the mussel to nearest half mm and record this on the field sheet (Appendix 2. Return umbo-end down (see Appendix 6) to the location they were found in (or as near as possible).
7. Measure the first 50 mussels of each species only. Then record the count for each species after that in the space provided on sheet (Appendix 2 attached). Once the 50 m reach has been surveyed then stop searching and record the finish time.

8. Add any relevant comments to the comments section.
9. Give the site a 'search efficiency' score (1= poor, 5 = good) taking into account visual clarity, depth and aquatic plant cover.
10. Finally fill out the Habitat Assessment Field Data Sheet (HAFDS) (N.B., slightly different forms need to be used for hard and soft bottomed streams; region specific, see Collier & Kelly 2005 for Waikato, or nationally used, Rapid Habitat Assessment see RHA, Clapcott 2015. Appendix 3).
11. If less than 50 mussels were found, complete a further 30 minute search (i.e., combined time spent by searchers) upstream in likely habitats to try and get 50 of each species for

size distribution data. Record these details on the field sheet (but don’t include these mussels in any population density estimates).

12. Decontaminate all gear using check, clean, dry protocols (https://www.mpi.govt.nz/travel-and-recreation/outdoor-activities/check-clean-dry/) and a suitable disinfectant to prevent the unwanted spread of bacteria, viruses fungi and other infections i.e. Trigene.

5 Protocol Three – Presence, density, size structure & habitat

Once a site is selected (see **Figure 2**), begin by undertaking a 30 minute visual survey with underwater viewers (N.B., if two people are present 2 x 15 = 30 minutes) targeting likely habitats (i.e., along banks/undercuts/macrophytes/shaded areas/logs) to establish presence or absence. A full equipment list for monitoring can be found in Appendix 5.

If no mussels are found, take a GPS reading of survey start and finish locations, take photos of the site and record that no mussels were present. Then take water quality measurements of temperature (˚C), dissolved oxygen (DO%, DO mg L^{-1}) and specific conductivity (µS/ cm^{-1}). Lastly, Field Assessment Forms (FAF, region specific, Collier & Kelly (2005)) are to be filled out before moving onto the next site.

If mussels are present the site should be surveyed more thoroughly. Survey steps are outlined below (also see Appendix 3).

1. Fill out a region-specific standard Field Assessment Form (FAF) for the site (canopy cover, fencing DO, conductivity etc.) – (N.B., region specific; Collier & Kelly (2005) for the Waikato).
2. Collect water samples for analysis (see Appendix 8 for complete parameter list).
3. Set up a 50 m reach, broken down into 5 x 10m subreaches from the point the first mussel was found. Take a GPS reading at start photos, take photos of the site and record the start time of searching.
4. At the start point and at the end of each subreach, record wetted width, thalweg depth and any areas that were unsearched due to depth etc.
5. Start an intensive search of the entire stream width slowly working your way upstream. Each time a mussel is found visually, remove it from the substrate and undertake a tactile (hand) search of the substrate one hand width around that mussel to detect any buried mussels nearby (also check the base and stalks of macrophytes for small juveniles with your hands). Record whether juvenile mussels were found by either “hand search” or “visual” if interested in juveniles, as little is known about usual location or habitat preferences in streams and rivers.
6. Identify the mussel species (using the photo guide and comments in Appendix 6).
7. Measure the length, width, depth of the mussel shell to nearest half mm using Vernier callipers and record this on the field sheet (Appendix 2). Record the condition of the mussel shell (% erosion), flow type found in, three habitat locations (see key with data sheet), substrate type, position and search type (refer to site sheet and key in Appendix 2). Return umbo-end down (see Appendix 6) to the location they were found in (or as near as possible).
8. Record any dead shells (both sides of the shell must be present to count), record whether it was in the stream or in the bank and if signs of predation are present (i.e., are shells bitten, do they look recently broken (by force), or do they have “new” holes in the sides of the shell? Are there bird or mammal tracks or other signs associated with the shells?).
9. At the end of each 10-m sub reach measure the wetted width, thalweg depth (maximum depth at that transect), % macrophyte cover for the 10 m reach, estimate % substrate (clay, silt, sand, small gravel etc. for the 10m reach), % shade using a densiometer, % wood and record any area that was unsearchable due to depth etc.
10. Repeat steps 5-9 for the next four sub reaches.
11. Measure the first 50 mussels of each species only. Then record the count for each species after that in the space provided on sheet (Appendix 2 attached).

12. Add any relevant comments to the comments section (i.e., if dead mussels are present are they freshly dead? Are there signs of recent flooding?).
13. Give the site a 'search efficiency' score (1= poor, 5 = good) taking into account visual clarity, depth and aquatic plant cover.
14. Finally fill out the Habitat Assessment Field Data Sheet (HAFDS) (N.B., slightly different forms need to be used for hard and soft bottomed streams; region specific, see Collier & Kelly 2005 for Waikato, or nationally used, Rapid Habitat Assessment see RHA, Clapcott 2015. Appendix 3).
15. If less than 50 mussels were found, complete a further 30 minute search (i.e., combined time spent by searchers) upstream in likely habitats to try and get 50 of each species for size distribution data. Record these details on the field sheet (but don't include these mussels in any population density estimates).
16. Decontaminate all gear using check, clean, dry protocols (https://www.mpi.govt.nz/travel-and-recreation/outdoor-activities/check-clean-dry/) and a suitable disinfectant to prevent the unwanted spread of bacteria, viruses fungi and other infections i.e. Trigene.

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Appendix 1 – Discovery phase trials

Discovery phase

Internationally, multiple mussel survey measures have been employed such as timed search approaches (Strayer & Smith 2003), while in New Zealand approaches used have included how long it takes to fill a bucket (Rainforth, 2008), how many are found in 30 minutes of hand-searching for community volunteer surveys (McEwan 2012, 2015), or per unit area measures using quadrats (McEwan 2012). The objective of the monitoring trial was to assess and monitor population size and density of the two species known to be present in our region, thus an area search method as recommended by Strayer & Smith (2003) was trialled. This method is understood to be better suited to assessing density and size demographics (Obermeyer 1998, Tiemann et al. 2009) within stream systems. Using this approach enables sites to be revisited in the future and allows comparisons of mussel populations from a known area to document the state of mussel populations and any recruitment and/or declines. Methods were initially trialled in the Maunurima Stream, a small 1-m wide stream located in the Raglan Harbour catchment. A number of methods were tested within a 100-m section of the stream which was on average 1.17 m wide (wetted), 0.20 m deep, flowing at 0.2 m s^{-1} and had a substrate comprised mainly of small gravel, clay and sand. The reach was open with little shading present from riparian plants although the incised banks provide some shade (**Figure 4**).

Figure 4: Maunurima Stream, Discovery phase study reach.

Within the study reach, methods such as visual and tactile searches, excavating and sieving substrate were trialled (**Figure 5**). Quadrats (0.25m^2) were excavated to a depth of approximately 5 cm and then the material was sieved through a 4-mm mesh. This method was selected because it was thought that it might detect small juvenile mussels and was trialled using 43 quadrats within the reach. No mussels large or small were found using this method so it was excluded from further use as it was time-consuming and did not appear to be effective. Following these trials, we attempted tactile hand searches through the substrate to about 8 cm depth. Using this method 14 mussels ranging from 40 mm to 97 mm in length were found. In contrast, 92 mussels were detected visually using underwater viewers. Based on this information, primarily searching for mussels using viewers was considered the best option, although when mussels were detected visually it was decided to supplement the visual search with a tactile hand search of that area to about 8cm depth, to identify any buried mussels nearby.

Mussel populations were also investigated using 0.25m^2 quadrats, visually searching transects and undertaking a full reach search. Simple random and systematic sampling designs from Strayer & Smith (2003) were undertaken to identify if these designs were the best fit to find mussels and account for their patchiness. The simple random design uses a random number generator or table to select a known number of transects or quadrats to sample within a reach. The systematic design uses a random start point and then repeats at a set interval up the reach. The ability of the survey methods to provide an accurate abundance estimate was 'ground-truthed' by carrying out full searches of the reach and obtaining a total count of the number of mussels which were actually present and mussels found were recorded every metre. The known mussel population (estimate) for the reach was used as the basis to test the simple random and systematic sampling designs from Strayer & Smith (2003). Retrospectively, the accuracy of these two methods at the test site was tested by applying them to the known numbers found in the full 100-m search of the reach. The length of time the various methods took was recorded. This was repeated with 3 random and 3 systematic design scenarios. The random design was found to have a lot of variability until approximately 50 transects had been undertaken (Figure 6). With the systematic design there was generally an overestimation of the mussel density and the confidence intervals did not decrease until approximately 65 transects had been searched (Figure 6).

Figure 5: A) Excavating and sieving (4 mm; Left photo), B) Tactile search of a quadrat (Middle photo), C) Visual search of transect (Right photo).

When comparing transects and quadrats it was found that randomised transects were more effective than randomised quadrats at detecting mussels (WRC Unpublished data). However, at least 50 transects would have to be surveyed to have any confidence in the data (Figure 6). The outcome of this testing highlighted that our survey would need to include at least 50, 1-m wide transects, across a 100-m reach to be confident of any mussel density estimate. Logistically this would be time-consuming and impractical. It was therefore decided that a full search of a 50-m section of stream would be undertaken to make it more feasible logistically and improve efficiency. Due to the search reach now being shorter, it was decided that employing an initial timed search to ascertain mussel presence would be beneficial before continuing with a full 50-m reach survey.

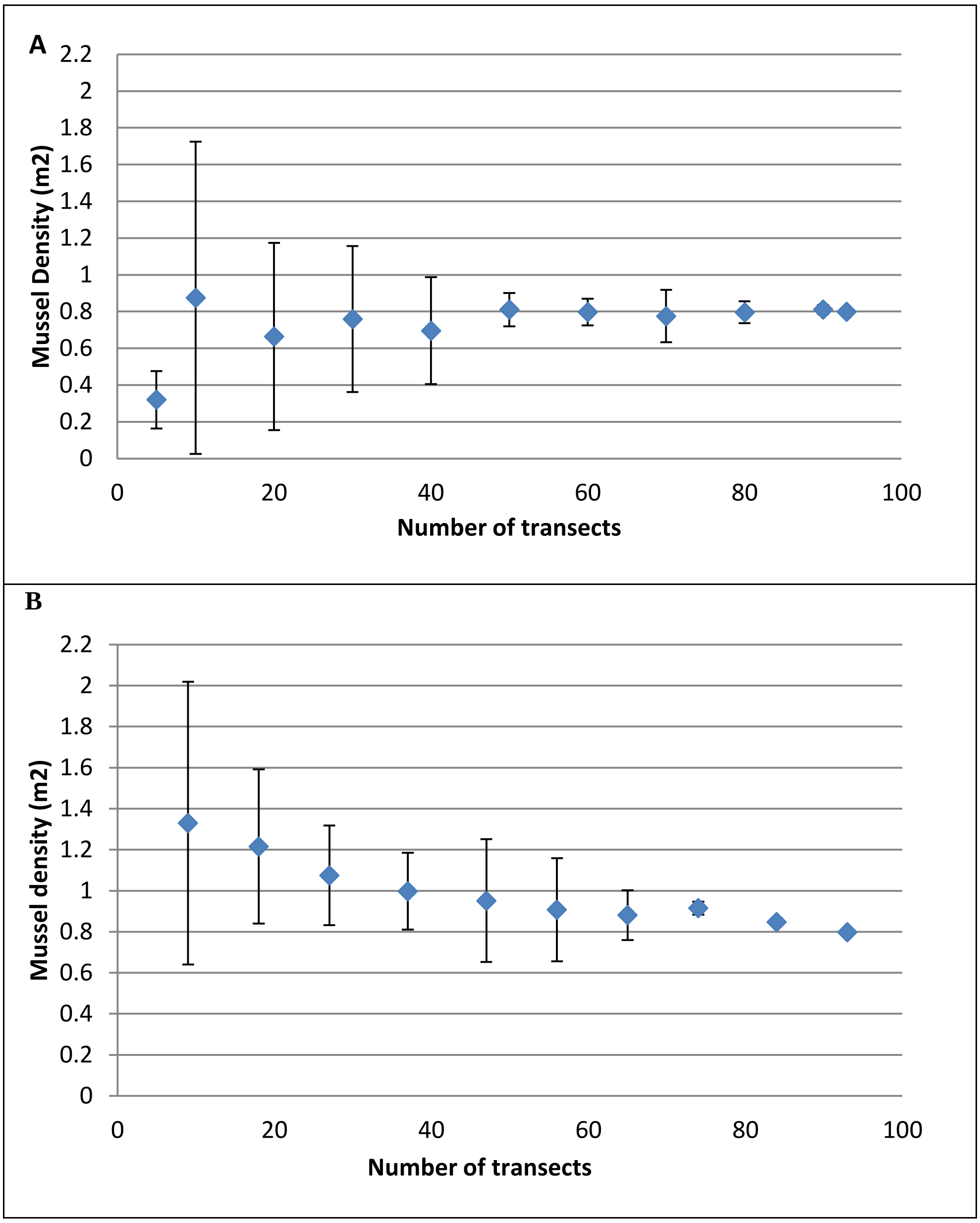

Figure 6: Density estimates across 100 reach using:
A) a randomised sampling design and
B) a systematic sampling design (see Strayer & Smith 2003). Points represent the average of 3 simulated samplings of an actual known population. Error bars = 95% confidence intervals.

The full search of the site was repeated over four seasons to understand the temporal variability of population estimates using this method. Mussels were replaced in the sediment at the same location after discovery. Mussel numbers varied across all four seasons surveyed, and two of the samplings occurred after large floods in which mussel numbers were the highest. As this trial stream was very narrow in width, average depth and located in a small catchment, we concluded that the seasonal assessments of mussel numbers may not be representative of large stream and rivers that would usually be surveyed. Based on this, we decided that we would undertake all our future mussel surveys in autumn as Waikato Regional Council's other fieldwork generally finishes at the end of March, and autumn typically has stable weather and low flows with few flood events which is important for accessing streams and for searching visually.

Appendix 2 – Freshwater mussel survey form

Date:				**Observer:**			
Catchment:				**Stream:**			
GPS D/S:				**Temp:**			
DO:		**%**		**mg/L**	**Cond:**		**µS/cm**
Start time:		**End time:**		**Length searched (m):**			

			Subreach					
		Start	**S1**	**S1**	**S1**	**S1**	**S1**	**Mean**
Width								
Depth								
Macrophyte cover %								
Large Wood %								
Area unsearched								
Substrate	Clay							
	Silt							
	Sand							
	Small G							
	Large G							
	Cobble							
	Boulder							
	Bedrock							
Densiometer	U/S							
	TL							
	D/S							
	TR							

Total Area Searched	
Overall Searching Efficiency	

1= poor → 5 = excellent

Comments:

Protocol 2 Data Survey Sheet

No.	Species A	Length	Width	Depth	Species B	Length	Width	Depth
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
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50								

Protocol 3 Data Survey Sheet (see key overleaf) Mussel Species:

No.	Sub reach	Length	Width	Depth	Condition	Flow	Hab 1	Hab 2	Hab 3	Substrate	Emerged	Search
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
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50												

Record number of dead shells	Count	Bank Middens	Rat Damage	Bird Damage

Extra Count:	S1	S2	S3	S4	S5

Key:

Condition	0 - no wear on shell surface, slight on beak		**Emerged**	Yes	
	1-25% surface worn, light wear			No	
	25-50% surface worn, light to some wear			Unknown	
	50-75% surface worn, some deep pitting		**Search**	Visual	
	75-100% surface worn, badly eroded			Hand search	
Habitat 1	TL	true left	**Subreach**	S1	
	TR	true right		S2	
	M	middle		S3	
Habitat 2	O	outside bend		S4	
	I	inside bend		S5	
	S	straight		E	End
Habitat 3[A]	Mac	Macrophyte	**Flow**	Riffle	
	W	Wood		Run	
	Sub	other inorganic substrate		Pool	
	BF	Bankfoot		Backwater	
	UC	Undercut bank			
	RM	Root Mat			
	BR	Sand Bar			
Substrate[B]	Cl	Clay (<0.004mm)			
	Si	Silt (0.004-0.06mm)			
	Sa	Sand (0.06-2mm)			
	SG	Small Gravel (2-16mm)			
	LG	Large Gravel (16-64mm)			
	Co	Cobble (64-256mm)			
	Bo	Boulder (>256mm)			
	Bed	Bedrock			

[A]Note the habitat type that the mussel was most closely associated with; [B]Select the most prevalent substrate type where the mussel was found.

Appendix 3 – Field and habitat assessment data sheets

Field Assessment Form (FAF), Collier & Kelly 2005

FIELD ASSESSMENT COVER FORM: (100m reach)

WADEABLE HARD-BOTTOMED AND SOFT-BOTTOMED STREAMS

STREAM NAME:		**ASSESSOR:**	
SITE NUMBER:	**SAMPLE NUMBER:**	**DATE:**	**TIME (NZST):**

GPS COORDINATES: **Downstream end of reach - Easting –** **Northing –**
Upstream end of reach - Easting – **Northing –**

Canopy Cover:
_ Open _ Partly shaded _ Significantly shaded

Riparian Fencing:
_ None/ ineffective
_ One side/ partial both sides
_ Complete both sides

Dominant rip.landuse (≤20m)(

_ Urban	_ Retired Grass
_ Parkland	_ Forestry
_ Crops/hort	_ Native shrub
_ Pasture	_ Native forest

INSTREAM HYDRAULIC CONDITIONS

Estimated reach average conditions:
Stream width (C) _________ m
Stream width (W) _________ m
Stream depth _________ m
Surface velocity _________ m/sec

Temperature: _________ ºC Conductivity: _______µS/cm _ Ambient
Dissolved Oxygen: _______ % _______ mg/L _ Adjusted to 25ºC
Turbidity: _ Clear _ Slightly turbid _ Highly turbid _ Stained _ Other______________

INORGANIC SUBSTRATE

Compaction
_ assorted sizes tightly packed &/or overlapping
_ moderately packed with some overlap
_ mostly a loose assortment with little overlap
_ no packing / loose assortment easily moved.

Embeddedness
_ <5% gravel-boulder particles covered by fine sediment
_ 5-24% covered by fine sediment
_ 25-49% covered by fine sediment
_ 50-75% covered by fine sediment
_ >75% covered by fine sediment

% surficial substrate size composition
(should sum to 100%)

Substrate type	**Diameter**	**Percentage**
Bedrock	-	
Boulder	> 256mm	
Cobble	>64 - 256mm	
Large Gravel	>16 - 64mm	
Small Gravel	>2 - 16mm	
Sand	>0.06 - 2mm	
Silt	0.004-0.06mm	
Clay	<0.004mm	

ORGANIC SUBSTRATE
(% cover in reach - need not sum to 100%)

Large wood (>10 cm diameter): _______%
Detritus (small wood, sticks, leaves etc > 1 mm): _____%
Muck/mud (fine organic matter < 1 mm): _______%

HABITAT TYPES SAMPLED (% of effort; each column should sum to 100%)

Stones: ______%
Wood: ______%
Macrophytes: ______%
Edges: ______%

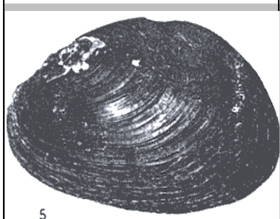
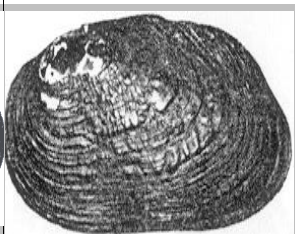
Riffles: ______%
Runs: ______%

COMMENTS

NO. INVERTEBRATES RETURNED TO STREAM

Koura: _______ Shrimps: _______
Crabs: _______ Mussels: _______
Others (specify) ___________________________

Species of mussel (tick)

E. menziesii	*E. aucklandica*
	
Shell smooth; up to 100mm long; curved shell shape	*Nodules and ridges on upper part of shell; straight lines*

WADEABLE SOFT-BOTTOMED STREAMS – 100 m reach

Qualitative Habitat Assessment Field Data Sheet

STREAM NAME:		**SITE NUMBER:**	
SAMPLE NUMBER:	**ASSESSOR:**	**DATE:**	

Habitat Parameter	Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
1. Riparian Vegetative Zone Width (score each bank riparian zone)	• Bankside vegetation buffer is >10m • Continuous and dense	• Bankside vegetation buffer is <10m • Mostly continuous	• Pathways present and/or stock access to stream • Mostly healed over	• Breaks frequent • Human activity obvious
SCORE ___(LB)	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
SCORE ___(RB)	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Mean LB&RB_____				
2. Vegetative Protection (score each bank) Note: determine left or right side by facing downstream.	• Bank surfaces and immediate riparian zones covered by native vegetation • Trees, understorey shrubs, or non-woody plants present • Vegetative disruption minimal	• Bank surfaces covered mainly by native vegetation • Disruption evident • Banks may be covered by exotic forestry	• Bank surfaces covered by a mixture of grasses/shrubs, blackberry, willow and introduced trees • Vegetation disruption obvious • Bare soil/closely cropped vegetation common	• Bank surfaces covered by grasses and shrubs • Disruption of streambank vegetation very high • Grass heavily grazed • Significant stock damage to the bank
SCORE ___(LB)	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
SCORE ___(RB)	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Mean LB&RB_____				
3. Bank Stability (score each bank) Note: determine left of right side by facing downstream	• Banks stable • Erosion/bank failure absent or minimal • <5% of bank affected	• Moderately stable • Infrequent, small areas of erosion mostly healed over • 5-30% of bank eroded	• Moderately unstable • 30-60% of bank in reach has areas of erosion • High erosion potential during floods	• Unstable • Many eroded areas • 60-100% of bank has erosional scars
SCORE ___(LB)	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
SCORE ___(RB)	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Mean LB&RB_____				
4. **Channel sinuousity**	• Bends increase stream length 3-4 times longer than if it was in a straight line	• Bends increase the stream length 2-3 times longer than if it was in a straight line	• Bends increase the stream length 1-2 times longer than if it was in a straight line	• Channel straight
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1

Habitat Parameter	*Category*			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
5. Channel Alteration	• Changes to channel/dredging absent or minimal • Stream with normal pattern	• Some changes to channel/dredging • Evidence of past channel/dredging • Recent channel/dredging not present	• Channel changes/dredging extensive • Embankments or shoring structures present on both banks • 40 to 80% of reach channelised and disrupted	• Banks shored with gabion or cement • >80% of the stream reach channelised and disrupted. • Instream habitat altered or absent
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
6. Sediment Deposition	• Little/no islands or point bars present • <20% of the bottom affected by sediment deposition	• New increase in bar formation, mostly from gravel, sand or fine sediment • 20-50% of the bottom affected; • Slight deposition in pools	• Some deposition of new gravel, sand or fine sediment on old and new bars • 50-80% of the bottom affected • Sediment deposits at obstructions, constrictions, and bends	• Heavy deposits of fine material • Increased bar development • >80% of the bottom changing frequently • pools almost absent due to sediment deposition
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
7. Pool Variability	• Pools evenly mixed • Large/shallow, Large/deep, Small/shallow, Small/deep	• Majority of pools large/deep • Very few shallow pools	• Prevalence shallow pools	• Majority of pools small/shallow
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
8. Abundance and Diversity of Habitat	• >50% substrate favourable for invertebrate colonisation and wide variety of woody debris, riffles, root mats, snags/submerged logs/undercut banks/cobbles. • Substrate provides abundant fish cover • Must be not new and not transient	• 30-50% substrate favourable for invertebrate colonisation • Snags/submerged logs/undercut banks/cobbles • Fish cover common • Moderate variety of habitat types. Can consist of some new material	• 10-30% substrate favourable for invertebrate colonisation • Fish cover patchy • 60-90% substrate easily moved by foot • Woody debris rare or may be smothered by sediment	• <10% substrate favourable for invertebrate colonisation • Fish cover rare or absent • Substrate unstable or lacking • Stable habitats lacking or limited to macrophytes
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
9. Periphyton	• Periphyton not visible on hand held stones • Stable substrate • Surfaces rough to touch	• Periphyton not visible on stones • Stable substrate • Periphyton obvious to touch	• Periphyton visible • <20% cover of available substrate	• Periphyton obvious and prolific • >20% cover of available substrate
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Total Score ____	NB: Use only means of LB and RB values			

Habitat Assessment Field Data Sheet (HAFDS) for hard-bottomed streams. Collier & Kelly 2005.

WADEABLE HARD-BOTTOMED STREAMS – 100 m reach **Qualitative Habitat Assessment Field Data Sheet**				
STREAM NAME:			**SITE NUMBER:**	
SAMPLE NUMBER:	**ASSESSOR:**		**DATE:**	
Habitat Parameter	**Category**			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
1. Riparian Vegetative Zone Width (score each bank riparian zone)	• Bankside vegetation buffer is >10m • Continuous and dense	• Bankside vegetation buffer is <10m • Mostly continuous	• Pathways present and/or stock access to stream • Mostly healed over	• Breaks frequent • Human activity obvious
SCORE ___(LB)	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
SCORE ___(RB)	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Mean LB&RB_____				
2. Vegetative Protection (score each bank) Note: determine left or right side by facing downstream.	• Bank surfaces and immediate riparian zones covered by native vegetation • Trees, understorey shrubs, or non-woody plants present • Vegetative disruption minimal	• Bank surfaces covered mainly by native vegetation • Disruption evident • Banks may be covered by exotic forestry	• Bank surfaces covered by a mixture of grasses/shrubs, blackberry, willow and introduced trees • Vegetation disruption obvious • Bare soil/closely cropped vegetation common	• Bank surfaces covered by grasses and shrubs • Disruption of streambank vegetation very high • Grass heavily grazed • Significant stock damage to the bank
SCORE ___(LB)	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
SCORE ___(RB)	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Mean LB&RB_____				
3. Bank Stability (score each bank) Note: determine left of right side by facing downstream	• Banks stable • Erosion/bank failure absent or minimal • <5% of bank affected	• Moderately stable • Infrequent, small areas of erosion mostly healed over • 5-30% of bank eroded	• Moderately unstable • 30-60% of bank in reach has areas of erosion • High erosion potential during floods	• Unstable • Many eroded areas • 60-100% of bank has erosional scars
SCORE ___(LB)	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
SCORE ___(RB)	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Mean LB&RB_____				
4. **Frequency of Riffles**	• Riffles relatively frequent • Distance between riffles divided by width of stream = 5-7 • Variety of habitat is key	• Occurrence of riffles infrequent • Distance between riffles divided by width of stream = 7-15	• Occassional riffle or run • Bottom contours provide some habitat • Distance between riffles divided by width of stream = 15-25	• Generally flat water, shallow riffles • Poor habitat • Distance between riffles divided by width of stream = >25
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1

Habitat Parameter	*Category*			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
5. Channel Alteration	• Changes to channel/dredging absent or minimal • Stream with normal pattern	• Some changes to channel/dredging • Evidence of past channel/dredging • Recent channel/dredging not present	• Channel changes/dredging extensive • Embankments or shoring structures present on both banks • 40 to 80% of reach channelised and disrupted	• Banks shored with gabion or cement • >80% of the stream reach channelised and disrupted. • Instream habitat altered or absent
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
6. Sediment Deposition (out of channel and in channel)	• Little/no islands or point bars present • <20% of the bottom affected by sediment deposition	• New increase in bar formation, mostly from gravel, sand or fine sediment • 20-50% of the bottom affected; • Slight deposition in pools	• Some deposition of new gravel, sand or fine sediment on old and new bars • 50-80% of the bottom affected • Sediment deposits at obstructions, constrictions, and bends	• Heavy deposits of fine material • Increased bar development • >80% of the bottom changing frequently • pools almost absent due to sediment deposition
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
7. Veloctity/Depth Regimes	• 4 velocity/depth regimes present • slow/deep, slow/shallow, fast/shallow, fast/deep	• 3 of 4 velocity/depth regimes present • If fast/shallow is missing then score lower	• 2 of 4 velocity/depth regimes present • If fast/shallow or slow/shallow are missing score low	• Dominated by 1 velocity/depth regime • Usually slow/deep
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
8. Abundance and Diversity of Habitat	• >50% substrate favourable for invertebrate colonisation and wide variety of woody debris, riffles, root mats, snags/submerged logs/undercut banks/cobbles. • Substrate provides abundant fish cover • Must be not new and not transient	• 30-50% substrate favourable for invertebrate colonisation • Snags/submerged logs/undercut banks/cobbles • Fish cover common • Moderate variety of habitat types. Can consist of some new material	• 10-30% substrate favourable for invertebrate colonisation • Fish cover patchy • 60-90% substrate easily moved by foot • Woody debris rare or may be smothered by sediment	• <10% substrate favourable for invertebrate colonisation • Fish cover rare or absent • Substrate unstable or lacking • Stable habitats lacking o limited to macrophytes
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
9. Periphyton	• Periphyton not visible on hand held stones • Stable substrate • Surfaces rough to touch	• Periphyton not visible on stones • Stable substrate • Periphyton obvious to touch	• Periphyton visible • <20% cover of available substrate	• Periphyton obvious and prolific • >20% cover of available substrate
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Total Score ____	NB: Use only means of LB and RB values			

Rapid Habitat Assessment (RHA). Nationally specific. Clapcott et al. 2015.

Habitat parameter	Condition category										SCORE
1. Deposited sediment	*The percentage of the stream bed covered by fine sediment.*										
	0	*5*	*10*	*15*	*20*	*30*	*40*	*50*	*60*	*≥ 75*	
SCORE	10	9	8	7	6	5	4	3	2	1	
2. Invertebrate habitat diversity	*The number of different substrate types such as boulders, cobbles, gravel, sand, wood, leaves, root mats, macrophytes, periphyton. Presence of interstitial space score higher.*										
	≥ 5	*5*	*5*	*4*	*4*	*3*	*3*	*2*	*2*	*1*	
SCORE	10	9	8	7	6	5	4	3	2	1	
3. Invertebrate habitat abundance	*The percentage of substrate favourable for EPT colonisation, for example flowing water over gravel-cobbles clear of filamentous algae/macrophytes.*										
	95	*75*	*70*	*60*	*50*	*40*	*30*	*25*	*15*	*5*	
SCORE	10	9	8	7	6	5	4	3	2	1	
4. Fish cover diversity	*The number of different substrate types such as woody debris, root mats, undercut banks, overhanging/encroaching vegetation, macrophytes, boulders, cobbles. Presence of substrates providing spatial complexity score higher.*										
	≥ 5	*5*	*5*	*4*	*4*	*3*	*3*	*2*	*2*	*1*	
SCORE	10	9	8	7	6	5	4	3	2	1	
5. Fish cover abundance	*The percentage of fish cover available.*										
	95	*75*	*60*	*50*	*40*	*30*	*20*	*10*	*5*	*0*	
SCORE	10	9	8	7	6	5	4	3	2	1	
6. Hydraulic heterogeneity	*The number of of hydraulic components such as pool, riffle, fast run, slow run, rapid, cascade/waterfall, turbulance, backwater. Presence of deep pools score higher.*										
	≥ 5	*5*	*4*	*4*	*3*	*3*	*2*	*2*	*2*	*1*	
SCORE	10	9	8	7	6	5	4	3	2	1	
7. Bank erosion	*The percentage of the stream bank recently/actively eroding due to scouring at the water line, slumping of the bank or stock pugging.*										
Left bank	*0*	*≤ 5*	*5*	*15*	*25*	*35*	*50*	*65*	*75*	*> 75*	
Right bank	*0*	*≤ 5*	*5*	*15*	*25*	*35*	*50*	*65*	*75*	*> 75*	
SCORE	10	9	8	7	6	5	4	3	2	1	
8. Bank vegetation	*The maturity, diversity and naturalness of bank vegetation.*										
Left bank AND Right bank	*Mature native trees with diverse and intact understorey*		*Regenerating native or flaxes/sedges/tussock > dense exotic*			*Mature shrubs, sparse tree cover > young exotic, long grass*			*Heavily grazed or mown grass > bare/impervious ground.*		
SCORE	10	9	8	7	6	5	4	3	2	1	
9. Riparian width	*The width (m) of the riparian buffer constrained by vegetation, fence or other structure(s).*										
Left bank	*≥ 30*	*15*	*10*	*7*	*5*	*4*	*3*	*2*	*1*	*0*	
Right bank	*≥ 30*	*15*	*10*	*7*	*5*	*4*	*3*	*2*	*1*	*0*	
SCORE	10	9	8	7	6	5	4	3	2	1	
10. Riparian shade	*The percentage of shading of the stream bed throughout the day due to vegetation, banks or other structure(s).*										
	≥ 90	*80*	*70*	*60*	*50*	*40*	*25*	*15*	*10*	*≤ 5*	
SCORE	10	9	8	7	6	5	4	3	2	1	
TOTAL	(Sum of parameters 1-10)										

Appendix 4 – Flow chart of methodology for Protocol 3

(modified from Neijenhuis 2015)

Appendix 5 – Equipment List

Figure 21 - Mussel survey equipment taken from Neijenhuis 2015.

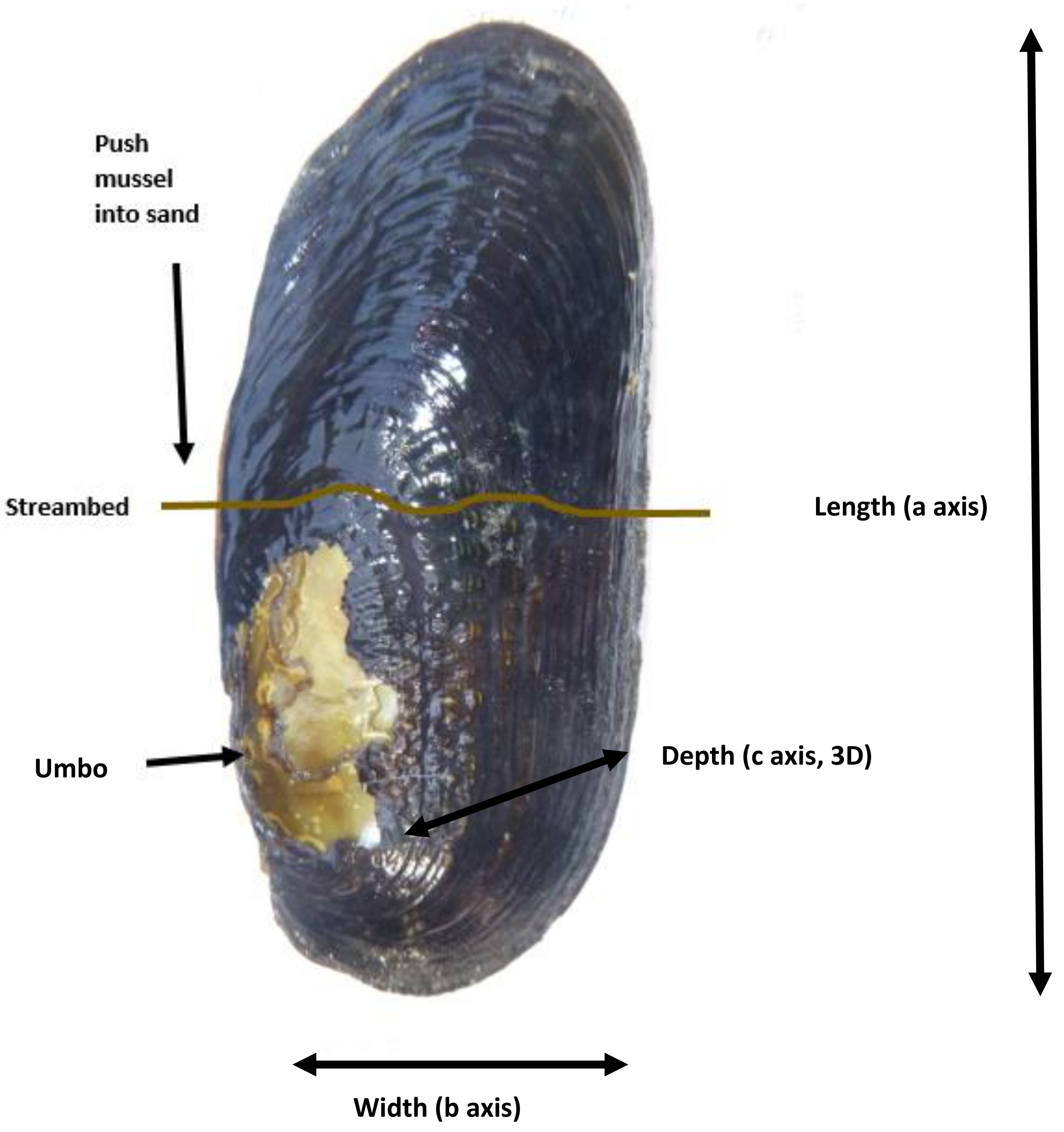
1. Chest waders (a wetsuit and mask/snorkel may be better, especially in summer).
2. Underwater viewer per person[1]; used to search for freshwater mussels and to determine the habitat structure.
3. Measuring tape (50m); used to indicate the 50 meter reach.
4. Measuring tape (30m); used to measure the wetted width of the stream.
5. Depth stick (1m); used to measure the depth of a stream.
6. Small buckets or mussel bag; used to collect mussels.
7. Multi-parameter meter; used for undertaking measurements of dissolved oxygen, conductivity and temperature in a stream (ensure that calibration is undertaken if needed, before use in the field).
8. GPS; used to determine the coordinates of a stream.
9. Densiometer[2]; used to determine the amount of canopy cover at a stream.
10. Vernier callipers; used to measure the size of the mussels.
11. Water bottles; used for taking water samples for analysis.
12. Chilly bin/ice; for keeping the water samples cool until delivery to analytical laboratory.
13. Tablet or other data capture device if available (preferably waterproof).
14. Clipboard; containing the freshwater mussel field survey form, mussel ID pictures, FAF and HAFDS, macrophyte identification form and maps of the area. Printed or downloaded maps are recommended when out of cell service areas.
15. Spray pump filled with detergent mixture, scrubbing brushes, fish bins; used to clean equipment after use in a stream – use away from the stream and drain contents into wastewater system if possible.
16. Camera (waterproof recommended); used for taking pictures of the streams.

Other recommended equipment includes: first aid kit.

N.B.[1] can be purchased from recreational boating suppliers, [2] can be purchased from Forestry suppliers Inc.

Appendix 6 - Returning Mussels to the stream

All mussels have an umbo, or shell origin - it is usually obvious as the shell will be eroded around it. Return mussels into the substrate by gently pushing the umbo end down into the sand/silt to half cover the mussel. It is important to put the correct end downward, because their siphons (used for filter-feeding) are located inside the top of the shell if positioned like the photo), and need to be oriented upward to filter-feed the water column. Mussels in streams need to be embedded so that they can maintain their position in suitable habitat and not become "washed out" when flows increase.



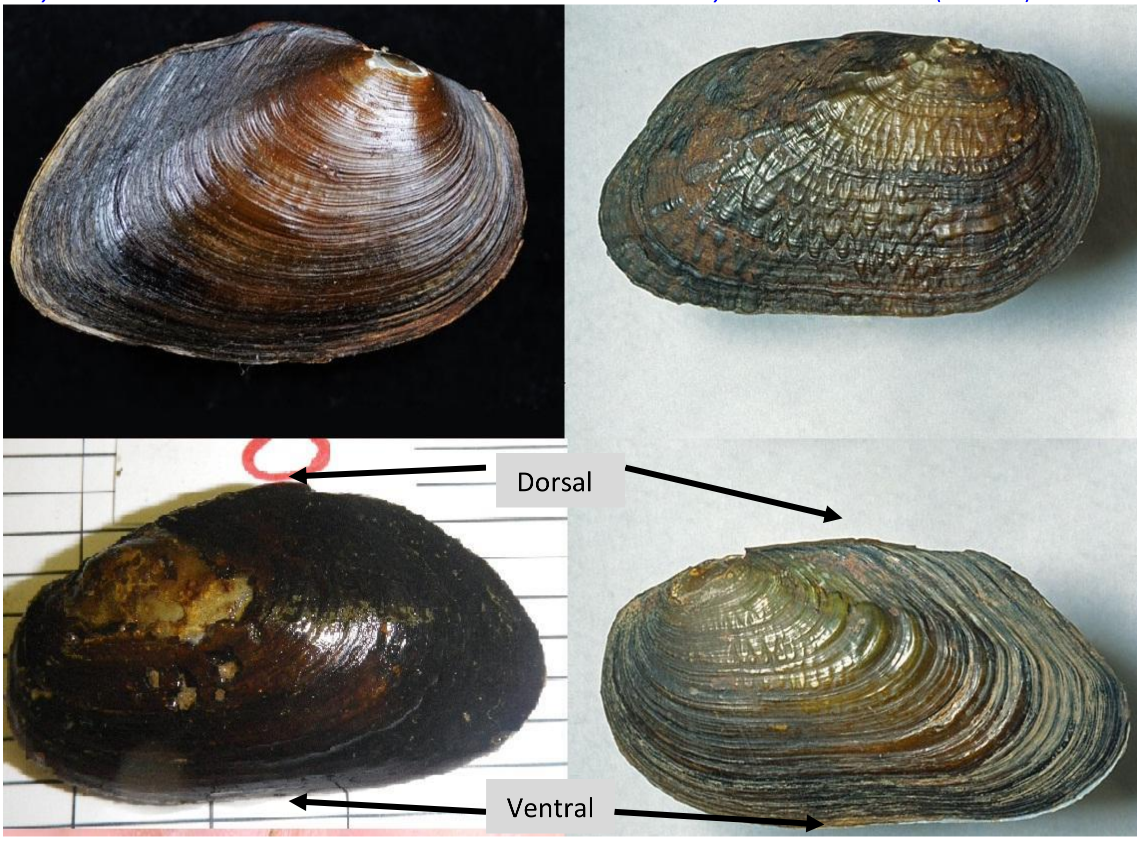
Appendix 7 – Freshwater mussel identification guide

Echyridella menziesii

Echyridella aucklandica (>80mm)

Echyridella menziesii

Echyridella aucklandica (>80mm)



Echyridella menziesii

Echyridella aucklandica (<80mm)
Images from www.mollusc.co.nz

Diagnostic features (see Marshall et al. 2014 for definitive descriptions):

Echyridella menziesii	***Echyridella aucklandica***
Curved outline (usually) to top of shell (i.e., the dorsal edge) – often the shell outline is quite round.	Dorsal and ventral shell margins are parallel. Mussels often appear quite tubular (i.e., long and thin, rather than round).
	Some large mussels can be “bent”, with a concave ventral margin (see top picture).
Growth lines curved up to 80mm	Growth lines up to 80mm are more or less parallel to dorsal and ventral margins
Weak nodules / ridges can be present	Nodules / ridges often obvious on upper (dorsal) part of shell
Maximum length is usually 80 mm, less commonly found 90-100 mm in length,	Generally grows to a larger size than *E. menziesii* and often >90 mm long.
Can be either brown or greenish in colour – colour does not distinguish the species	
Shells of both species can have no erosion or severe erosion and deformation	

Appendix 8 – Suite of analyses to be included in water sample testing for Protocol 3

Parameter	**Units**
Turbidity	NTU
pH	pH units
Total Hardness	g/m^3 as $CaCO_3$
Electrical Conductiviy (EC)	mS/m
Dissolved Calcium	g/m^3
Dissolved Copper	g/m^3
Dissolved Magnesium	g/m^3
Dissolved Zinc	g/m^3
Total Ammoniacal-N	g/m^3
Nitrate-N + Nitrite-N	g/m^3
Total Kjeldahl Nitrogen (TKN)	g/m^3
Dissolved Reactive Phosphorus	g/m^3
Total Phosphorus	g/m^3