Locating lamprey

Guidelines for identification of critical habitats and population monitoring

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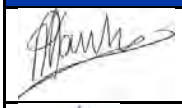	Reviewed by:	Paul Franklin
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Contents

Tables

Figures

Introduction & lamprey ecology

1 Introduction

The pouched lamprey, also known as piharau in the North Island and kanakana in the South Island, can be regarded as a "living fossil", with a lineage dating back around 500 million years (Janvier 2007). Aside from their intrinsic value for native biodiversity, lamprey are a very important mahinga kai for Māori communities. However, populations are in decline and, presently, lamprey are classified as "Threatened - Nationally Vulnerable" in the New Zealand Threat Classification (Dunn et al. 2018). The major threats identified in the decline of lamprey are climate change, land development (including dredging and excavation), pollution, anthropogenic migration barriers, altered flow regimes, reduced water quality, predation, and disease (Maitland et al. 2015, Williams et al. 2017, Clemens et al. 2021; Egan et al. 2020).

Lamprey is one of four species in the Department of Conservation's (DOC) Migratory Fish Programme. The programme seeks to protect and conserve key migratory species that are nationally declining or threatened. Additionally, under the Conservation Act (1987), DOC have specific responsibilities towards freshwater fish populations. In particular:

- Section 6 (ab) requires DOC to preserve so far as is practicable all indigenous freshwater fisheries and protect recreational freshwater fisheries and freshwater fish habitats.
- Under section 26ZJ, it is an offence to disturb or injure the eggs or larvae of any freshwater fish.

Consequently, methods to identify the location of lamprey habitats and monitor temporal trends in their populations will be critical for DOC to ensure management interventions are meeting legislative requirements, improving the security of the species and successfully protecting declining populations. However, lamprey is a cryptic species that is not routinely captured using standard fish survey techniques. Since 2016, NIWA's Ministry of Business Innovation and Employment (MBIE) funded Endeavour research programme (Contract C01X1615) has focused on developing methods to identify the location of critical life-stage habitats of lamprey, particularly spawning habitats. The methods and tools developed can be used to determine the distribution of lamprey within a waterway, locate critical habitats, and monitor populations over time.

To support DOC in meeting their legislative requirements and to protect threatened lamprey populations, NIWA were contracted to provide a guidance manual on how to effectively locate larval and adult lamprey in fresh water. The manual outlines:

- Biology of the species and the general habitats used by both larvae and adult lamprey, as well as what spawning habitat looks like and how adaptable lamprey are.
- Using passive sampling methods to determine presence/absence of lamprey within a catchment and to identify key spawning streams.
- Tagging and tracking options for pinpointing spawning locations once key tributaries are identified.
- A standardised electric fishing method for monitoring larval densities, which can be used as a proxy for inferring key spawning streams.

In addition to DOC's legislative requirements, in September 2020, new national policies took effect; the National Policy Statement for Freshwater Management 2020 (NPS-FM) and the National Environmental Standards for Freshwater (NES-FW). These new policies require every regional council to identify and protect the habitats of threatened species. Therefore, the methods to determine the distribution of lamprey within a waterway and locate critical habitats that are outlined in this manual will also support regional councils in meeting their obligations under the NPS-FM threatened species compulsory value.

This is a living document that was prepared using the best available information at the time of writing. It is envisaged that the guidance document will be updated as techniques and methods evolve and develop.

1.1 Consideration of mātauranga Māori and partnership with iwi/hapū/whānau

Piharau/kanakana are a taonga to many iwi/hapū/whānau, contributing to cultural identity, cohesion and as a vehicle for intergenerational knowledge transfer (e.g., Kitson et al. 2012). The customary importance of this species has been recognised in contemporary legislation resulting from treaty settlements and other customary management mechanisms such as mātaitai (Almeida et al. 2021). For example, the Ngāi Tahu Claims Settlement Act prohibits the targeted commercial harvest of "Kanakana/Ute – southern lamprey (*Geotria australis*)". In Southland, two mātaitai that encompass fresh water (Mataura and Waikawa/Tumu Toka) were put in place over areas of significant kanakana customary fisheries and bylaws for the Mataura River Mātaitai Reserve prohibit the taking of kanakana without customary authorisation from the reserve's tangata tiaki/kaitiaki.

The social and economic wellbeing of Māori has long relied on the sustainable utilisation (including trade), and management of their local natural resources, where fisheries like piharau/kanakana, tuna, kōura, kōaro and kākahi were once key components of local and regional economies and helped to sustain communities with food and resources. Utilisation meant that whānau fishers engaged with their waterways in all weathers, in different seasons, and observed the environment in its many different states. Changing behaviours in response to changing conditions meant that whānau were well versed in the principles and practice of adaptive management. Through intergenerational interaction a wealth of knowledge and experiences that should be used to inform management and restoration has been generated.

Engagement with mana whenua prior to any piharau/kanakana research and/or monitoring (i.e., early in the process) is essential. One of the hardest factors in identifying lamprey spawning habitat is knowing where and when to capture adult lamprey for tracking (see Section 5). The mātauranga of customary kanakana/piharau fishers can provide valuable information, for example, about catchment-specific harvest sites and the conditions and timing of adult entry into freshwater. Concern over the state of the customary fishery has led some mana whenua to examine customary harvest methods as a way to monitor lamprey abundance (e.g., Te Ao Marama Inc and Waikawa Whanau 2010; Kitson et al. 2012).Therefore, alongside mana whenua, customary fishing methods could potentially be reinvigorated as a means of monitoring adult lamprey populations.

A multi-agency group (e.g., iwi representatives, MfE, DOC, NIWA), facilitated by Te Wai Māori Trust (https://waimaori.maori.nz/), have developed a Piharau/Kanakana Restoration Strategy that seeks to support iwi/hapū involvement in the management and restoration of this fishery. This group includes experienced kanakana/piharau fishers and researchers who can also provide expert advice and guidance.

2 Current knowledge on lamprey biology and ecology

New Zealand's pouched lamprey have an anadromous life cycle with three key life stages (Figure 2-1). Larvae, or ammocoetes, begin their life as blind filter feeders in fresh water. Based on growth rates and length-frequency distributions, the larval phase is estimated to take between 3 and 4.5 years (Potter et al. 1986; Potter & Hilliard 1986; Todd & Kelso 1993). After completing metamorphosis (change colour and turn into the adult form), the resultant juveniles, or macrophthalmia, migrate to the ocean and feed parasitically on fish and marine mammals for 3 to 4 years. Between April and October, adult lamprey return to fresh water and travel upstream to their spawning areas. It is thought that adults select spawning streams by detecting the scent (or pheromones) released by larvae resident upstream (NIWA 2013; Johnson et al. 2015). The adults do not feed once in fresh water and they will spend up to 18 months maturing in their stream of choice before they spawn and die. Spawning has been documented to occur in October and November the year following entry to freshwater.

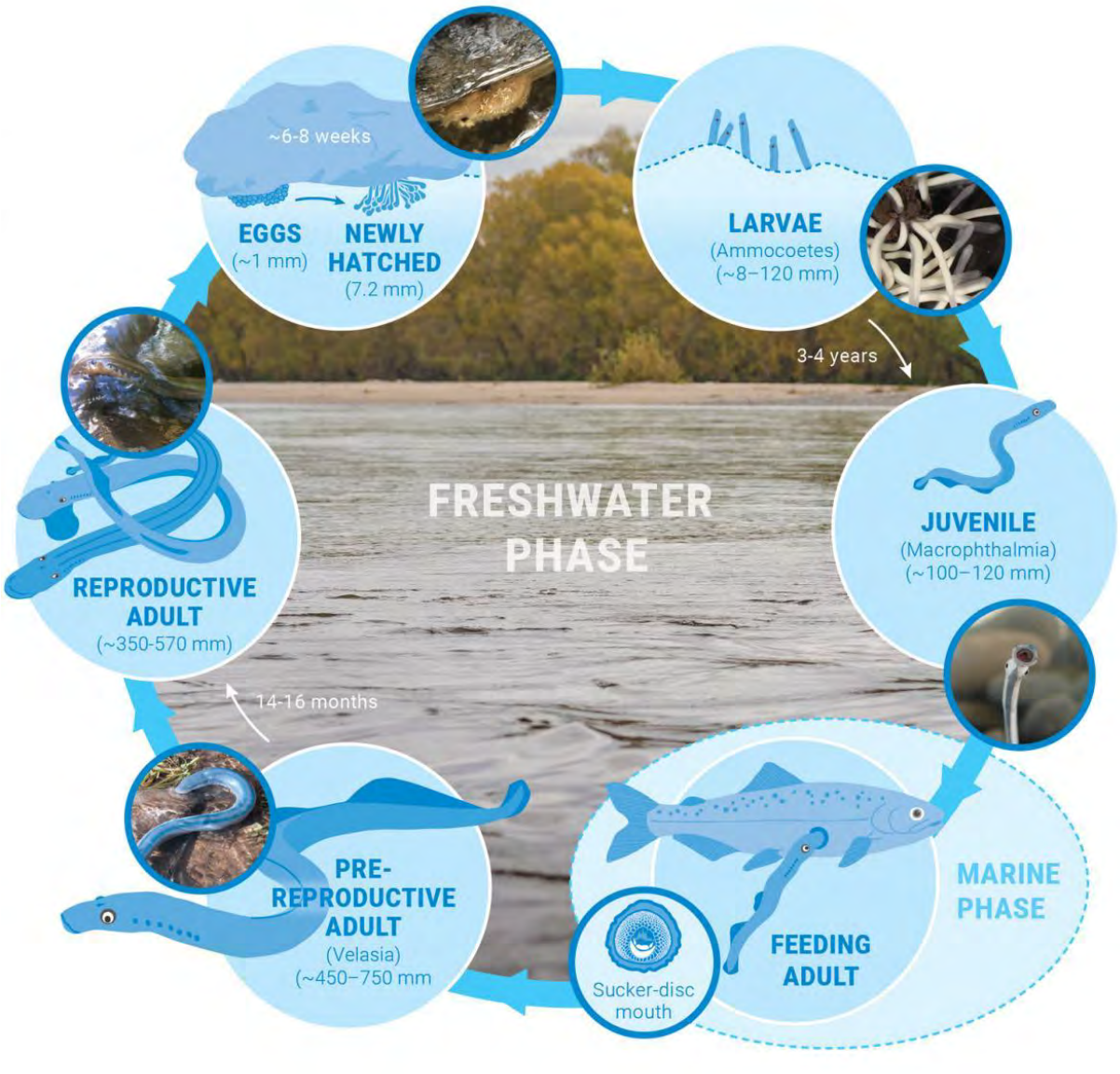

Figure 2-1: Lifecycle of lamprey (piharau/kanakana).

2.1 Habitat used by larval lamprey

Lamprey larvae generally burrow and reside in soft, sandy substrates, with a low water velocity, and some amount of organic detritus (Figure 2-2 & Figure 2-3). Potter et al. (1986) found the highest densities of larval lamprey in medium sand (0.2-0.6 mm in diameter) and Jellyman and Glova (2002) found larval lamprey preferred sediments < 1 mm in diameter. Although the fine sand/silt substrates are preferred by larvae, they will also occur in fine and coarse gravels (Figure 2-4 & Figure 2-5). Habitat suitable for larval lamprey often occurs in eddies, backwaters, pools and along stream margins. Depositional areas downstream of boulders, logs and other obstructions to flow can also provide pockets of sediment that ammocoetes exploit (Figure 2-5).

Figure 2-2: Fine sand habitat preferred by larval lamprey in the lower reaches of the Canal Reserve Drain (Styx River). Inset shows two larvae burrowing into the sediment. Ammocoetes recorded in densities of 54 per m^2.

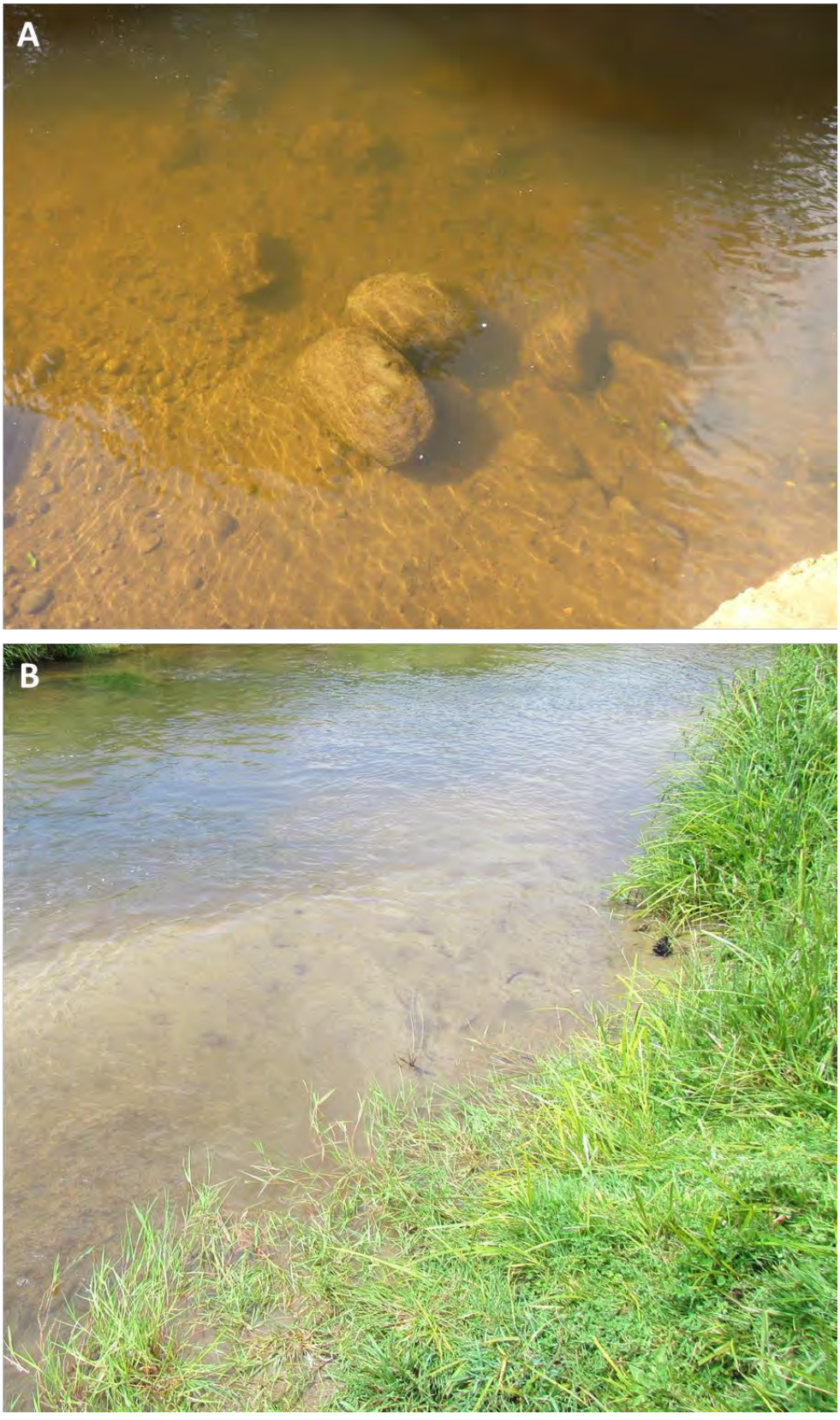

Figure 2-3: Depositional areas of silt (A) and sand (B) preferred by ammocoetes.

Figure 2-4: Ammocoete burrowing into fine (A) and coarse (B) substrates in Kaniwhaniwha Stream after being removed from the area during electric fishing. The blue arrows indicate the ammocoete in each picture.

Figure 2-5: Larval lamprey habitat in Kaniwhaniwha Stream (indicated by the red ellipses).

The habitats selected by larval lamprey are more commonly associated with low water velocities (<0.1 m s^{-1}; Jellyman & Glova 2002), but the range of water depths utilised are not well understood. All surveys of lamprey habitat use have been carried out using backpack electric fishing in shallow wadeable water less than one meter deep. In contrast, using deep water electric fishing, Pacific lamprey (*Entosphenus tridentatus*) and *Lampetra* species have been located at depths up to 16 m (Jolley et al. 2012; Silver et al. 2008). Consequently, the range of water depths larval lamprey have been found at (up to 0.6 m; Jellyman & Glova 2002) may be biased by limitations in sampling rather than reflecting the actual depth range utilised, especially in large rivers.

2.2 Migration cues utilised by adult lamprey

The upstream spawning migration of anadromous lampreys is known to be triggered by flow variations and water temperatures (see review by Moser et al. 2015). For lamprey, data indicates a key migration cue is increased flow (Jellyman et al. 2002; Kitson et al. 2012). Between July and December 2012, migratory adult lamprey in the Okuti River and Kinloch Stream were tagged with Passive Integrated Transponders (PIT) and their migrations monitored using fixed PIT antennae. Most migrations occurred on the receding limb of flood or higher flow events, but no statistical relationship could be determined with respect to the magnitude or duration of the flood events (Figure 2-6).

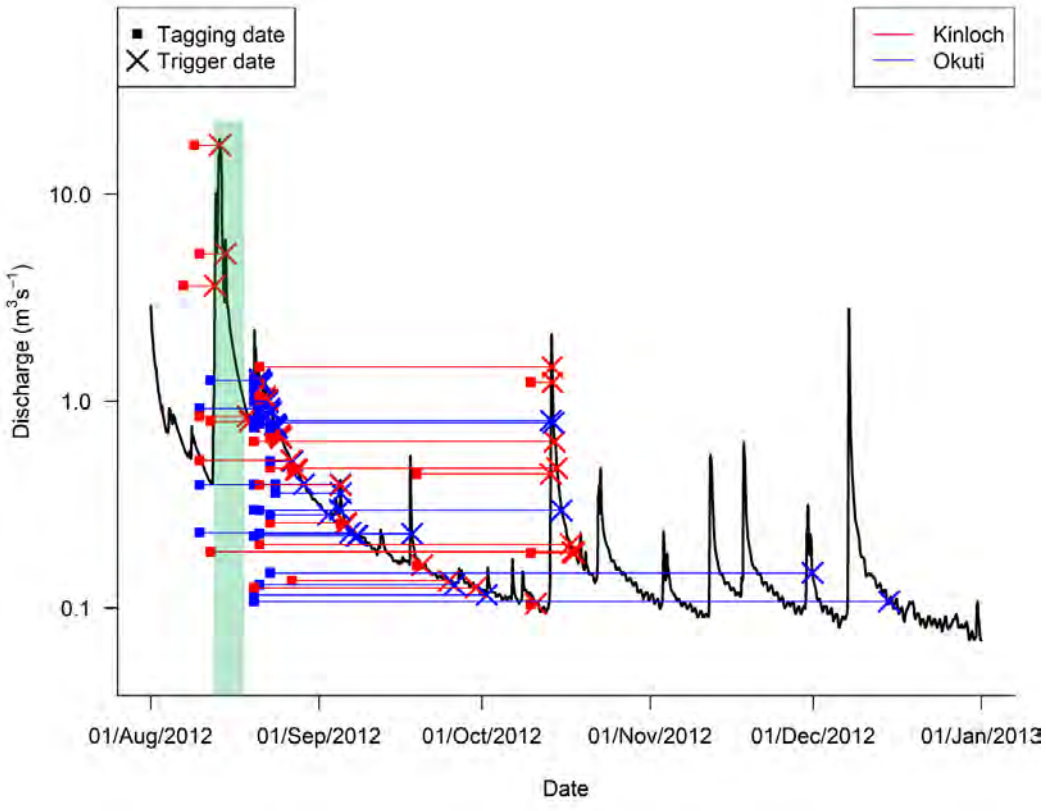

Figure 2-6: Discharge in the Okana River between July and December 2012, superimposed with lamprey movements. No flow data were available for the Okuti River and Kinloch Stream so flow records from the Okana River, a major tributary in the Okuti River catchment in close proximity to the lamprey capture and release sites, was used as a proxy. The date each lamprey was tagged and released in the Okuti River and Kinloch Stream is indicated by blue and red points, respectively, with the date each lamprey was detected on one of the fixed PIT antennae indicated by the cross of the same colour. The green shaded region indicates when the fixed antenna in the Okuti River was damaged by the large flood and was not functional.

In the Okuti and Mataura rivers, Jellyman et al. (2002) found upstream movement of adult lamprey was stimulated by increased flow only up to a point, with large floods decreasing migratory activity. Recent investigations on the timing of lamprey migrations are in contrast to the findings of Jellyman et al. (2002), indicating flood flows of all sizes stimulated upstream migration in the Okuti and Waikawa river catchments (NIWA unpublished data; Figure 2-6). In the Waikawa River, Southland, a large migration run of adult lamprey was recorded on a flood peaking at 106.7 $m^3 s^{-1}$, which is the third highest flood on record causing the river to rise 2.4 m above normal (equates to a 20% AEP (Annual Exceedance Probability)).

The diel timing of migrations by PIT tagged adult lamprey in the Okuti River catchment was also investigated. After tagging, 54% of lamprey migrated upriver within two days, while others did not continue migrating until 50 – 120 days post-tagging. The tagged lamprey mostly migrated at night (70%) with peak movements between the hours of 9 pm and 2 am (Figure 2-7). Some movements (30%) occurred during daylight hours, which was consistent with visual observations of untagged migratory lamprey during daytime surveys.

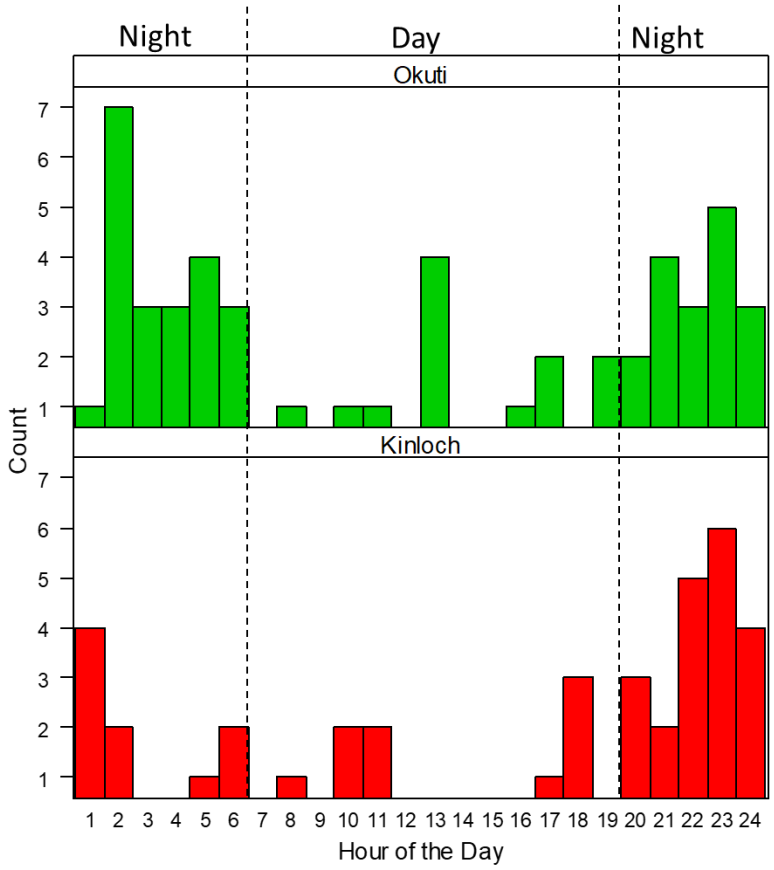

Figure 2-7: Diel timing of PIT tagged lamprey moving past instream antennae in the Okuti River and Kinloch Stream. Hour of the day is represented by the 24-hour clock, i.e. 1 = 1 am and 24 = midnight.

The scent (or pheromones) released by upstream resident lamprey are thought to drive stream or river selection in migratory lampreys (see review by Johnson et al. 2015). It has been well established that Northern Hemisphere adult lampreys are attracted to migratory pheromones released by larvae (Fine et al. 2004; Sorensen and Hoye 2007) and to mating pheromones released by adults (Johnson et al. 2015; Moser et al. 2015).

However, our understanding of the role of pheromones as a migratory and mating cue for pouched lamprey is still limited. Buchinger et al. (2017) found New Zealand post-spawning male pouched lamprey did not release high volumes of 3-keto petromyzonol sulphate (3kPS), the mating pheromone identified in sea lamprey (*Petromyzon marinus*). However, larval pouched lamprey in New Zealand have been shown to release petromyzonol sulphate (PS), petromyzonamine disulfate (PADS), allocholic acid, 3-keto allocholic acid and 3kPS (Baker et al. 2009; Stewart & Baker 2012; author's unpublished data), which are key components of the sea lamprey migration pheromone. In addition, using a choice chamber NIWA (2013) found adult migratory pouched lamprey showed a preference towards the odour of their own larvae as well as towards a mixture of seven sea lamprey pheromones.

Monitoring of stream selection by PIT tagged adult lamprey in conjunction with deploying NIWA's lamprey Polar Organic Chemical Integrated Samplers (POCIS) that detect the larval pheromone PS, has shown that the proportion of adults selecting a stream correlates with the strength of the larval odours (Figure 2-8). Across two seasons in the Waikawa River catchment around 70% of PIT tagged adult lamprey chose the stream branch with the highest pheromone cue (Figure 2-8). A similar result was found in the Okuti River catchment in 2012, when 74% of PIT tagged lamprey continued migrating up the Okuti River after release, and 26% choosing to enter Kinloch Stream. Where Kinloch Stream feeds into the Okuti River, the concentration of PS was 3.6 times higher in the mainstem of the Okuti River than Kinloch Stream. Although the pheromone samplers only detect PS, and adult lamprey are undoubtedly responding to a bouquet of odours, the field studies indicate that the concentration of PS is a good proxy for predicting stream choice by migratory adult lamprey.

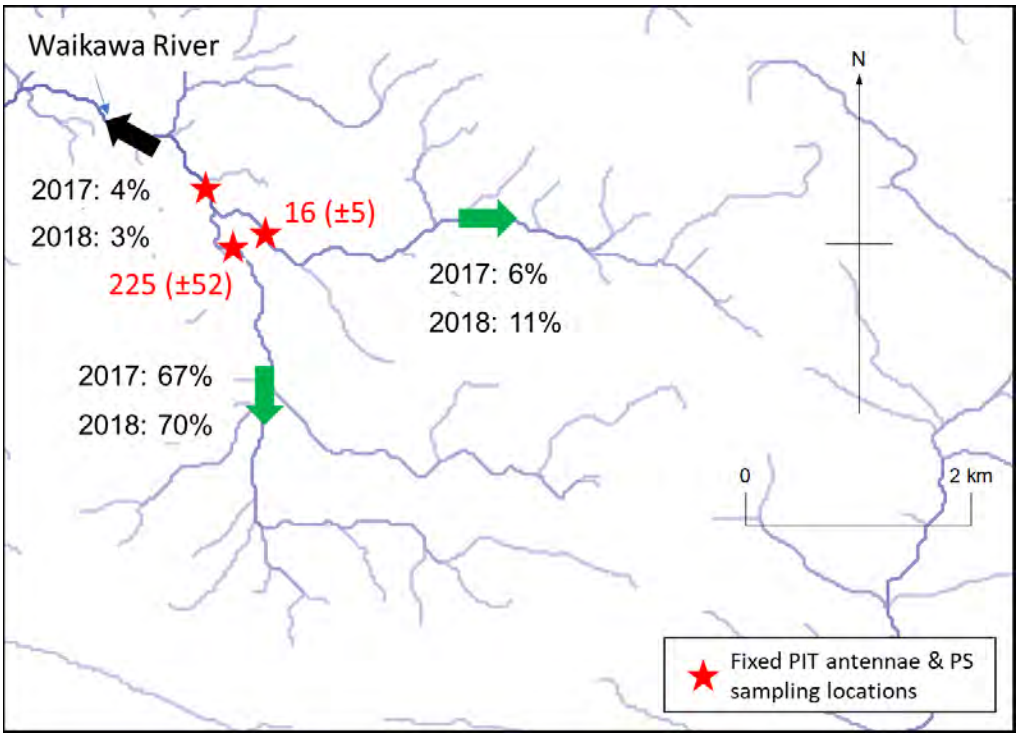

Figure 2-8: Percentage of PIT tagged adult lamprey selecting each stream branch, or moving downstream (black arrow) in the east branch of the Waikawa River. In 2017, 191 lamprey were releases and in 2018, 239 lamprey were released. Numbers in red indicate the mean femtomolar concentrations (10^{-15} Molar) of Petromyzonol sulphate (PS; ±1 standard error in brackets).

2.3 Maturation and spawning habitat used by adult lamprey

In contrast to Northern Hemisphere lampreys, pouched lamprey spawn exclusively in concealed cavities. Adult lamprey form spawning pairs in cavities that minimise predation, with good water flow, and that have hard surfaces for egg laying. Presently, mature and post-spawning lamprey have been located in three different stream types. Although the microhabitat adult lamprey are choosing for spawning is consistent between streams (i.e., cavities with good flow and hard surfaces), the structure or substrate utilised varies with what is available in each stream type.

In Kinloch Stream, Banks Peninsula, lamprey pairs spawned beneath boulders located in backwaters adjacent to riffle habitat, and amidst boulder clusters within shallow riffles (Baker et al. 2017a; Figure 2-9). The boulders chosen for spawning beneath were on average 0.70 x 0.49 m (l x w), whereas during their 12-14 month maturation period prior to spawning, adult lamprey utilised smaller boulders for cover (average size 0.44 x 0.32 m; l x w). The boulders were not always visibly breaking the water surface in the way the spawning boulder marked with an X in Figure 2-9 does. Instead, they could be buried beneath smaller cobbles, in areas that visually did not appear to contain spawning boulders (Figure 2-10).

Figure 2-9: X marks the spot! Typical spawning habitat utilised by adult lamprey in Kinloch Stream, Banks Peninsula, with a nest located under the boulder marked with an "X" in the foreground.

Figure 2-10: Habitat where lamprey nests were located beneath hidden boulders. A, stick points to the edge of a buried boulder that extends well under the visible cobbles and gravels. B) filming a nest beneath a hidden boulder.

In the Waikawa River, Southland, lamprey pairs were located underneath bedrock slabs or in cavities within the consolidated clay lining of the stream banks (Figure 2-11). Where smaller substrates were dominant in the river, nests were often located in cavities formed within the stream banks (Figure 2-12). Again, spawning habitats selected by lamprey could not be visually identified and without tagged adult lamprey, locating nests was considered near impossible (Figure 2-13).

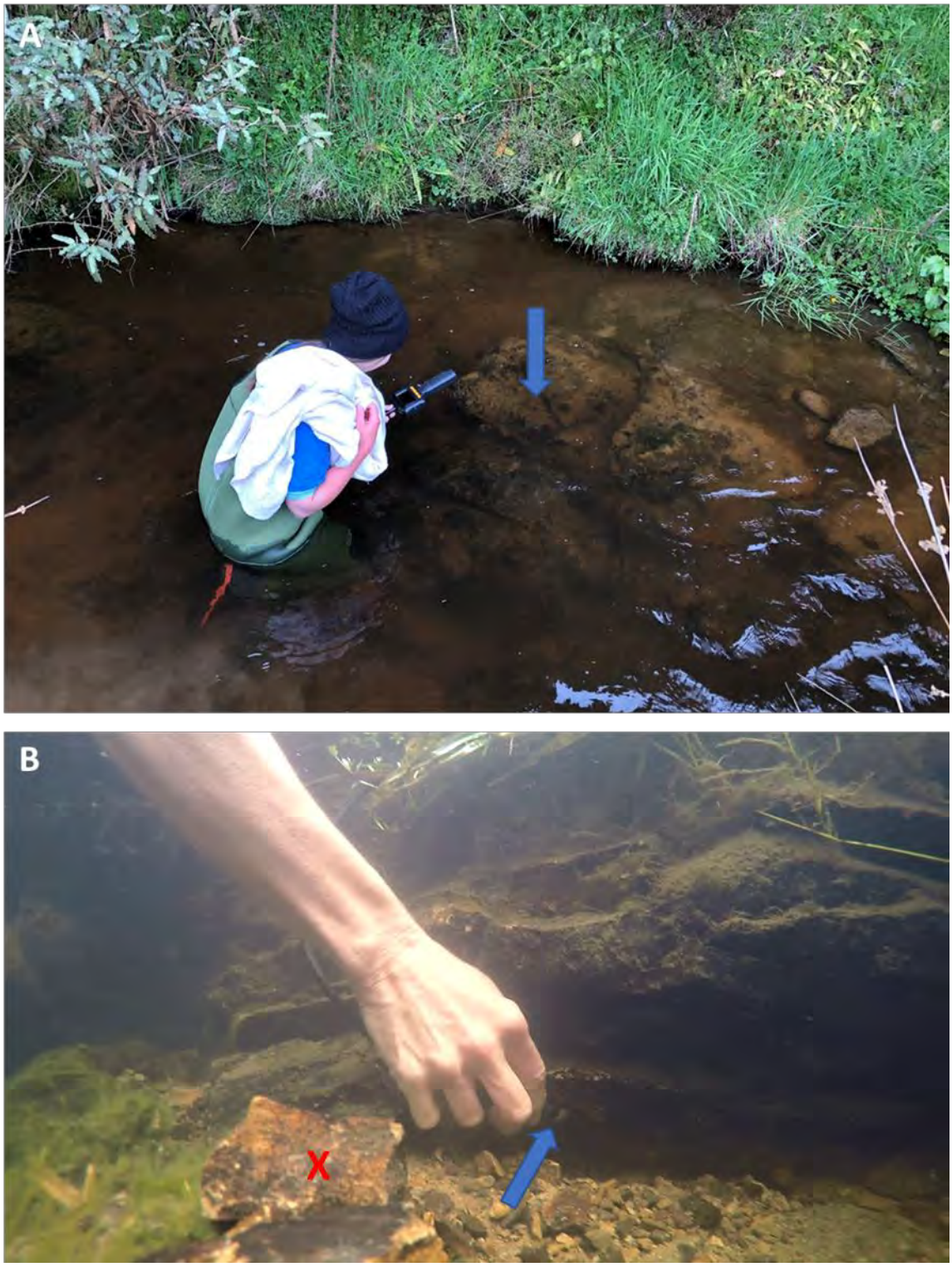

Figure 2-11: Typical spawning habitat utilised by adult lamprey in the Waikawa River, Southland, New Zealand A, a nest located under a bedrock slab (depicted by blue arrow); B, inserting an endoscope camera into a lamprey nest located under the consolidated clay lining the river bank (blue arrow indicated entrance to the nest). The red X marks a piece of consolidated clay that was broken by hand from the stream bank to enable camera entry to the nest. Breaking into nest sites is often necessary as openings are difficult to find.

Figure 2-12: Viewing a lamprey nest situated within a cavity at the base of the stream bank.

Figure 2-13: Further examples of spawning habitat in the Waikawa River, Southland. A, small headwater tributary subjected to harvesting for forestry with a nest located in the stream bank. B, a deep, swift run with a nest underneath a consolidated clay slab, which formed the substrate over most of the run.

In the Canal Reserve Drain (CRD), a highly modified box drain feeding into the Styx River, Christchurch, large substrates and natural stream banks are completely absent. However, electric fishing survey data indicates that lamprey are successfully and consistently spawning within the drain. To determine the habitat utilised during maturation and spawning within the CRD, NIWA radio tagged 10 adult lamprey in August and September 2018 and monitored their movements until the tag batteries died in January 2019.

Results to date have shown that adult lamprey are maturing in cavities behind the wooden boards lining the drain (Figure 2-14). These cavities tend to be associated with small tributaries, or seeps entering the drain (Figure 2-15) and both immature adults and mature adult lamprey have been located inside the cavities (Figure 2-15). Presently, no eggs have been located to confirm spawning has taken place but the hidden cavities provide a solid surface with continuous water flow that adult lamprey seek and utilise for spawning. Of interest is the behaviour observed in untagged adult lamprey moving upstream within the drain. Untagged lamprey were observed to undertake a searching behaviour when reaching a tributary or seep entering the drain through small openings in the boards. The lamprey would move slowly upstream and then back downstream, frequently turning and facing the boards. Given all lamprey species are known to use pheromones to select spawning streams, mates and spawning locations, it's possible that the observed lamprey were detecting the scent of conspecifics in the seep and were searching for the entrance.

Figure 2-14: Searching for tagged lamprey at a small inflow within the Canal Reserve Drain.

Overall, the spawning surveys carried out under NIWA's MBIE research programme highlight the difficulty in visually predicting and locating lamprey spawning habitat. Lamprey appear adaptable in the spawning habitat utilised, however, the concealed cavities are well hidden and for complex rivers such as the Waikawa River, Southland, pinpointing spawning locations would be near impossible without tracking tagged lamprey.

Figure 2-15: Cavities located behind the timber lining (A) and mature male lamprey (B) captured within the cavities during November 2019 in Canal Reserve Drain.

2.4 Egg development and hatching

Within the nest, eggs were spawned as a coagulated cluster that adhered both to each other and to the underside of the boulder (Figure 2-16). The fecundity has only been calculated from one female, which was 56,100 eggs with a mean egg diameter of 1.18 mm (SE = 0.02 mm; Baker et al. 2017). After spawning, larval development took approximately 5 – 7 weeks before hatching occurred. Variations in water temperature between sites and years are thought to be the main driver of observed differences in embryonic development time of larvae (Johnson et al. 2015).

Baker et al. (2017) found that post-hatched larvae formed clusters within the nest, adhering to the subsurface of the boulders and were not displaced even when disturbed by adult movements (Figure 2-17). Baker et al. (2017) observed the caudal (tail) region of each larva was adhesive and the larvae would readily attach to debris or any solid surface they were exposed to in the field and the laboratory (Figure 2-18). After hatching, larvae remained within the nests for approximately two weeks before leaving the nest to burrow into Type I habitats within streams (see Appendix E for habitat classifications). Retention in the nest at the larval stage is not seen in Northern Hemisphere Petromyzontid lampreys (Johnson et al. 2015) and appears unique to pouched lamprey.

From laboratory observations, at 15 days post-hatching (the timeframe when larvae observed in-situ were leaving the nests), the mean larval size was 7.2 mm (±0.30 mm S.E.; Baker et al. 2017). Rearing larval lamprey in the laboratory under a constant temperature of 18°C, found that at two months post-hatching, larvae were approximately 15 mm in length (Figure 2-19). At this stage, the larvae were still translucent and had not fully pigmented into their brown ammocoete colouring. Presently, data indicates that these <15 mm larvae do not drift far from the nest before recruiting into suitable rearing habitat. As such, recording these translucent larvae during electric fishing surveys indicates the spawning site will be in close proximity.

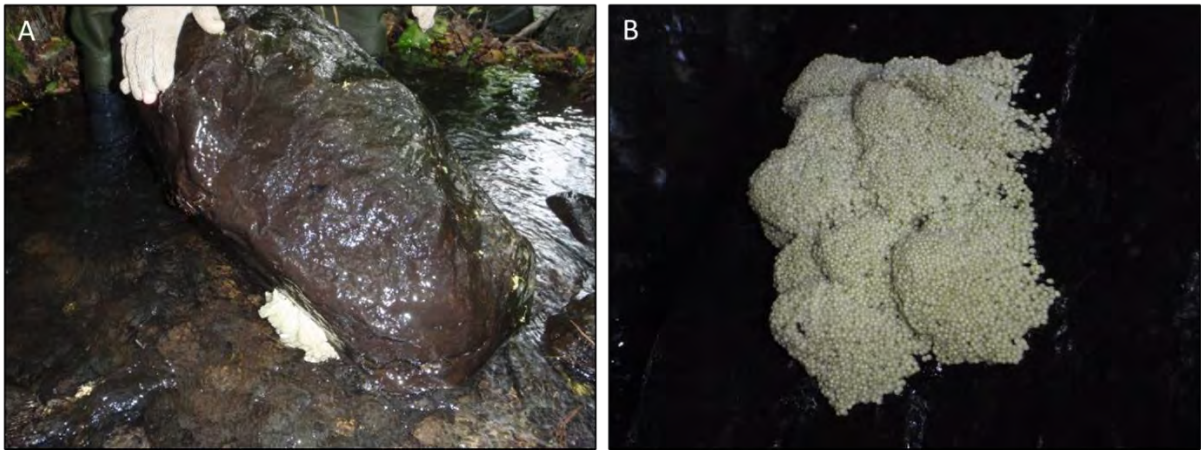

Figure 2-16: Nest located underneath a boulder in Kinloch Stream, Banks Peninsula. A, showing eggs attached to the undersurface of the boulder. B, coagulated mass of lamprey eggs.

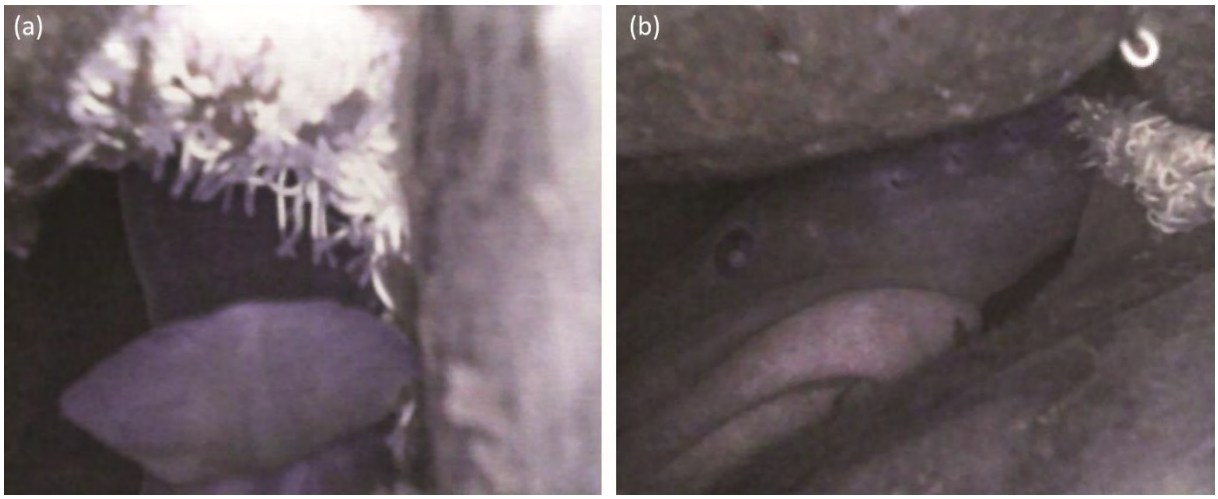

Figure 2-17: Newly hatched lamprey larvae. (a) clustered between boulders 10 days post-hatching and (b) still attached to boulders 2 weeks post-hatching, adjacent to the male lamprey, whose folded gular pouch is evident. Reproduced from Baker et al. (2017).

Figure 2-18: Cluster of lamprey larvae 10 days post-hatching showing the caudal region of larvae adhering to debris. Reproduced from Baker et al. (2017).

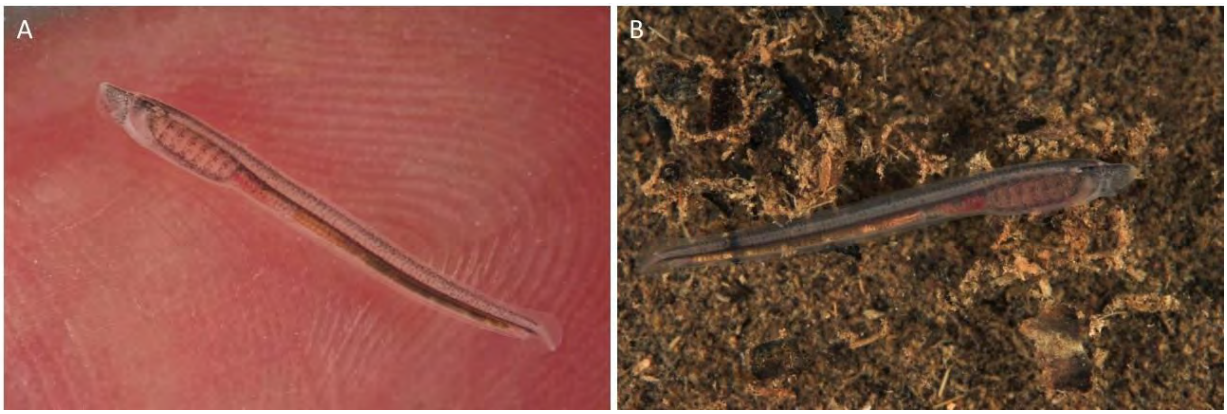

Figure 2-19: lamprey larva approximately 2 months post hatching. A, on a fingertip. B, against natural substrate. Larva is approximately 15 mm in length. Photographs courtesy of Rod Morris.

Methods to locate lamprey habitats

3 Methods to locate critical freshwater habitats utilised by lamprey

Management of freshwater lamprey populations requires knowledge of the critical life-stage habitats utilised by adult and larval lamprey. Several methods are available to identify adult spawning habitat and larval rearing habitats, which enable:

- The spatial distribution of lamprey within a waterbody to be identified.
- The identification of key spawning streams used by lamprey within a catchment.
- Temporal trends in abundance to be monitored.
- The response of lamprey populations to management interventions to be measured.

The methodologies employed will be contingent upon management objectives and habitat types present. Figure 3-1 can assist in determining the appropriate approach to achieve specific goals for each catchment.

Regardless of the objective, the first step is to determine the spatial distribution of lamprey within the catchment of interest. Here, passive tools will provide the most cost-effective approach (see Section 4). An alternative to using passive sampling to locate key streams utilised for spawning and rearing by lamprey is to carry out spot electric fishing using the standardised lamprey method (see Section 6). It is important to remember that passive tools will indicate where the majority of lamprey are in a catchment. Data to date indicate that electric fishing Type I habitats is a more sensitive method of locating low density lamprey populations than using passive tools.

If pinpointing precise locations of lamprey spawning nests within the catchment is a priority, tracking of adult lamprey will be necessary (see Section 5). The key areas for tracking will be delineated by the passive sampling or electric fishing results. Where objectives are to identify and protect key spawning areas within a catchment, pinpointing the precise location of lamprey spawning nests is not always necessary. Instead, standardised assessments of larval density is the recommended approach (see Section 6).

Using larval densities as a proxy to identify and protect lamprey spawning areas is recommended for three reasons:

1. Larval lamprey are present in the stream year-round and are easily monitored with targeted electric fishing surveys, whereas catching and tracking adult lamprey is more logistically challenging, labour intensive and requires specialist expertise.
2. Larval lamprey recruit into streams close to spawning areas and as they develop, they progressively move downstream closer to the coast (Kelso & Todd 1993). Consequently, for a given stream, the upper limit of adult lamprey spawning can be predicted by the upper limit of larvae.
3. Monitoring to date has found lamprey spawning sites have been spread across the stream length investigated and did not display any spatial clustering. Consequently, the distribution of larvae in spawning streams will approximate the general distribution of spawning areas.

In this regard, the distribution and density of larvae within a stream will provide a more cost-effective means for inferring important lamprey spawning areas within streams compared to pinpointing nest locations. Monitoring larval growth through time, particularly the size structure of populations can also be a useful indicator of spawning and recruitment success. Such information can

be used to predict occupancy and help inform and update the conservation status of lamprey in different regions.

Detailed guidance on each method is provided in Sections 4 – 6 of this document.

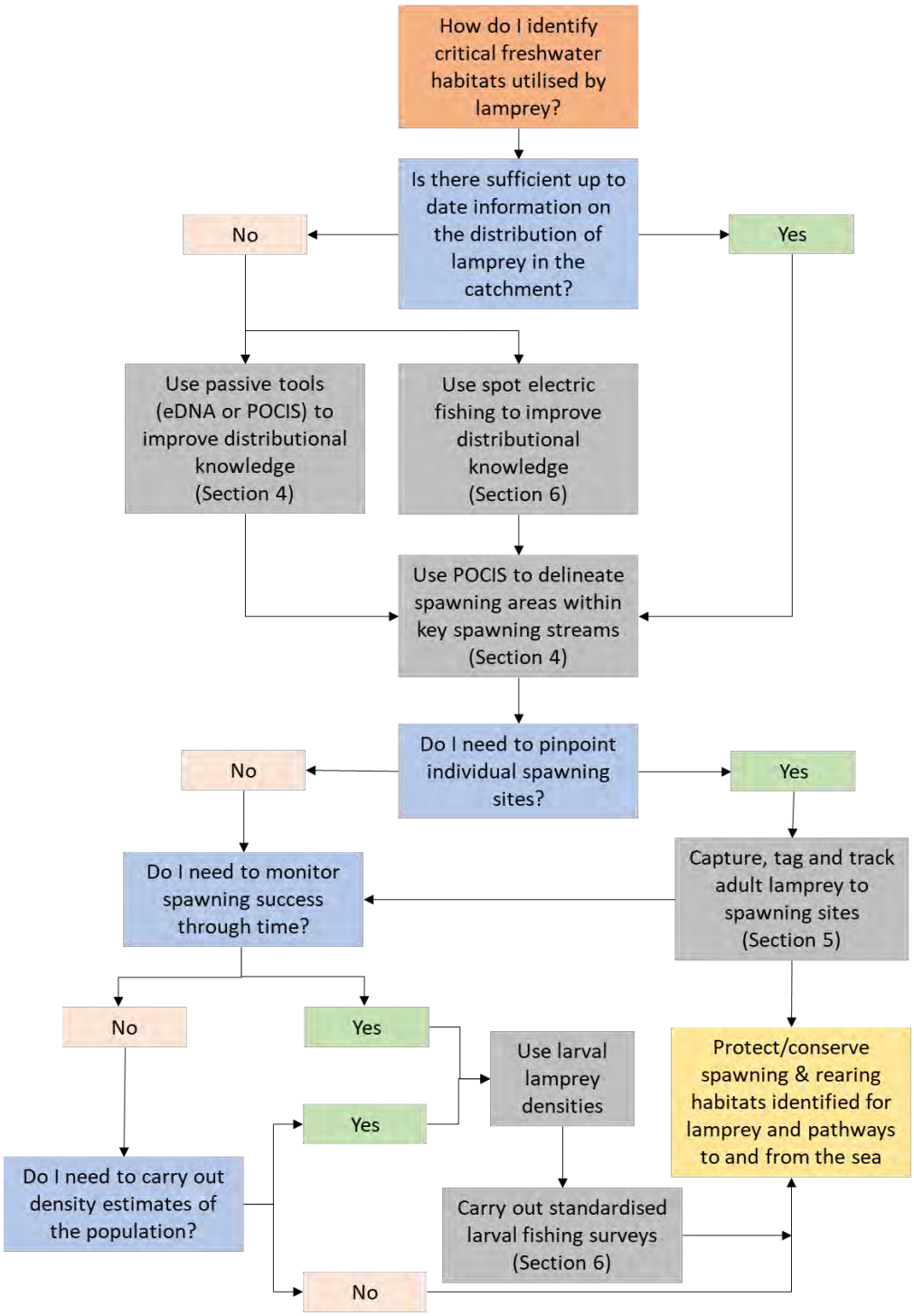

Figure 3-1: Flowchart to guide methodology selection contingent upon management objectives.

Passive sampling

4 Utilising passive sampling to locate key streams used by lamprey

A passive sampling tool is one where biota are not physically captured or observed, but instead their presence is detected by sampling stream water for a specific analyte released only by the species of interest. The development of passive sampling tools enables us to examine the distribution of species within a catchment quickly and cost-effectively. Presently, two passive tools are available for detecting lamprey presence, environmental DNA sampling (eDNA) and lamprey Polar Organic Chemical Integrative Samplers (POCIS).

It is important to remember that a negative result from either eDNA passive samplers or POCIS does not necessarily mean lamprey are absent in the waterway. Instead, lamprey may still be present in the stream or river surveyed but were below the detection limit of the sampling method. Consequently, both eDNA and POCIS can provide false negatives, but both methods generally indicate where the **majority** of lamprey are in a catchment. In this regard, spot electric fishing (see Section 6.3) is also a valid approach to identifying the spatial distribution of lamprey in a catchment.

4.1 An overview of POCIS and eDNA passive sampling techniques

POCIS samplers developed by NIWA absorb the lamprey pheromone petromyzonol sulphate (PS) (Stewart et al. 2011; Stewart & Baker 2012), which is thought to form one of the pheromone cues used by adult lamprey to select spawning streams during their upriver migrations. PS is primarily released by larval lamprey as a metabolic by-product during feeding (Baker et al. 2009), with adult lamprey releasing negligible quantities (author's unpublished data). As POCIS accumulate PS over time, they can be used to provide a crude estimate of larval lamprey abundance upstream of the sampling point. In addition, because adult lamprey are thought to select spawning streams based on the presence and abundance of larval lamprey, the identification of streams containing high densities of larvae can be used to infer the importance of these areas for adult spawning.

Outside of POCIS sampling, eDNA is the only other field-based water sampling tool developed for detecting the presence of lamprey, although eDNA can also detect a wide range of other freshwater fish, invertebrate and plant species. POCIS sampling examines organic compounds released by feeding lamprey larvae, whereas eDNA focuses on examining water samples for one or a few gene sequences that can be used to identify aquatic species present in the waterway. As such, eDNA detects both live and dead animals of all life stages.

There are currently two eDNA sampling methods available in New Zealand, a syringe kit that samples discrete volumes of water, and eDNA passive samplers that are left in-situ overnight. Field testing of the species detection efficacy between active syringe sampling and passive samplers indicated no clear advantage to using passive sampling for cryptic species such as lamprey (Melchior & Baker 2023). Consequently, either active or passive eDNA sampling can be undertaken. Based on validation trials of eDNA, six sample replicates should be undertaken at each site regardless of whether passive or active sampling is carried out (Melchior & Baker 2023). This should detect around 90% of fish species present in the vicinity of sampling.

It is important to remember that both POCIS and eDNA sampling have limitations. For any analytical method to detect fish species from water samples, a threshold concentration of the analyte is needed. The concentration of PS and DNA in the water is not only contingent upon the presence of lamprey, but is also dependent upon a wide variety of factors including water temperature, river flow, water velocities within the waterbody, release rate of the biological material being detected,

and the nature of the organism (i.e., is it buried in substrate or swimming actively in the waterway). As both larval and adult lamprey are cryptic and bury themselves in substrates, they will need to be present in higher abundances for clear detection from pheromones compared to fish swimming freely in the water column. Similarly, the shedding rate of DNA from pelagic fish compared to a burrowing species like lamprey is likely to be different but has not been quantified.

The advantages and disadvantages of each passive sampling method are outlined in Table 4-1.

Table 4-1: The advantages and disadvantages of using eDNA and POCIS sampling to detect lamprey presence in a waterway . Abbreviations: PS, petromyzonol sulphate.

eDNA		POCIS samplers	
Advantages	**Disadvantages**	**Advantages**	**Disadvantages**
Easy to use with no lengthy deployment period (syringes - instant, passive samplers - overnight)	Six replicates at each site	Easy to use with one sampler at each site usually effective	Longer deployment period (2-3 weeks) means floods and fouling of the sampler can reduce sampling efficiency and result in a lower signal detected
Highly selective and sensitive, detects all life stages	Detects dead animals as well as live (e.g., cormorant regurgitates, or eel faeces can result in positive hits for streams with no lamprey)	Only detects living animals and, therefore, a positive signal indicates a feeding population of lamprey	Potentially higher detection threshold than eDNA as predominantly detecting the larval life stage
	Can't currently quantify the abundance of lamprey and detections haven't been correlated with adult spawning stream selection	The relative concentration of the pheromone PS has been correlated with stream selection by adult lamprey	

Based on the current information returned by passive sampling methods, eDNA sampling is recommended for use as a broad screening tool as it is not selective to lamprey life-stage and results have not been validated to adult stream choice. As the abundance of PS has been shown to correlate with stream selection by adult lamprey (see Figure 2-8), POCIS can provide an indication of live larval presence, and is recommended for use in inferring key spawning streams within a catchment. Because of the spatial and temporal variability in PS, and limitations of the technique, the relative abundance estimates should never be used to compare POCIS data between catchments or POCIS data in the same catchment over a different time period. An exception to this, however, is examining temporal variability at the same site within a catchment.

4.2 Recommended passive sampling strategy for lamprey

How eDNA and POCIS are utilised will largely be determined by budget and objectives. Figure 4-1 outlines the recommended approach for using passive tools as a first step in identifying key spawning streams used by lamprey in a catchment.

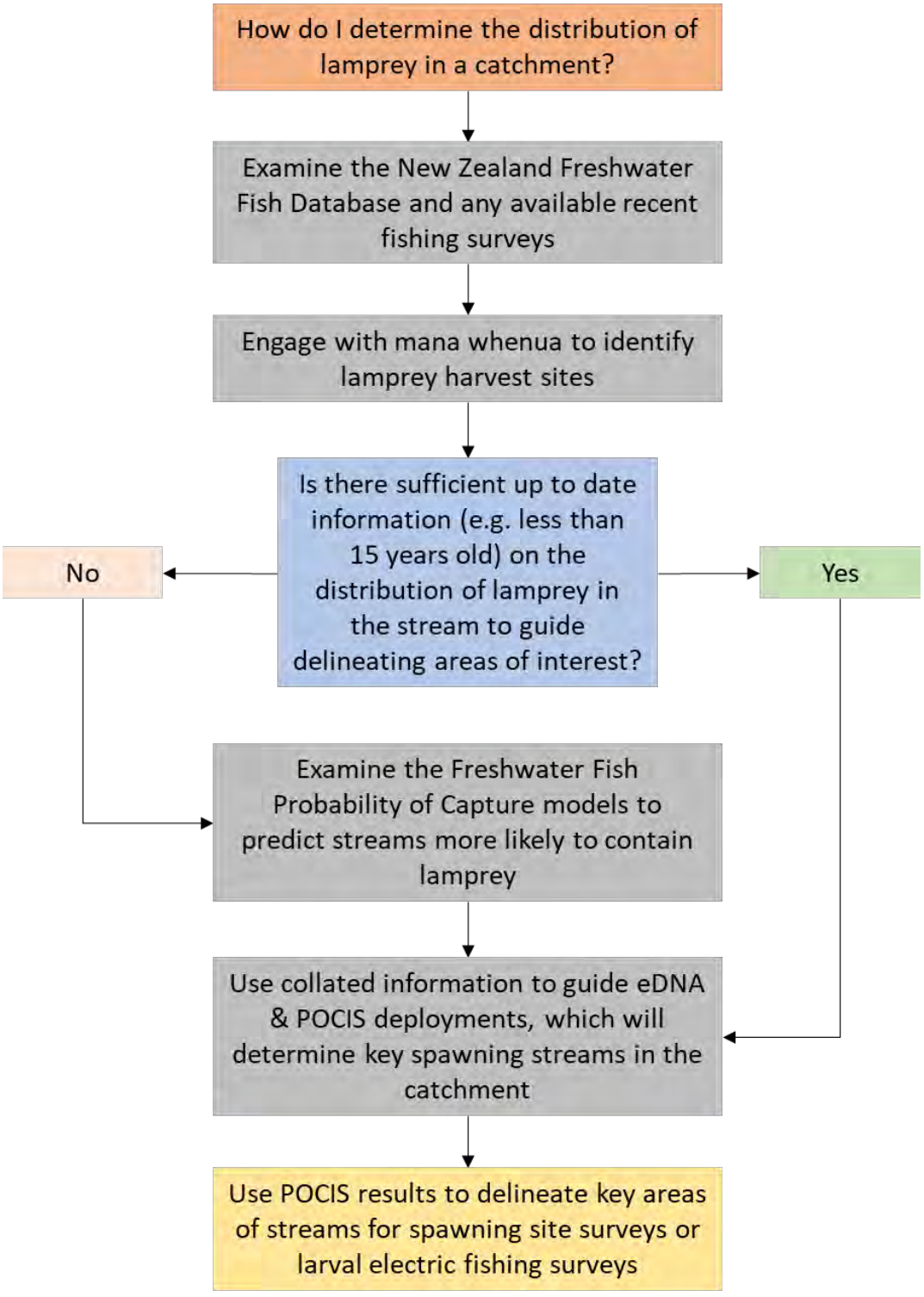

Figure 4-1: Recommended steps for using eDNA to determine the distribution of lamprey in a catchment and POCIS to delineate key areas of streams to locate and monitor lamprey.

Additional guidance on carrying out each step is outlined below, followed by a hypothetical worked example in Section 4.3.

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

2. **Engage with mana whenua to identify harvesting sites**. Discussions with mana whenua will identify streams that were known to historically contain lamprey and these should be prioritised for sampling.

3. **Spatial coverage of information**. For the catchment of interest, do the recent surveys, the NZFFDB records and mātauranga from mana whenua provide up to date information on lamprey presence/absence in all key tributaries feeding into the mainstem? Key tributaries would usually contain at least two stream orders. Short first order tributaries feeding into a fourth or higher order mainstem are less likely to represent key habitat for lamprey in the catchment. If so, going straight to Step 6 and deploying POCIS to delineate spawning and rearing areas within tributaries containing lamprey is recommended.

4. **Examine the Freshwater Fish Probability of Capture models.** Leathwick et al. (2008) used the NZFFDB records and the River Environment Classification (REC1) to generate spatial predictions of freshwater fish probability of capture across New Zealand. Crow et al. (2014) updated the model of Leathwick et al. (2008) using the REC2 and where data was available, completed separate probability of capture models for different fishing methods. For lamprey, Crow et al. (2014) used electric fishing records to develop the probability of capture model. Both the Leathwick and Crow models can be used to predict tributary streams in a catchment with a higher probability of lamprey occurrence. The model of Crow et al. (2014) can be accessed through NZ River Maps. However, DOC have a GIS layer available to staff.

   The model of Crow et al. (2014) also includes a classification threshold based on Cohen's Kappa. For each species in the model, the Cohen's Kappa value represents the probability threshold above which a prediction value is considered more likely than not to indicate a species could be present. The Cohen's Kappa value for lamprey is 0.51 ± 0.011 (1 standard deviation; Crow et al. 2014). For any stream segment if the predicted probability of capture is higher that the Cohen's Kappa value plus one standard deviation (i.e. higher than 0.521) then lamprey are most likely to be present. Similarly, if the predicted probability of capture is lower that the Cohen's Kappa value minus one standard deviation (i.e. lower than 0.499) then lamprey is most likely to be absent. Values within the range of the Kappa value plus or minus the standard deviation have a varying degree of confidence in both categories and should be assigned as indeterminate.

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

slight differences in predictions, combining the results of both models would maximise the predictive accuracy.

5. **Choose suitable locations for using eDNA or POCIS sampling**. Here, either eDNA or POCIS sampling can be used to investigate the distribution of lamprey in a catchment. If budget allows, the first deployment of eDNA or POCIS sampling should be as extensive as possible to gain the most knowledge on the distribution of lamprey in the target catchment. However, in reality, budget, access or other factors can limit the number of sites that can feasibly be surveyed. In this regard, the information gleaned from Steps 1 through 3, can be used to prioritise tributaries in the catchment for eDNA or POCIS sampling.

   Regardless of method used, collection sites should be focused at the base of tributaries to sample as much water as possible. Although contingent upon stream flow, current investigations indicate that eDNA syringe kits can detect species presence across 4 - 5 km of linear stream length (Josh Smith, Waikato Regional Council, pers. comm.). The detection range of eDNA passive samplers has not been tested, however, it would intuitively be further than discrete spot sampling using the syringes. Consequently, in small sub-catchments, setting passive samplers at the base of second or third order tributaries may also sample water from first order tributaries.

6. **Refine understanding of key spawning streams using POCIS deployments**. This step uses POCIS deployments to pinpoint tributaries or branches of tributaries where lamprey are spawning. The concentration of PS detected at each site will indicate which sites have a higher lamprey abundance and this will delineate key lamprey habitats.

   For streams or sub-catchments selected from eDNA or POCIS sampling in Step 5, a POCIS sampler should be set at the first monitoring location. This is used as a positive control to gauge pheromone levels higher in the catchment relative to that at the base of the stream. Contingent upon spawning areas, stream branches in the upper river can often have higher concentrations of PS than lower river sites. This helps identify tributaries or key areas of the sub-catchment with higher abundances of larvae.

   POCIS sites should focus on sampling usable tributaries within the target sub-catchment. This is where the freshwater fish probability of capture model and examining stream gradient and elevation (see optional step below) can help prioritise tributaries for POCIS deployments. POCIS should also be deployed in the mainstem of the target sub-catchment to determine the uppermost penetration of lamprey and which areas of the mainstem have the highest abundance of lamprey.

4.2.1 Use of stream gradient and elevation to inform POCIS deployment

This optional step utilises the gradient and elevation in tributary streams determined from the REC database to help inform the choice of locations to deploy POCIS samplers. If you do not have access to a GIS application or experience using the software, the NZ species DB programme[1] is a simple and easy to use alternative. This step is not critical but can be helpful for determining which parts of streams are at an altitude too high for lamprey to utilise, or where a possible migration impediment/waterfall could be. In addition, many short, first order tributaries could be present that have extremely steep gradients that would not be suitable for lamprey.

[1] Link to download the software from Jowett Consulting is in Step 1. A detailed instruction guide is downloadable from the site.

Presently, only 1% of all lamprey records in the NZFFDB are above 300 m in elevation. Consequently, focusing POCIS deployments on stream sections below 300 m in elevation is recommended. As lamprey are adept climbers, the assessment of stream gradient is subjective, and a steep gradient does not mean lamprey will be restricted in passage. However, it provides an indication of likely habitat type (e.g. higher prevalence of fast flowing sections and large substrates). It also indicates the presence of large waterfalls, which are signified by a sharp increase in elevation with little to no change in distance inland. In general, large waterfalls at elevations greater than 200 m will reduce lamprey penetration and steep gradients (c. 100 m increase in elevation across 1 km distance inland) will reduce the availability of fine sediment deposition for larval nursery sites. In this regard, setting a POCIS around 1 km below any perceived sharp elevation change will determine if that upper stream section requires further assessments for either larvae (electric fishing, see Section 6) or adults (manual searches, see Section 5). In Section 4.3, Figure 4-3 and Figure 4-4 provide examples of how stream gradient was used to determine appropriate POCIS deployment points in the lower Patea River tributaries.

4.2.2 Microhabitat selection and POCIS setting within streams

Appendix B and F provide an overview of the deployment methodology and equipment needed, respectively, for setting and retrieving POCIS. Key reminders are outlined below.

- Transport POCIS to and from the site within a plastic bag inside a chilly bin containing ice packs. Before deployment several POCIS can be inside a single bag but **upon retrieval, each POCIS must be in a separate plastic bag**.
- Ensure each POCIS is attached securely to the **downstream side of the waratah** and **positioned with the mesh sides parallel to the flow** – not perpendicular (see Appendix B.1).
- When **setting each POCIS, stand on the downstream side or adjacent to the waratah** and sampler. **Do not stand upstream** as water containing the lamprey pheromone can be washed off waders and contaminate the sampler.
- Upon retrieval, ensure **each POCIS has a waterproof label inside the plastic bag and the site and collection date is also clearly labelled on the outside of the bag**.
- When couriering the POCIS back to the NIWA laboratory ensure all individual POCIS bags are inside a larger plastic bag to protect labelling[2].
- Always courier the POCIS in a chilly bin with perishables labelled. Do not put them in a courier bag as they will not be delivered to NIWA as a perishable item.

Microhabitat selection

In general, POCIS should be set in runs rather than pools or riffles. This is because water velocities can be excessively high in riffles and pools tend to be slow flowing or have backflow, which may reduce the volume of water sampled. When selecting a location to locate the sampler within a run, it is important to think about potential changes in flow. That is, ensure that the sampler will not become dry when water levels drop and, when water levels increase, the water velocities where the

[2] Many ice packs condensate inside the chilly bin during transport and site labels on the outside of POCIS bags are lost. These samplers have not had internal labels and then they cannot be linked back to a collection site.

sampler is set do not become too high (>0.8 m s^{-1}). Ideally, water velocities should remain between 0.3 and 0.6 m s^{-1}. Appendix B.4 describes how to measure water velocities if a velocity meter is not available.

Below are some examples of POCIS site selection in both large and small rivers and streams.

- In large rivers set the POCIS closer to the margins.
- If possible, set downstream of bank outcrops/bends that will help direct faster waters in flood flows around the sampler.

- Set POCIS on the edge of runs that are adjacent to backwaters and/or associated with open floodplains. Here increased flows can spread across the channel more readily meaning increases in water velocity will be reduced.

- Set POCIS in wider stream sections, downstream of riffles or channelised stream sections. Here wider bank margins help protect the sampler from being exposed to excessive water velocities during freshes or floods.

- In streams with uniform widths and habitats, site selection is usually best mid-stream.
- Where POCIS are set mid-stream, a weather watch is pertinent to ensure the sampler is removed early if flood waters are expected.

- Where streams are shallow and comprised mostly of shallow, riffle habitat, set POCIS on the edge of pool habitat to ensure the sampler does not get exposed if water levels drop.
- If possible, set the POCIS behind large boulders for some protection from increased water velocities during higher flows.

4.2.3 Timing

To maximise lamprey detection using eDNA and POCIS it is recommended to sample between **December and March (inclusive)**.

POCIS

The December to March summer/early autumn window is when ammocoetes are actively feeding and metabolic rates for creating and excreting PS will be highest. Examination of monthly POCIS data between May 2019 and February 2020, from two streams in the Waipa catchment, indicated that flow and temperature had a significant effect on the detection of PS. Highest detection of PS was at low flows and peak detection occurred in the warmer summer months (NIWA, unpublished data). Consequently, we **recommend setting POCIS in January or February**.

POCIS should be set for between 14 – 21 days. If the stream is flashy and flow can increase quickly with rainfall, or has high turbidity, then the POCIS should be set for 14 days. In more stable streams, or streams with high visual clarity, the POCIS can be set for 21 days. Once set, if a large or severe storm is forecast, a precautionary approach is advised and the POCIS can be removed any time after 7 days to ensure it is not compromised/lost.

If lamprey have previously been detected in the waterway using either passive tools or physical capture methods then POCIS can be set for a reduced timeframe. **The minimum recommended deployment time for POCIS is 7 days**.

eDNA

Examination of monthly eDNA[3] sampling between May 2019 and February 2020, from the same two streams in the Waipa catchment as for POCIS investigations, found flow was the main factor influencing lamprey detection (NIWA unpublished data). There was a clear negative trend between eDNA detection/concentration and increasing stream discharge. The temporal trend for eDNA detection was less clear than for POCIS (e.g. not statistically significant) but peak detection of lamprey was during early summer. Consequently, we **recommend sampling for lamprey eDNA in December or January**.

[3] A qPCR assay for *Geotria australis* developed by the University of Manitoba was utilised to examine eDNA concentrations in the NIWA study. Therefore, results may differ to the metabarcoding analyses offered by Wilderlab.

4.3 Example: Pātea River

The Pātea River provides an example catchment for using passive methods to sample lamprey following the approach outlined above. Only the lower Pātea River below the Pātea Hydro-electric Dam is used in the example as the Pātea Dam represents a passage barrier to lamprey.

1. **Examine fishing records from recent surveys and the New Zealand Freshwater Fish Database**. The NZFFDB only has records of lamprey prior to 1995, from two streams above the Pātea Dam. As such, there is no recent information on the density and distribution of lamprey in the Pātea River with which to plan passive sampling deployments.

2. **Engage with mana whenua to identify harvesting sites**. Piharau were historically harvested from streams feeding into the upper Pātea River (now Lake Rotorangi) by Ngāti Ruanui. After commissioning Pātea Hydro-electric Power Station in 1984, adult piharau were gathered by iwi from the tailrace, but it appeared that very few, if any, were seen from the mid-1990s. Discussions with mana whenua would need to be carried out.

3. **Spatial coverage of information**. For the lower Patea River, there are no recent surveys or NZFFDB records. Mātauranga from mana whenua would highlight historic harvest sites but this would not provide adequate knowledge of the current distribution of lamprey in the catchment. Therefore, Steps 4 and 5 should be undertaken.

4. **Examine the Freshwater Fish Probability of Capture models.** In the Crow et al. (2014) model, all habitat downstream of Pātea Dam was below the Kappa value minus one standard deviation (0.499). However, the model of Leathwick et al. (2008) suggested that the mainstem of the Pātea River and mainstem of larger tributary streams were likely to be more suitable for lamprey than most smaller first order tributary streams (Figure 4-2).

5. **Determine suitable locations for deploying eDNA passive samplers.** Figure 4-2 depicts a recommended approach for deploying eDNA passive samplers to survey for lamprey presence in the lower Pātea River. Larger tributaries are targeted over small first order streams. As it is not realistic to sample every single tributary, four sites in the mainstem of the Pātea (red circles; Sites 1, 2, 3, 4) at approximately 7, 15, 22 and 35 km upriver from the sea, are included as check points. The spacing of the mainstem samplers was set to broadly cover unsampled tributaries alongside those where samplers were set. For example, if lamprey DNA is not detected in Kuranui Stream (Site 5) but is detected in the mainstem of the Pātea River at Site 1, this verifies lamprey are present in tributaries above Site 1 that were not sampled. Similarly, if Site 2 in the mainstem detected lamprey DNA but lamprey were not detected at Sites 6 – 11, then the smaller first order tributaries will require sampling. If the mainstem of the Pātea River and upstream tributary sites do not have detectable lamprey DNA, this provides two lines of evidence for lamprey being in low abundance or absent in that section of the catchment.

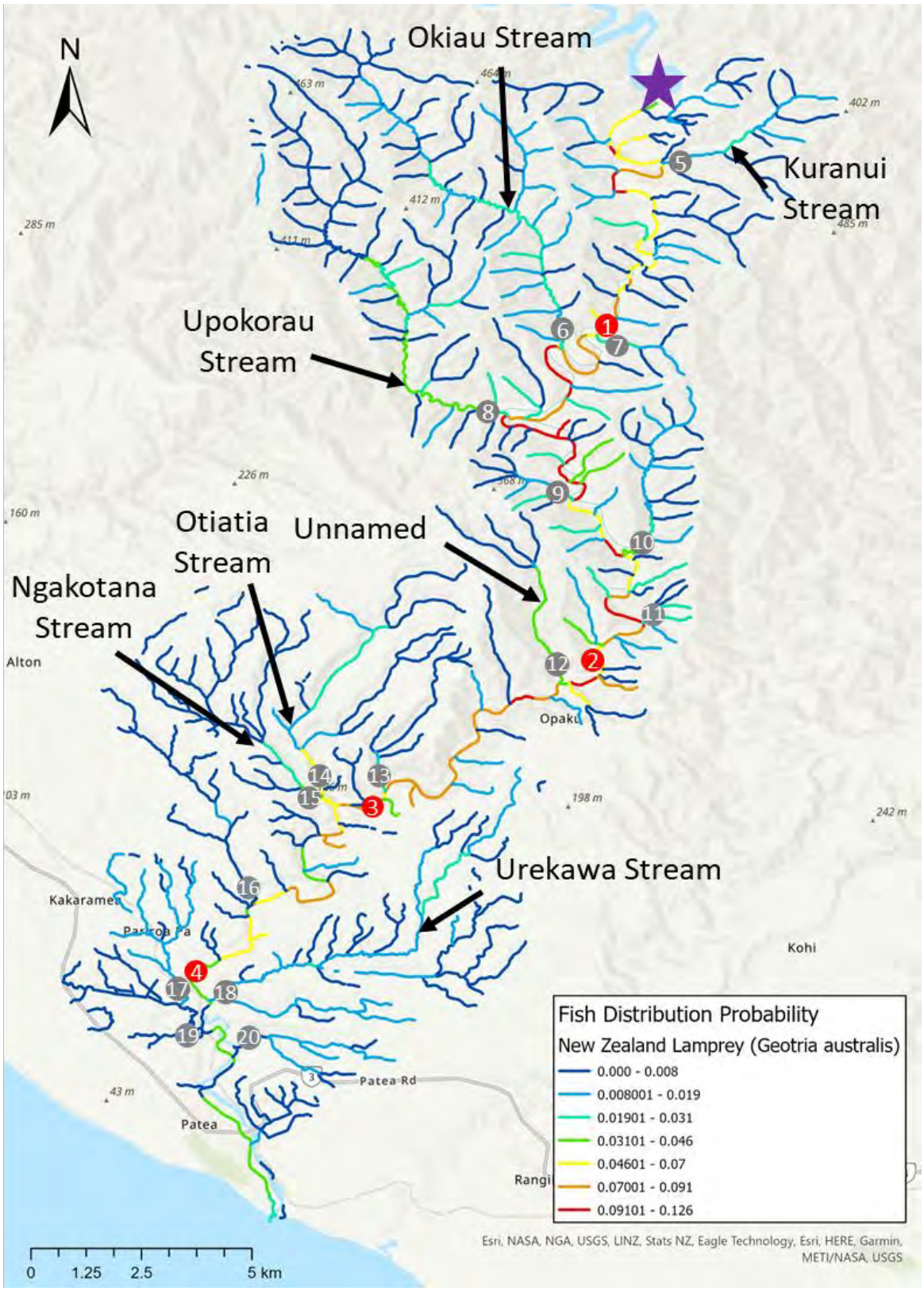

Figure 4-2: Proposed eDNA monitoring locations to screen for tributaries containing lamprey. The purple star signifies the location of Pātea Hydro-electric Power Station. The red circles indicate samplers in the mainstem of the Pātea River and grey circles are tributary deployments. Stream segments are colour coded relative to the probability that lamprey will be present using the Freshwater Fish Probability of Capture model of Leathwick et al. (2008).

6. **Refining understanding of key spawning streams using POCIS deployments**. There are many hypothetical outcomes from the suggested deployment of eDNA passive samplers in Figure 4-2, contingent upon where lamprey DNA is or is not detected. To cover options for every scenario is beyond the scope of this manual. Consequently, the example POCIS deployment is based on two key streams returning positive detections for lamprey DNA; the Upokorau Stream and the Otiatia Stream (Figure 4-3 & Figure 4-4).

   For both the Upokorau and Otiatia streams, the first POCIS sampling site (1) repeats the eDNA monitoring location (Sites 8 and 14, respectively; Figure 4-2) as a positive control. Using the REC database, in the Upokorau Stream, many short first order tributaries are present that are deemed to be unsuitable for lamprey based on size and gradient. As such, POCIS 2 and 3 focus on usable tributaries containing a low gradient section of stream for around 1 km before steep gradients occur (Figure 4-3).

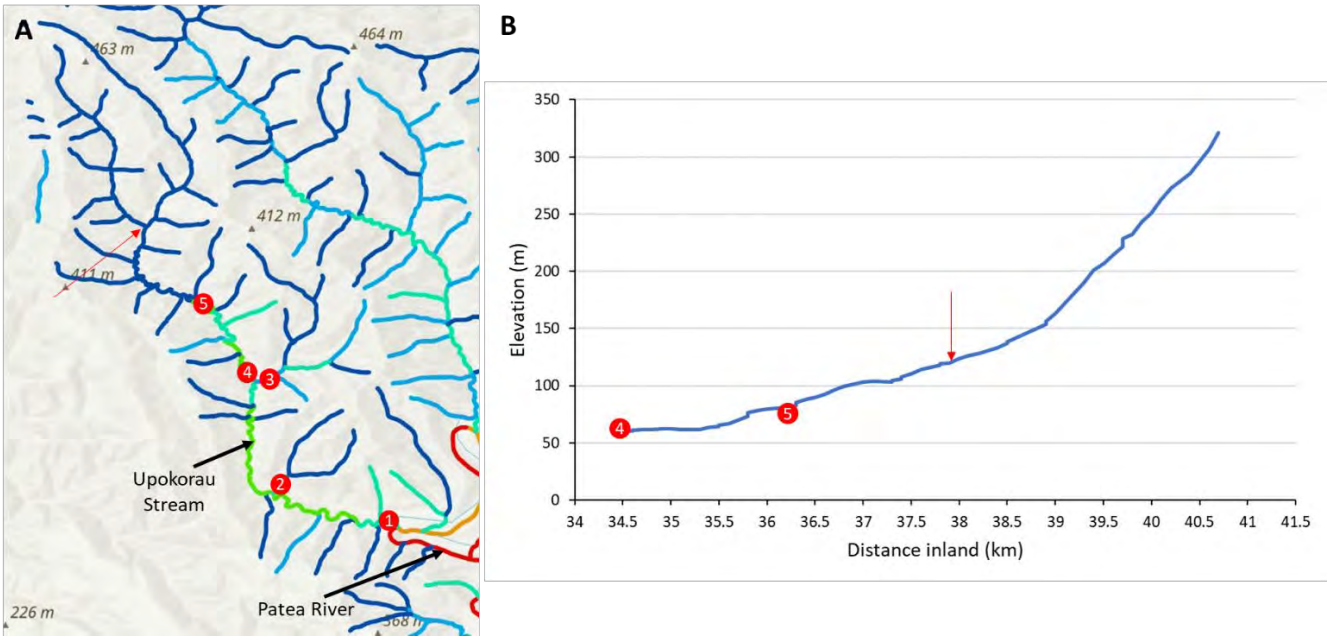

Figure 4-3: Proposed monitoring locations (red circles) for utilising POCIS as a secondary screening tool in the Upokorau Stream to pinpoint key sections likely to be used by spawning lamprey. A, overview of the stream and POCIS locations, as per Figure 4-2, stream segments are colour coded relative to the probability that lamprey will be present using the Freshwater Fish Probability of Capture model of Leathwick et al. (2008). B, Gradient of Upokorau Stream between POCIS 4 and the headwaters. The position of POCIS 4 and 5 along the mainstem are indicated. In both A and B, the red arrow points to where the mainstream branches, and the gradient shown in B represents the true right headwater tributary. Stream location in the Patea River catchment shown in Figure 4-2.

Based on stream gradient and elevation, POCIS 4 and 5 are positioned in the mainstem of the Upokorau to determine the likelihood of lamprey presence in the upper reaches of the stream (Figure 4-3). Upstream of both POCIS 4 and 5, low gradient, relatively flat sections of stream are present at elevations that adult lamprey could easily access and that both adult and larval life stages could utilise (Figure 4-3). Where the stream branches above POCIS sampler 5 (indicated by the red arrow in Figure 4-3), the gradient of the headwater tributaries increase rapidly (approximately 100 m elevation gain over 1 km distance inland) to reach over 300 m in elevation and if lamprey were present their abundance would likely reduce rapidly with increasing distance upstream (Figure 4-3).

The same approach was followed for determining POCIS placement in the Otiatia Stream, whereby each significant stream branch is sampled (samplers 2, 5, and 6; Figure 4-4). POCIS 2 and 3 separate the lower stream branch where a change in probability of capture occurs. The gradient of Otiatia Stream upstream of POCIS 4 is shown in Figure 4-4. The tributary sampled by POCIS 5 has a similar gradient to the mainstem upstream of POCIS 6, with both of these streams below 300 m in elevation. Upstream of POCIS 5 and 6, an elevation gain of approximately 50 m occurs over 1 km distance inland, whereas the tributary sampled by POCIS 7 exhibits an elevation gain of approximately 75 m per kilometre. Consequently, rather than grouping headwater branches (POCIS 6 and 7) into one sampling site, they have been separated to determine if lamprey show a preference between these stream branches and help determine the maximum distance of lamprey penetration upstream of POCIS 2 and 4 (Figure 4-4).

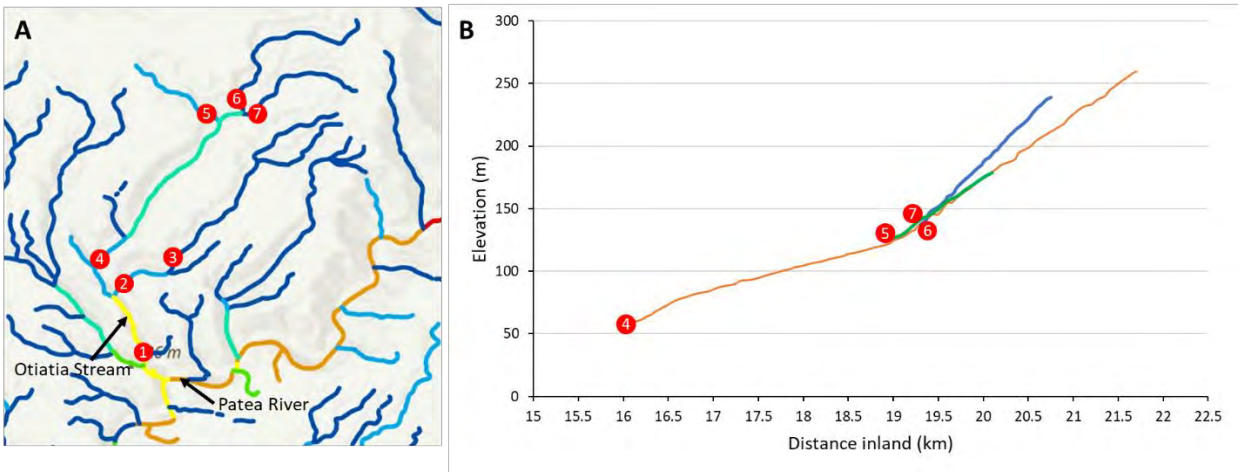

Figure 4-4: Proposed monitoring locations (red circles) for utilising POCIS as a secondary screening tool in the Otiatia Stream to pinpoint key sections likely to be used by spawning lamprey. A, overview of the stream and POCIS locations, as per Figure 4-2, stream segments are colour coded relative to the probability that lamprey will be present using the Freshwater Fish Probability of Capture model of Leathwick et al. (2008). B, Gradient of Otiatia Stream between POCIS 4 and the headwaters. The blue line signifies the gradient of the stream branch above POCIS sampler 7. The green line signifies the gradient of the tributary upstream of POCIS sampler 5. The location of POCIS sampler 6 is shown on the mainstem branch indicated by the orange line. Stream location in the Patea River catchment shown in Figure 4-2.

The combined results of the eDNA and POCIS deployments will provide information to characterise the broad scale distribution of lamprey within the catchment and to pinpoint key tributaries/habitats that may be targeted for further investigations, or can inform spatial conservation planning. Sections 5 and 6 outline approaches to manual track adult lamprey to successfully locate spawning sites in the area delineated by the POCIS deployments, and standardised electric fishing methods to estimate larval densities, respectively.

Locating spawning sites

5 Determining spawning sites within a catchment

Spawning surveys undertaken to date indicate that visually identifying lamprey spawning habitat will be difficult, if not impossible. Waikawa River surveys have found that although boulders are utilised for spawning, when the stream contains boulders and an array of other habitat types, lamprey will utilise cavities formed in other habitats as well as cavities under boulders. Consequently, by targeting just boulder habitat, the spawning sites used by lamprey will likely be underestimated. From a management perspective, this is problematic as key stream areas used by lamprey for spawning may be missed in protection and conservation plans.

The best approach for pinpointing lamprey spawning locations is outlined in Figure 5-1.

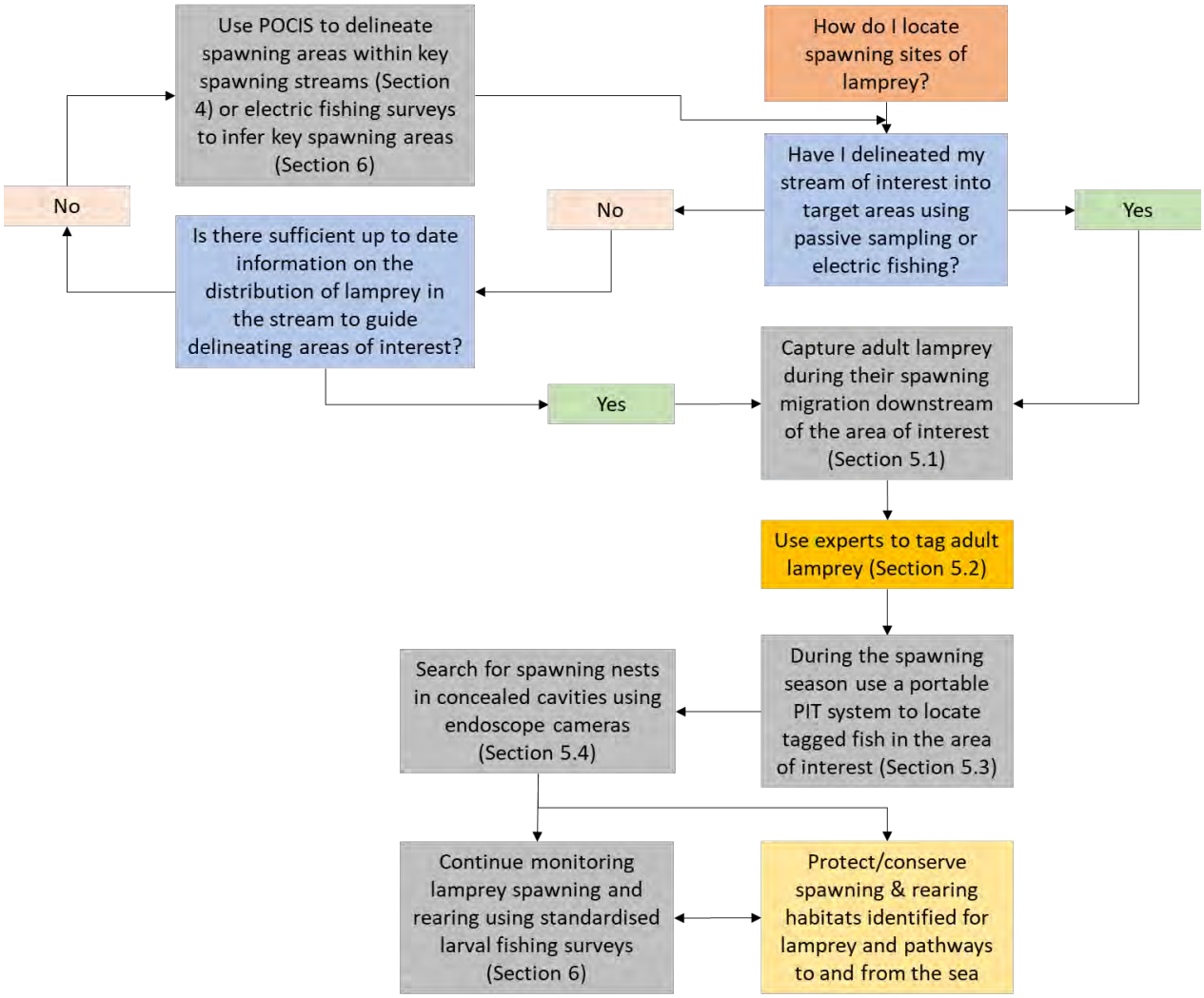

Figure 5-1: Recommended steps to follow to locate lamprey spawning nests.

Assuming key areas have already been determined using eDNA and POCIS deployments or knowledge of where larval lamprey reside is known from electric fishing surveys, the following sections outline methods for capturing, tagging and tracking adult lamprey, along with undertaking spawning surveys to locate nesting sites. A caveat of any manual tracking method is that it becomes more difficult in large, deep rivers. Consequently, until different or better technology is developed for locating lamprey spawning sites, spawning habitat surveys will be limited to wadeable waters.

5.1 Capturing adult lamprey

The migration window for adult lamprey spans autumn, winter and spring, with migratory lamprey captured anytime between April and October. In some North and South Island locations, the peak movements occur primarily June through August so these would be key months to target.

5.1.1 Hand capture

The easiest method to capture migratory adult lamprey is by hand from an instream migration impediment (e.g., a waterfall, weir or instream structure), as lamprey will leave the water and climb over the structure (Figure 5-2). The climbing behaviour at impediments, slows progress and results in lamprey being visible and easily captured. For any migration impediment where adults have not been captured previously, during the migration window, repeat visits at night, on the receding limb of floods will determine the appropriate timing and flood conditions when adult migrations occur.

Whilst capturing adult lamprey from instream impediments during high water levels, stringent health and safety practices are essential. The following is by no means exhaustive but instead covers key safety equipment and practices that can be added to protocols specific to each agency/organisation. A life jacket and sturdy waders or drysuit/wetsuit should always be worn. Each fisher in the water should use a waterproof head torch to spot lamprey to ensure both hands are free for use in traversing slippery surfaces. A safety observer who stays on land is essential and the safety person should carry a throw rope to rescue a fallen fisher. A cell phone or communication device should also be carried by the safety observer, even if walking or driving to reception from the fishing location is required.

Figure 5-2: Collecting migratory adult lamprey from a weir by hand (left) and a group of lamprey attempting to pass a rock weir (right). Red arrow depicts adult lamprey.

5.1.2 Fyke nets

When an impediment to migration is not present, fyke nets can be utilised. Fyke nets are most effective at capturing migratory adult lamprey when set across the entire river (Figure 5-3). In large fast flowing rivers, fyke nets can be effective when set facing downstream adjacent to high velocity waters where lamprey attempting to swim around the fyke net end up pushed inside by the fast water (Figure 5-4). For any fyke net set to capture adult lamprey, the most important factor is the addition of eel excluders, as in our experience, once eels enter a fyke net they will either eat all lamprey present or deter lamprey from entering the nets. Cable tying a piece of plastic mesh at the fyke net entrance (no larger than 40 x 40 mm squares) is effective at excluding large eels.

Figure 5-3: Double winged fyke nets set in the Okuti River to capture migratory adult lamprey.

Figure 5-4: Single leader fyke net set in the Santa Cruz River, Argentina targeting migratory *Geotria macrostoma*.

5.1.3 Pipe traps

If instream obstacles are present, as well as spotlighting for hand collection, they provide the opportunity to set traps for migratory adult lamprey. In particular, pipe traps have been devised as an effective means of capturing adult lamprey at obstructions (Morris & Maitland 1987; see Appendix C for design details). NIWA have successfully caught adult lamprey utilising these traps below a weir in Kinloch Stream and within a culvert in Canal Reserve Drain (Figure 5-5). As lamprey appear to enter the trap for refuge whilst migrating past an impediment, they can be left in situ for several days before checking. In contrast, fyke nets require checking on a daily basis to ensure debris loads and high velocity waters don't compromise the net.

Figure 5-5: Pipe traps set within a box culvert in Canal Reserve Drain, Christchurch (A) and below a weir in Kinloch Stream, Banks Peninsula (B). Red arrows indicate one of the conduit pipe traps.

5.1.4 Mātauranga Māori

In catchments where mana whenua have historically harvested piharau/kanakana, working alongside local iwi/hapū is the best approach for capturing migratory adults as they will know the locations, timing and conditions when migratory adult lamprey are present and can be captured. When working with mana whenua, mātauranga can be reinvigorated to capture adult lamprey using a variety of different methods which may include elaborate weirs made of wood or stone, hand and hood retrieval from holes, bracken fern bundles and hinaki nets (Almeida et al. 2021).

5.1.5 Sample size

The number of adults required for tagging is contingent upon stream size and length. In Kinloch Stream, Banks Peninsula, where we were interested in <2 km of stream length, with an average wetted width of 2.4 m (during base flow), c. 50 adult lamprey were tagged each study year. In the Waikawa River, Southland, between 100 and 300 adult lamprey were tagged to assess 8 – 10 km of stream, with an average wetted width of 3.6 m (during base flow). Sample size will be limited by the number of adult lamprey that can be captured, however, the more fish tagged, the higher the chances of pinpointing multiple spawning sites.

5.2 Tagging adult lamprey

Tracking lamprey during their freshwater migration to document spawning habitats is difficult due to their biology. This is the main reason the reproductive ecology has only recently been documented. Pre-reproductive adults entering freshwater have limited space in the visceral cavity (where fish are typically tagged) and are unable to fit transmitters larger than 12 mm (Baker et al. 2017a). Currently, there are three feasible options for tagging and tracking adult lamprey:

- 12 mm PIT tags
- 12 mm Eel/Lamprey Acoustic Tag (ELAT)
- Externally mounted radio tags

NIWA recommend the use of 12 mm PIT tags if pinpointing spawning sites is the objective, or externally mounted radio tags if understanding maturation habitat and stream choice is the objective. An overview of each method is provided below.

Surgically implanting a PIT tag or mounting an external radio tag on a lamprey is a difficult procedure and requires appropriate permits and approvals. For these reasons, detailed methods on the tagging procedures are not provided as they should only be implemented by a trained expert.

5.2.1 PIT tags – long term monitoring

PIT tags have no battery and last indefinitely, which enables lamprey to be tracked to their spawning locations. NIWA have developed custom-made fixed and portable PIT systems to successfully track adult lamprey implanted with 12 mm Half Duplex (HDX) PIT tags across their 15-16 month spawning migration (Baker et al. 2017a & b). Half duplex PIT tags are recommended as the technology is cheaper and larger antennae can be more easily built compared to full duplex tags. Consequently, PIT tagging adult lamprey with HDX tags is the primary method recommended for locating spawning sites (Figure 5-6).

5.2.2 Externally mounted radio tags – short term monitoring

Externally mounted tags are problematic as lamprey bury themselves amongst large substrates and quickly shed the tag (Jellyman et al. 2002; Figure 5-7). In addition, the battery life of radio tags that lamprey are able to carry (based on body weight) is too short to remain active until spawning. For these reasons radio tags are only recommended for tracking lamprey over short periods of 1 – 2 months to determine stream choice or habitat used during maturation as they cannot be used to pinpoint spawning locations. NIWA and potentially other agencies hold ATS receivers that can be used for tracking the externally mounted transmitters (Figure 5-7).

5.2.3 ELAT acoustic tags – potential technology in the future

The ELAT internally implanted tags also have a short battery life. The estimated battery life is ~30 days at a 5 second pulse rate. Extending the pulse rate could enable the tag to remain active for up to half a year, but it is unlikely to be effective for the necessary 15 - 16 months between when adults enter freshwater and then spawn. Presently, no agency in New Zealand holds receivers that will detect the acoustic signal (417 Hz) and the ELAT tag is not commercially available, it can only be purchased through collaborations with the Pacific Northwest National Laboratory. Consequently, both cost and availability currently restrict the use of the ELAT tags in New Zealand.

Figure 5-6: Inserting an Oregon RFID 12 mm Half Duplex (HDX) PIT tag into an adult lamprey. Inset shows the 12 mm tag.

Figure 5-7: ATS external radio tag (F1960) mounted on an adult lamprey. Inset shows the attachment harness.

5.3 Tracking adult lamprey to identify spawning locations

Either fixed or portable antennae/receivers can be used to track any tagged animals. In order to use fixed receiver stations, knowledge of where the animal is going is necessary. As the objective is to identify unknown locations, where lamprey spawning sites will be numerous and likely dispersed throughout the stream, mobile tracking is recommended. For active radio tags tracking can occur either by plane/helicopter or walking the stream. For acoustic tags, fixed receivers set to triangulate the tag's signal is the usual method employed to understand fish movements, however, walking the stream with a hydrophone can also be effective. NIWA have found tracking PIT tagged fish to be the most successful and cost-effective method of locating lamprey spawning sites.

5.3.1 Timing of tracking surveys

Across the three years monitoring spawning sites with PIT tagged lamprey in the Waikawa River, it was found that frequent tracking of the lamprey was not necessary. After releasing tagged fish, the same number of nests were found the following year by either manually tracking lamprey locations every few months or just carrying out a single tracking event at the time of the spawning surveys. Therefore, if adult lamprey can be easily captured during migration, PIT tagging fish and carrying out one tracking event during spawning surveys should be effective for locating nest sites.

If PIT tracking is incorporated with spawning surveys, the recommended timing would be between mid-November and late-December for South Island locations and mid-October to late November for North Island locations. These timeframes are based on a window of 9 – 10 weeks to conclusively document spawning sites by observing eggs and/or larvae (e.g. Baker et al. 2017a).

5.3.2 Tracking surveys

In choosing a portable half duplex PIT system for tracking lamprey there are limited options available. Portable PIT readers are commercially available from Oregon RFID but these were designed for 23 mm tags and don't have the largest read range for the 12 mm tag. NIWA have upgraded the portable wand available from Oregon RFID to improve its read range for 12 mm tags (details on the wand design are provided in Appendix D).

1. Delineate the target stream length using passive sampling results (Section 4)
2. Track the entire stream reach during the spawning window.
3. To track effectively, the stream is scanned in either an upstream or downstream direction. Going with the flow is generally less effort. For the main stem channel, the antenna should be horizontal (Figure 5-8) and scanned across the channel as close to the substrate as possible.

Figure 5-8: Basic search method using the portable PIT system in a stream channel.

For the stream banks and instream structures (e.g. waterfalls) the antenna is held vertically (Figure 5-9).

Figure 5-9: Portable PIT system search method for waterfalls and weirs.

For areas of the stream with large structures (e.g., boulders, tree roots, under-cut banks or wood/instream objects) that lamprey could utilise as cover, the antenna should be moved and held in a variety of directions (Figure 5-10).

Figure 5-10: Portable PIT system search method for areas of streams with boulders, undercut banks or other features lamprey could use as cover.

Changing orientation of the antenna during scanning is important to help maximise the read range on the tagged fish. This is because the tag could be in a variety of positions, which influences its read range between optimal and sub-optimal orientations (see Appendix D).

4. Once a tagged fish is detected, record the GPS location and continue scanning the entire stream reach recording the location of successive lamprey tags detected.

5.4 Spawning site surveys

1. Use the portable PIT antenna to narrow down the tag location prior to attempting to access the nest. To help pinpoint a tagged fish, the antenna is folded against the wand handle to minimise its detection range. From the vertical folded position, with the antenna edge parallel to the stream bank, the antenna is moved back and forth (perpendicular to the stream bank) across the area containing the tagged fish until the tag is detected (Figure 5-11). The position is noted and then the wand is turned 90° so the edge is now perpendicular to the stream bank and moved forward and back over the area where the tag was just detected (Figure 5-11). The cross over point where the tag is detected by the antenna in each of the two orientations, is where searches are focused to try and locate the lamprey nest.

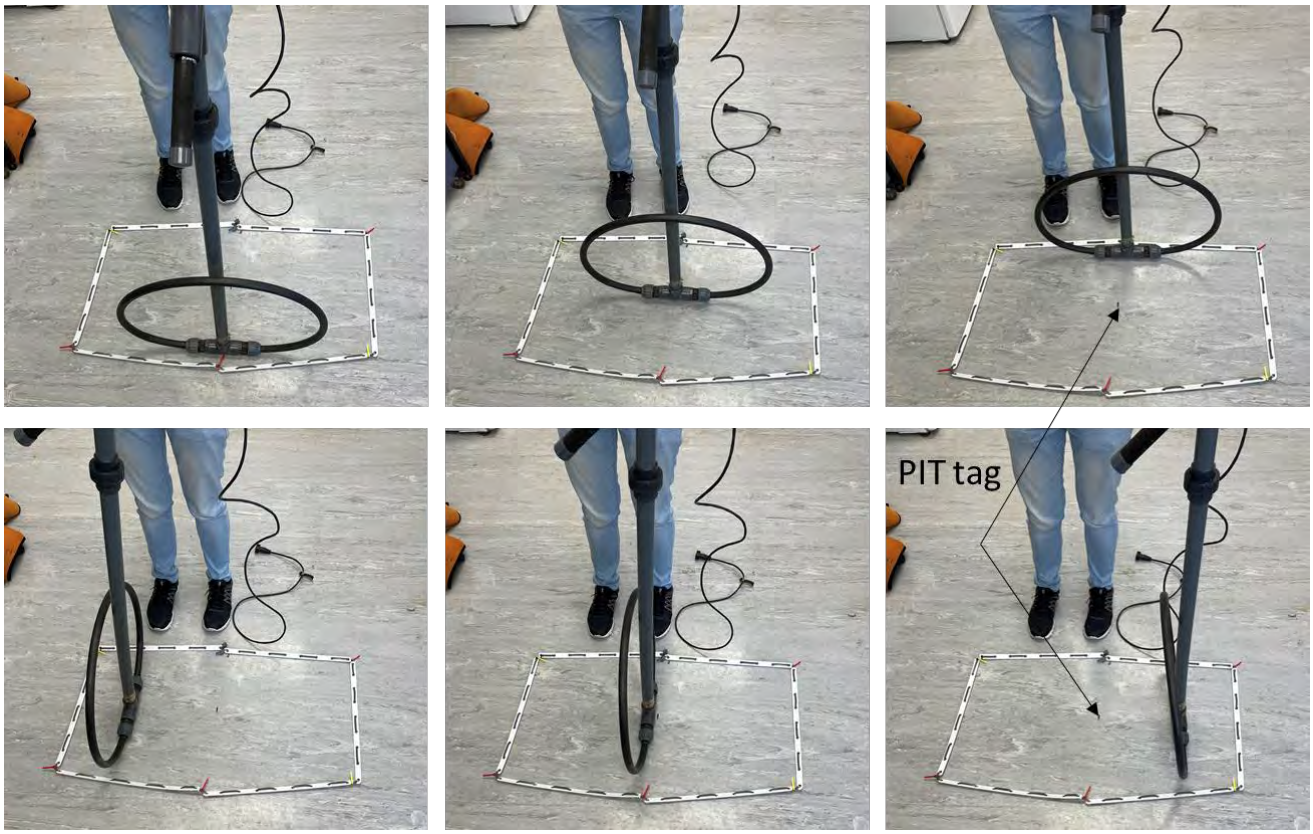

Figure 5-11: Pinpointing a tagged fish using the antenna in its weakest orientation. Hypothetical area where tag is detected is indicated by the square

For boulders, pinpointing the tag may not be feasible as the boulder itself dampens the read range of the antenna. Consequently, in its folded position the field may not be strong enough to activate the tag underneath a large boulder. However, once a tag is associated with a boulder it is generally easy to search underneath using an endoscope camera (see next step).

2. Once a tag has been pinpointed searching for nests is undertaken. This requires a crowbar, spade and endoscope camera[4]. Wearing neoprene or leather gloves is recommended for hand protection.

3. To locate a spawning nest beneath a boulder, the edges are searched by hand for an area of fine substrate or small cobbles that can be cleared to create a hole for the endoscope camera. If access underneath cannot be found through manual excavation of smaller substrates, a crowbar is used to gently lift the boulder by an inch (no more) to allow camera access (Figure 5-12). During this process it is important to watch any holes created for disturbed lamprey that flee the nest. Should a panicked lamprey swim out, they tend to stay in close proximity to the nest and can be picked up and manually put back inside their nest. If this occurs when you put the boulder back in place, the opening created is too large and should be filled with cobbles or the boulder gently lowered a fraction.

Figure 5-12: Searching for tagged lamprey beneath boulders.

4. Once the camera is underneath the boulder, undertake a thorough search for the egg mass and lamprey, which can be easily spotted using the inspection screen (Figure 5-13 & Figure 5-14). Once the egg mass is found, in our experience if the camera is held focused on the eggs, both the male and female lamprey will actively and aggressively approach the camera to guard the nest (Figure 5-13). NIWA has observed biting of the camera, stone throwing, sediment being spat at the camera and the lamprey wrapping themselves around the egg mass.

[4] NIWA primarily uses two models of endoscope cameras that are both IP67 rated, contain an LCD screen and the video footage can be saved to file. We have found the models that currently provide the best contrast, clarity and image quality when lamprey are moving, are the Aerpro Bullant G5000 inspection camera (https://aerpro.com/products/inspection-cameras) and the Teslong NTS300 series endoscopes (https://teslong.com/products/nts300-industrial-endoscope). It is recommended that two cameras be purchased so one is available as a back-up during spawning season should the primary camera get broken or damaged.

Figure 5-13: Inside a nest, the egg mass is clearly visible and both the male and female lamprey will actively approach the camera to protect their nest.

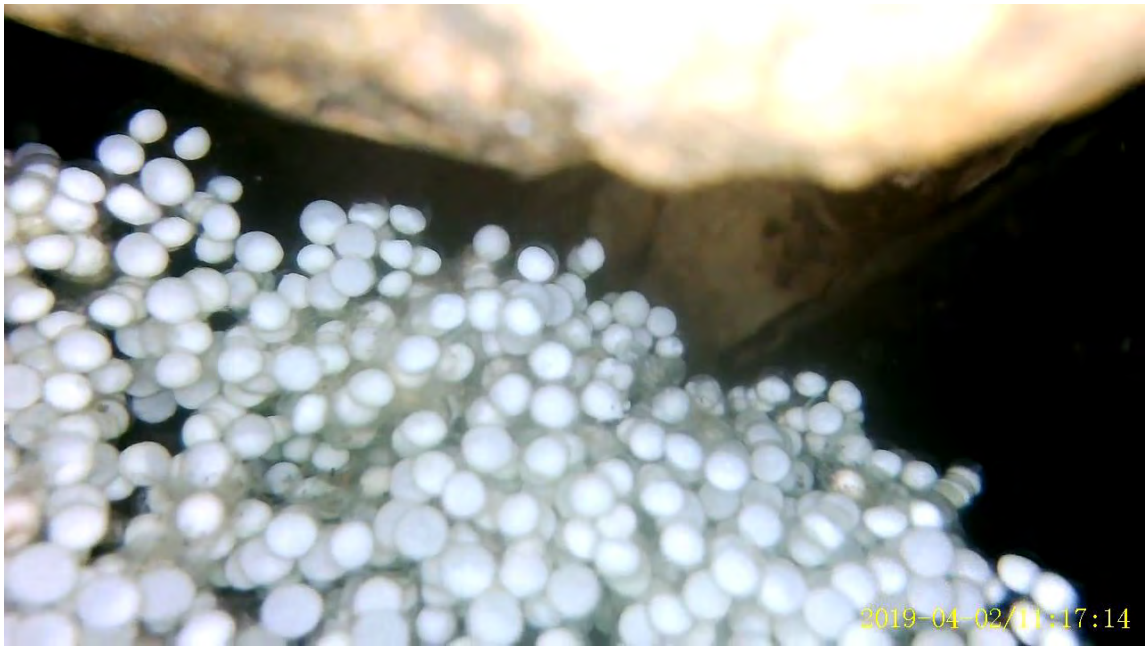

Figure 5-14: Image quality of Teslong endoscope camera showing newly fertilised, developing lamprey eggs.

5. For tags located in areas that do not contain boulders, searching for the nest site is more difficult. In our experience, not all nests can be accessed as they have either been in water deeper than arms can reach to locate entrances, or the entrances beneath the bedrock just cannot be found. However, where possible, the habitat is manually searched for cavity entrances and fine substrates are excavated to try and access openings. Generally, a solid stream bank or area of solid substrate (e.g., bedrock, consolidated clay etc) is present in the area where the tag is pinpointed. Using a spade and crowbar, bits of the stream bank or substrate are cut to access the spawning nest (Figure 5-15).

Figure 5-15: Searching for nest entrances in the stream bank (left) and under the river bed (right top and bottom).

6. Sometimes a lamprey can drop the PIT tag during maturation and tags are detected in areas containing only finer substrates with no large structure. To verify these dropped tags (occasionally there can be large structures and a nest beneath finer substrates), a stick antenna with a very small read range is used to search through the substrate (Figure 5-16). Finer gravels can be removed using a spade and the spoil searched for the PIT tag.

Figure 5-16: Verifying a dropped PIT tag in finer substrates using the stick antenna.

5.5 Caveats

A caveat of removing sections of substrate or stream bank to access lamprey spawning sites is that the method can be destructive to the habitat if too larger area is opened. The cost of accidentally destroying the nest site should be considered before attempting to access each of the tags detected as lamprey are a threatened species and spawning represents the culmination of their lifecycle after surviving the array of stressors to reach this final stage. It is also important to remember that under section 26ZJ of the Conservation Act (1987), it is an offence to disturb or injure the eggs or larvae of any freshwater fish. Consequently, it is best to confirm nests using cameras and non-invasive methods.

5.6 Alternative approach to determining lamprey spawning locations

Within the study streams examined for lamprey spawning sites, nests have been distributed widely throughout the area of stream surveyed (Figure 5-17). No spatial aggregation of sites has been seen. For this reason and the fact that documenting spawning sites can be destructive to a threatened species, inferring spawning areas using POCIS deployments and then electric fishing for larvae may provide a practical approach for identifying the broad scale distribution of spawning habitats within a catchment. If protection of lamprey critical life-stage habitats is the goal, the stream areas used by both larval and adult lamprey should be conserved.

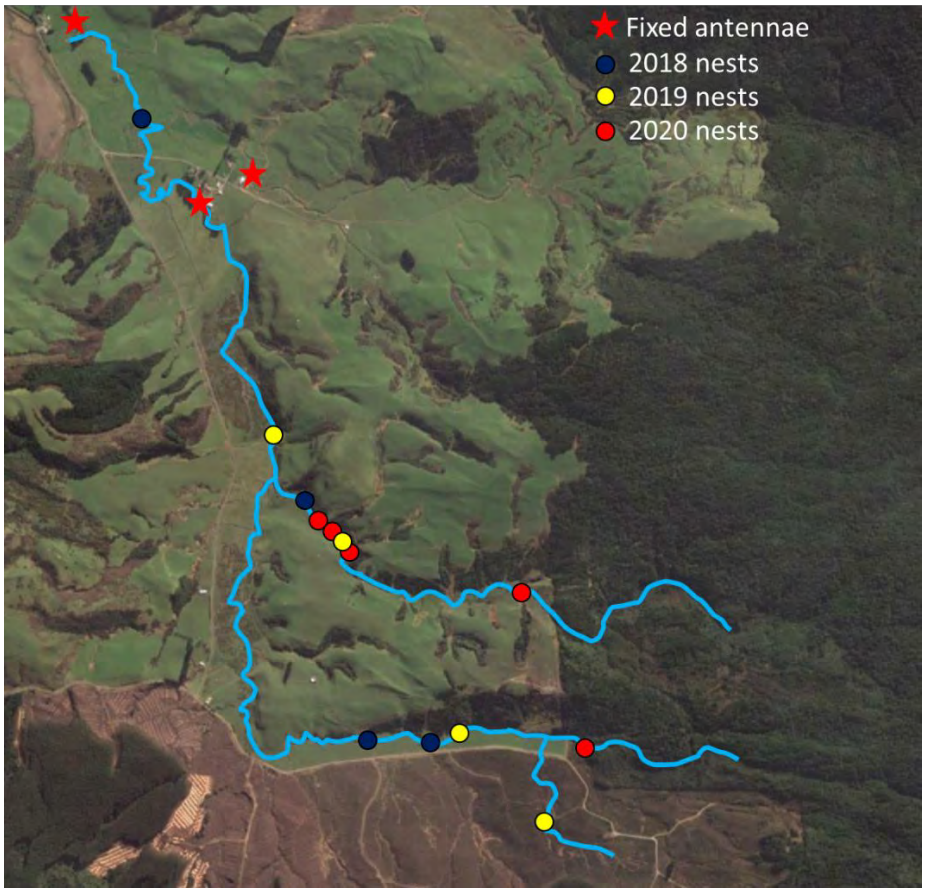

Figure 5-17: Distribution of 13 lamprey spawning nests located across three years in the Waikawa River. Study reaches of the river indicated in blue.

Piharau/kanakana (lamprey) standardised electric fishing methodology

6 Standardised electric fishing method for lamprey

In general, standard fish survey techniques are ineffective at capturing larval and adult lamprey. This is because larvae require a pulsed current over an extended timeframe to effectively draw them out of sediments meaning that standard electric fishing protocols tend to underestimate larval lamprey presence and abundance. The December to March timing of most ecological surveys is also not appropriate for capturing adult lamprey as, although adults spend over a year in freshwater before spawning, they are cryptic and cannot be captured in nets or through electric fishing outside of a small window during their upriver migration, which usually occurs in winter. In addition, seine nets and minnow traps are not effective fishing techniques for larval or adult lamprey.

To enable quantitative assessments of larval lamprey densities, NIWA has developed a standardised method of electric fishing targeting the ammocoete stage of the life cycle. Density estimates can be used to monitor temporal trends, assess spatial distributions of larvae (particularly using the spot fishing method), and infer which streams in a catchment have high numbers of adults spawning and/or successful larval recruitment.

Based on international approaches, particularly that used by Yakama Nation Fisheries, Oregon, a standardised electric fishing methodology has been developed by NIWA and field tested between January 2022 and March 2023.

6.1 Timing

To maximise lamprey capture efficacy, it is recommended to **sample between December and March (inclusive)**. This summer/early autumn window is when ammocoetes are actively feeding and river flows should be lower and more stable. For any stream where repeat fishing is undertaken, repeat surveys should always be carried out in the same calendar month as the original survey (between December and March).

6.2 Methodology for monitoring larval lamprey populations

If feasible, **surveys should be undertaken using a Kainga EFM300** backpack electric fishing machine as this will enable results to be comparable to other sites across New Zealand. Having data collected with the same fishing machine and using a consistent sampling methodology will build a robust dataset that can form the basis of distribution and occupancy models.

Using a Kainga EFM300 backpack electric fishing machine, **larvae will be sampled by manually pulsing** the electro-shocker, with c. **5 seconds on and 2 seconds off frequency**. If a Smith Root backpack electric fishing machine is utilised it includes a “lamprey” setting that automatically pulses the current. This setting, however, drains lead-acid batteries in minutes and lithium batteries are needed to effectively fish using the lamprey setting.

Set the EFM300 on 60 pps with a 3 ms pulse width. Test the electric fishing machine outside of the selected survey reach in each stream to determine the appropriate voltage. The voltage will depend on conductivity but will generally be between 100-300v. Ensure settings are such that larval lamprey leaving the sediment are stunned to the point of stopping swimming when the anode is near and the machine is on. If lamprey are not captured/present during testing, ensure invertebrates or small fish species are being adequately stunned.

Using the habitat and data sheets in Appendices E & F:

1. In each tributary stream, identify at least a 50 m reach with suitable Type I and Type IIA habitat (see Appendix E). Use aerial images from Google Earth to pre-select suitable sites and ground-truth habitat upon arrival. Select reaches that contain the largest amount of Type I habitat within the stream. These fine silt/sand habitats can be visibly recognised as light brown coloured areas along river margins, inside meanders, backwaters, confluence of side channels and downstream of large structures (e.g., wood and boulders; Figure 6-1).

Figure 6-1: Aerial image from Topo GPS (phone app) showing likely Type I habitats.

2. The number of reaches to be surveyed in each stream will be contingent upon stream length and the availability of Type I habitat. A rough guide is:
    - If streams are < 2 km in linear length and do not have prevalent Type I habitat, then select one reach. If Type I habitat is prevalent and 10 plots can be found in a 50 m reach, then survey two reaches.
    - If streams are > 2 km in linear length, then select two reaches.
3. **Reach length** is contingent upon availability of Type I and IIA habitat. **A minimum of 50 m** is to be surveyed but **length can extend to any distance if larval habitat is sparse**. If this occurs continue fishing until the minimum plot number (Step 4) is achieved and also record the final reach length.
4. In **Type I and Type IIA habitat, plots of between 1 and 1.5 m^2** will be selected across the reach and electric fished. Where possible fish **10 x Type I habitat plots and 5 x Type IIA**

habitat plots. If Type I and IIA habitats are not consistently present in the 50 m reach then the number of plots fished can be reduced to **a minimum number of 5 x Type I habitat plots and 3 x Type IIA habitat plots fished**.

5. **Extend reach length if needed** to obtain the minimum number of plots.

6. Visually **estimate and record the proportion of Type I, Type II and Type III habitat across the whole reach** surveyed.

7. Use a 3 m long weighted rope with 500 mm increments clearly marked to delineate each plot and enable the area fished to be calculated (Figure 6-2 & Figure 6-3). As the size and shape of each Type I habitat varies, **shape the fishing area using the weighted rope to what is feasible for each plot, aiming for between 1 and 1.5 m^2**. For example, plots may consist of 2.5 x 0.5 m = 1.25 m^2, 1.5 x 1 m = 1.5 m^2, 1 x 1 m = 1.0 m^2 etc (Figure 6-3). Make sure the length and width of each plot are multiplied together correctly to calculate total area fished for each individual plot.

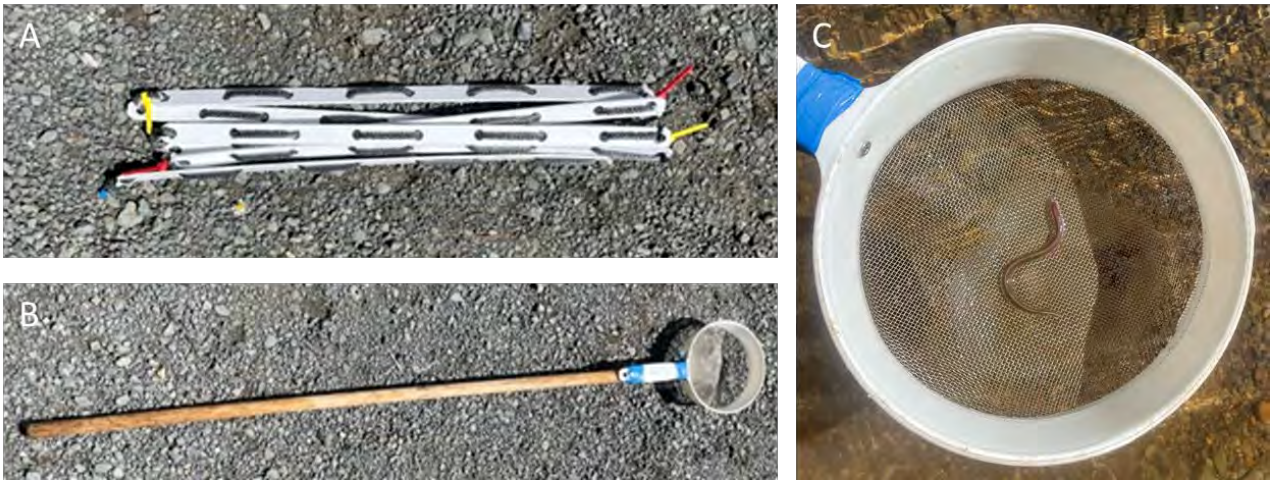

Figure 6-2: Recommended equipment for delineating plot and capturing lamprey. A, 3 m long weighted rope with articulated lengths of 500 mm. B, Dip net made from a kitchen sieve and broom handle. C, close up of sieve with an ammocoete inside. Mesh size is approximately 1 mm x 1 mm (l x w).

8. **Each plot will be fished using manual pulsing (c. 5 seconds on and 2 seconds off) for a total of 300 seconds**. In each 5 second pulse of the machine, sweep the entire plot area to ensure that all habitat within the plot is fished for 300 seconds (rather than splitting time on subsections within the plot). To maximise capture of larvae whilst minimising effects on non-target species outside of the plot, **place the cathode in a zig-zag formation along the base of each plot** (Figure 6-4).

9. Use a dip net with a maximum mesh size of 1 mm x 1 mm (length and height of mesh; Figure 6-2) to capture larvae. Lamprey larvae can disappear rapidly after exiting the substrate so having 1 – 2 dedicated catchers is recommended to quickly target larvae appearing in all sections of the plot (Figure 6-4).

Figure 6-3: Examples of Type I (left and centre) and Type II (right) habitat with different shaped plots fished.

Figure 6-4: Set-up of fishing plot showing zig-zag formation of the cathode along the base of weighted rope. Lamprey larvae are quick to appear then dart away so having dedicated catchers alongside the fisher is an effective way to quickly capture larvae from all sections of the plot.

10. **For each plot, record the total number of larval lamprey collected in each 60 seconds fished across the 300 seconds fishing time** (as per the number of larvae data sheet in Appendix F). Also record missed lamprey for each 60 seconds fished (i.e., larvae observed exiting the sediment but not captured).

11. **For each reach, measure the total length of 50 larvae and the weight of 25 larvae**. **Ensure the minimum and maximum sized larvae captured are measured and weighed** (see Biological data sheet in Appendix F). Make sure those captured from Type I and Type IIA habitat are kept separate to ensure lamprey size and weight can be recorded against habitat type.

12. Larvae can be collated across each plot fished (within a habitat type) and measured and weighed at the end of fishing the survey reach or they can be processed after each plot fished. Using the latter method, larvae can be returned to their capture location.

13. Processing larvae without anaesthetic is recommended as it is quicker and less invasive for the larval lamprey. We recommend using a photarium to measure unanaesthetised larvae (Figure 6-5). To enable unanaesthetised larvae to be accurately weighed, place each ammocoete in a small pottle and utilise scales accurate to 0.01 g (Figure 6-5). The larvae chosen for measuring should be a representative mix of those drawn from Type I and Type IIA habitats.

14. If using a photarium, larvae swim rapidly at first but will quickly settle to lie flat on the base of the tank. Always ensure the photarium is placed on the ground on a solid surface as when the photarium is held, larvae can take an extended time to settle.

Figure 6-5: Measuring and weighing larval lamprey without anaesthetic. A, NIWA Photarium containing a live ammocoete and macrophthalmia. B, pocket scales and tube for containing live ammocoete.

6.2.1 Density and condition estimates

Lamprey density estimates for the reach can be calculated for each habitat type (Type I or IIA) by dividing the total number of lamprey observed in Type I and Type IIA habitats (summing captured and missed lamprey counts) by the total area fished for each habitat type.

The following lamprey specific condition factor (modified from Fulton's condition factor) can be calculated for all lampreys with length and weight measurements (Lampman et al. 2016):

$K = W \times 10^5 / L^{2.6}$

where, K = lamprey specific condition factor, W = weight (g) L = total length (mm).

6.3 Spot fishing for determining the spatial distribution of larval lamprey

The standardised sampling method can also be used to investigate the spatial distribution of larval lamprey within a catchment. This method can be used as an alternative to eDNA and POCIS sampling to locate key streams utilised by lamprey. Data to date indicate that electric fishing Type I habitats is a more sensitive method of locating low density lamprey populations than using passive tools. **Surveys should be undertaken using a Kainga EFM300** backpack electric fishing machine to ensure density estimates are comparable to other sites across New Zealand. The machine settings and equipment are the same as for that outlined in Section 6.2.

Visually assess all habitat into Type I, II and III as outlined in Appendix E. **Fish only Type I habitats**, where larval lamprey densities should be the highest.

6.3.1 Survey protocol

1. For each stream, **record the GPS coordinates for the start and finish of the reach fished**.
2. Start as close to the base/confluence of the stream as feasible and move in an upstream direction.
3. At each area of Type I habitat located, fish one plot.
4. Use the 3 m long weighted rope with 500 mm increments to delineate a plot **aiming for around 1 m^2.** Sample larvae by manually pulsing the electro-shocker, with c. **5 seconds on and 2 seconds off frequency**.
5. **Fish each plot for 120 seconds**. Fishing time is reduced over the monitoring methodology (Section 6.2) to enable more ground to be covered and when mapping the distribution of lamprey, population estimates are less vital.
6. To enable density estimates to be calculated it is *vital* that **the area of each plot and the fishing time are recorded along with the number of lamprey observed/captured**.
7. Measure a representative selection of ammocoetes and macrophthalmia, counting any that are not measured. Processing larvae without anaesthetic using a photarium is recommended as it is quicker and less invasive for the larval lamprey (Figure 6-5).
8. Continue fishing each area of Type I habitat until either a larval lamprey is located or 500 m of stream has been traversed. There is no set number of plots to fish or maximum reach length for each stream so a larger distance can be surveyed. However, we recommend a minimum distance of 500 m is surveyed in each stream.

In large mainstem rivers or streams where water depths are above those suitable for electric fishing, the spot fishing method outlined above should still be carried out in Type I habitats along the river margins. Keep a note of which streams have all Type I habitats accessible versus those where only marginal waters can be surveyed.

6.4 Data collected to date

Presently, 45 sites have been fished across 27 streams to test the standardised fishing method. Of these streams, 13 are in the Waiau River catchment, Southland, and 14 are in the Waipa River

catchment, Waikato. For the purposes of this report, the figures presented below are from the Waipa River streams, which were carried out using DOC and NIWA funding.

In 2022, one key finding was that during the fifth minute of fishing, around 12% of the total catch of lamprey were still being drawn from the sediment, which suggests that 300 seconds of fishing time per plot is not effectively determining the population estimate for the plot (Figure 6-6). In 2023, further trials were carried out in eight streams within the Waipa River catchment that had varying ammocoete densities to determine how many minutes need to be fished to reliably return a zero catch. Results were similar for Type I and II habitats, with up to 16 minutes of fishing required to effectively return a zero catch in two consecutive minutes of fishing (Figure 6-7). Data indicates that 300 seconds of fishing represents approximately 86% of the total catch present in the plot (Figure 6-7).

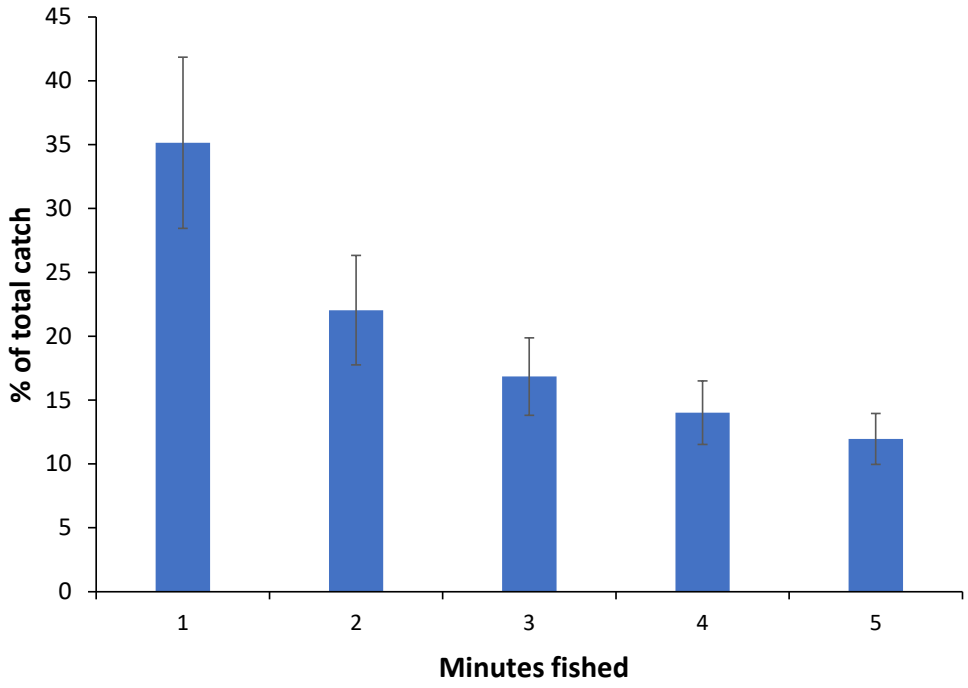

Figure 6-6: Mean percentage of ammocoetes captured during each minute of fishing Type I habitats across all stream types. Error bars represent ± 1 standard error.

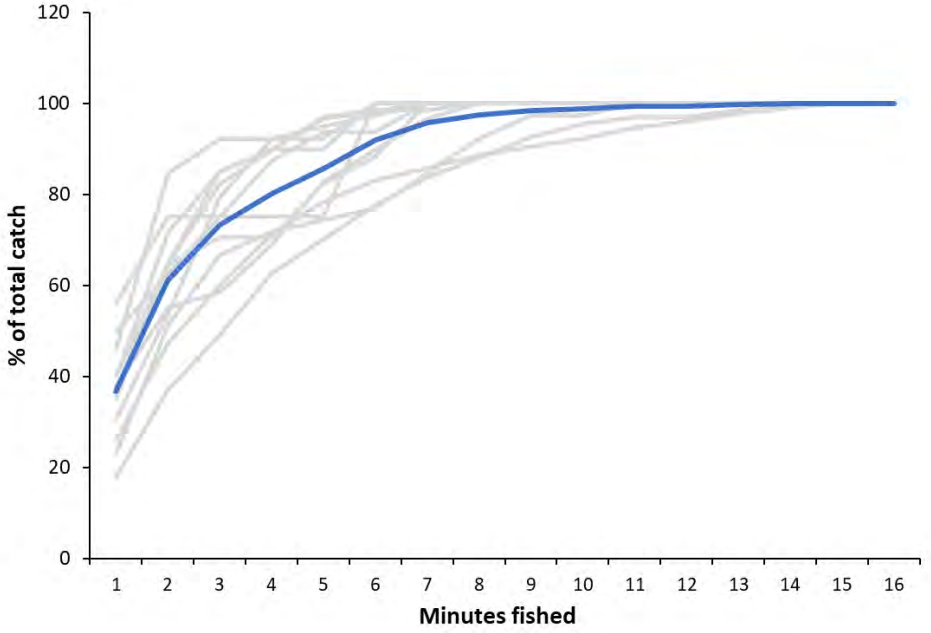

Figure 6-7: Cumulative percentage of larvae captured per minute fishing in Type I and II habitats. Individual site data shown in grey.

The test streams have included a range of large and small tributaries, with mean wetted widths between 2 and 28 m. The densities of juvenile lamprey based on habitat type and the condition factor of both ammocoete and macrophthalmia life stages are shown in Figure 6-8 & Figure 6-9. Presently, no data analyses have been carried out to examine juvenile density and size relative to the habitat variables measured.

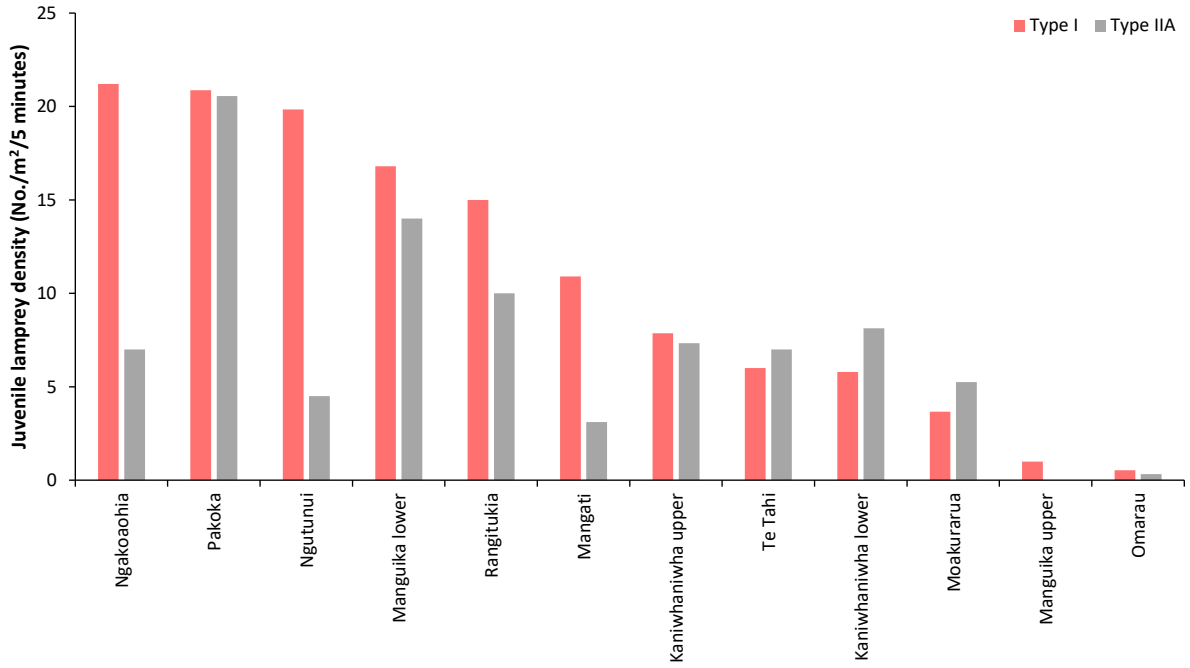

Figure 6-8: Density of juvenile lamprey (ammocoete & macrophthalmia combined) captured in each stream reach for Type I and Type IIA habitats fished. In each catchment streams are ordered by highest to lowest density of lamprey in Type I habitats.

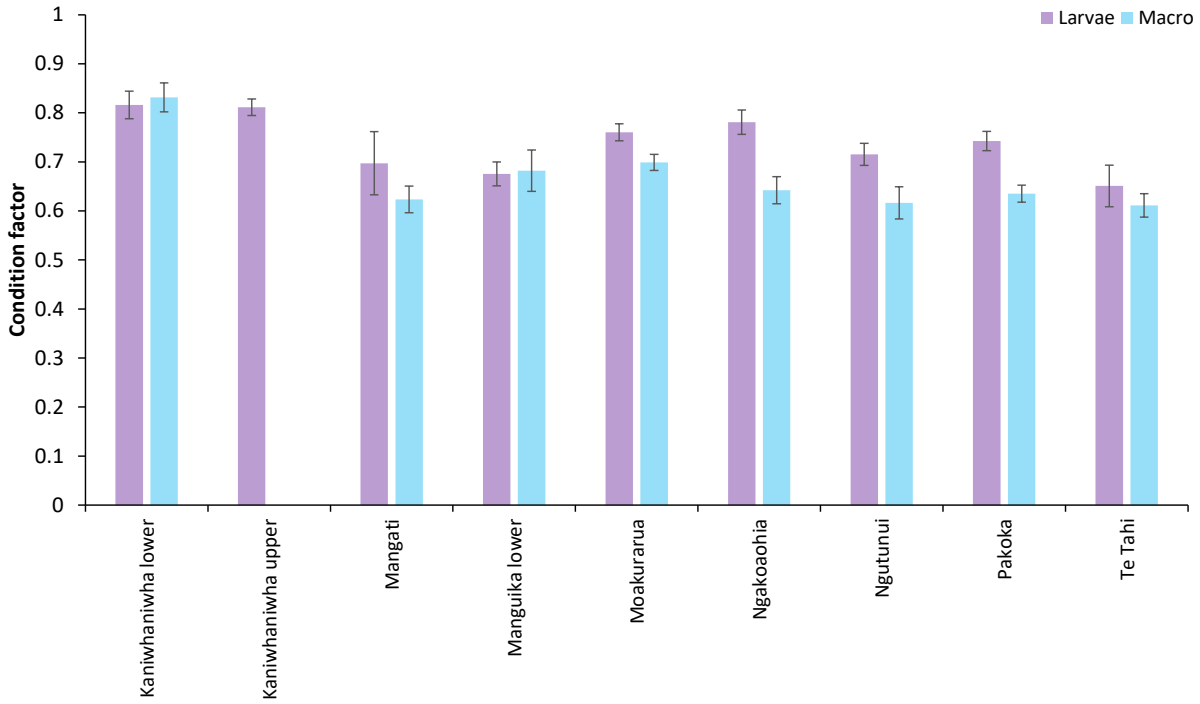

Figure 6-9: Average condition factor of ammocoetes (larvae) and macrophthalmia (macro) captured across all habitat types in the survey streams. Error bars represent ± 1 standard error. Data represent streams where greater than five larvae or macrophthalmia were captured in the reach fished.

6.5 Data repository

All survey data should be submitted to the New Zealand Freshwater Fish Database (NIWA NZFFDMS). The New Zealand Freshwater Fish Database (NZFFD) records the occurrence of fish in fresh waters of New Zealand, including major offshore islands. Data stored include the site location, the species present, their abundance and size, as well as information such as the fishing method used and a physical description of the site.

To contribute new freshwater fish records request an account by sending an email to fwdba@niwa.co.nz.

To develop new, and improve upon existing species distribution models, NIWA is interested in collecting raw standardised fishing data for lamprey (using either the spot or monitoring methodologies). Please contact **Cindy.baker@niwa.co.nz** if fishing data can be shared and utilised by NIWA.

Summary & conclusions

7 Summary and Conclusions

In order for DOC and other organisations (e.g., regional councils) to meet their legislative requirements and to protect threatened lamprey populations and spawning sites, knowledge of the spatial distribution of lamprey within catchments and the habitats utilised by both larvae and adult life stages is crucial. As lamprey is a threatened species, monitoring population structure and abundance will be necessary to ensure management interventions and conservation measures are effectively protecting populations.

This manual provides both passive and active methods for identifying critical freshwater habitats utilised by lamprey and monitoring temporal trends in the population. For all objectives, knowledge of the spatial distribution of lamprey within the catchment is necessary, which is best achieved using a combination of eDNA sampling, POCIS deployments and spot electric fishing. Where management objectives are to identify and protect lamprey habitats, pinpointing lamprey spawning sites is not always necessary. Instead, standardised assessments of larvae is recommended as larval density estimates are a valid proxy for inferring the location of spawning reaches and spawning success, and to monitor spatial and temporal trends in lamprey abundance.

7.1 Protection and conservation of lamprey populations

Policies and Plans will mandate how protection of threatened species is implemented by different organisations. However, based on the unique ecology of lamprey, we recommend using a stream wide approach to protection and conservation of populations. By using the methods outlined in this manual, identifying tributaries with the highest abundances of larval lamprey and then protecting the stream from its lower to upper limit of larvae will likely offer a greater level of protection than protecting sections of streams across a larger number of tributaries. This is because all habitat utilised by adult lamprey during their maturation in freshwater is pertinent to their spawning success. Unlike other fish species whose feeding and rearing areas can be delineated from their spawning habitat, in the c. 18 months adult lamprey spend in freshwater maturing and spawning, they will move between habitat types as they migrate to spawning locations. Consequently, after selecting a spawning stream, all habitats utilised should be considered under the umbrella of spawning habitat for this unique species. In addition, spawning sites do not show spatial clustering within a spawning stream. Consequently, within any tributary stream used for spawning, the distribution of larval lamprey will essentially mirror the distribution of spawning adults and the size structure of larvae can be used to infer proximity to spawning areas. Finally, as lamprey are obligate migratory species, ensuring free passage between spawning and rearing sites in fresh water and the sea is critical to protecting this threatened species.

8 Acknowledgements

We would like to thank all Waikawa mana whenua involved in catching, tracking and locating lamprey spawning sites in the Waikawa River, especially Jeremy Leith and Marcus Tuwairua. We also wish to thank tangata whenua Riki Parata and Calvin Russell. We also appreciate the support of local landowners George and Jeanette Buckingham, Geoff and Rachel Buckingham and Darryl and Carmen Stratford. We also wish to thank Jane Kitson as a project partner in the MBIE Endeavour Habitat Bottlenecks research programme. We would also like to thank reviewers of the report, particularly Paul Franklin and Erica Williams (NIWA) and Emily Funnell and Sjaan Bowie (DOC).

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Appendix A eDNA methodology

A.1 Active sampling protocols

eDNA Mini Kit Instructions

WILDERLAB

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

1

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

2

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

3

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

4

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

5

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

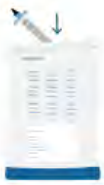
6

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

7

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

8



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

9

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

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

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

Send the samples by standard courier to:

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

Small packages can be sent by post to:

Wilderlab NZ Ltd
PO Box 15059
Miramar
Wellington 6243

A.2 Passive sampling protocols

eDNA Manifold-mount Instructions

WILDERLAB

Manifold set up:

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

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

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

Deploying the passive sampler:

1

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

2

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

Leave the sampler deployed for 24 hours.

Retrieving the passive sampler:

1

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

2

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

3

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

4

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

5

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

6

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

7

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

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

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

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

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Small packages can be sent by post to:

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PO Box 15059
Miramar
Wellington 6243

A.3 Purchasing eDNA kits and analyses

Wilderlab NZ limited are a key provider for the sampling kits, instructions for use, and analysis for eDNA in New Zealand. For general enquiries email info@wilderlab.co.nz. Sampling kits can be ordered through the Wilderlab website (wilderlab). Presently, the comprehensive eDNA package is $290 each + GST. This includes the sampling kit and a panel of assays for detecting fish, mammals, birds, reptiles, amphibians, macroinvertebrates, land and aquatic plants, algae, zooplankton, microorganisms and the invasive golden clam (*Corbicula* sp.). As six replicates are recommended per site (Melchior & Baker 2023), a discounted rate of $1540 + GST is available for 6-replicate sampling kits[5]. This applies to both syringe kits and passive samplers. Results are provided in an Excel spreadsheet as eDNA sequence counts and are typically available within 2-3 weeks. A database of publicly available eDNA data can be viewed at Explore — wilderlab.

Replicate number

For both active and passive eDNA sampling, six sample replicates should be undertaken at each site.

Appendix A provides field methods for active syringe sampling and deploying and retrieving eDNA passive samplers. Presently, data indicates a similar performance between active syringe and passive samplers (Melchior & Baker 2023), with there being no clear advantage to using passive sampling for cryptic species such as lamprey.

[5] This represents Wilderlab's commercial rate at 13 June 2024, temporal variations in costs could occur before version updates to the present manual.

Appendix B POCIS deployment methodology

B.1 Polar Organic Chemical Integrative Sampler (POCIS) methodology

The POCIS samplers absorb lamprey pheromones released by stream resident fish. The sampler is encased in a stainless-steel housing to protect it from physical debris (Figure B-1).

Figure B-1: POCIS samplers used for stream sampling of lamprey pheromones.

Methodology.

1. A 1.5 m or 1.8 m waratah is driven into the stream bed in flowing water where water velocities are between 0.3 and 0.6 m s^{-1} (Figure B-2).
2. To ensure the sampler remains underwater but is not destroyed by fast water velocities during deployment, it is important to assess how an increase or decrease in flow will affect the water depth and velocity at each site.
3. The waratah is placed at an angle of approximately 30 degrees in the direction of the stream flow (angled downstream to reduce debris collecting on the waratah; Figure B-3).
4. Each sampler is attached (using cable ties) at mid-water column on the downstream side of the waratah (Figure B-3). It is important that the sampler is tightly attached with no wobble (see Appendix B-2). Snip ends of cable ties to reduce debris attachment points.
5. Gloves are not necessary when deploying and retrieving the sampler, however, always hold the sampler using the solid metal plate and do not touch the steel mesh area.
6. **For each sampler deployed, record the date, time and GPS location**.
7. Leave each sampler in-situ for between **2 and 3 weeks**.
8. Upon collection, for each sampler record the date and time of retrieval.
9. **Place each sampler in a separate labelled (site name and date) plastic bag and immediately store on ice. Also include a waterproof label inside the sampler bag to ensure site details are not removed by water during transit.**
10. Retain all samplers in the -20°C freezer until all are collected (if collections are across subsequent days). A -20°C freezer is a standard household freezer; however, frost-free freezers are not suitable as they modulate temperature to prevent ice build-up. Therefore, a non frost-free or commercial freezer is necessary.
11. **Courier all samplers back to the NIWA Hamilton laboratory on ice in a chilly bin clearly marked as perishables (not a courier bag).**

Figure B-2: Picture of POCIS sampling position within small streams (left top and bottom) and a large river (top right). Cable tie configuration depicted bottom right. White arrow indicates direction of flow.

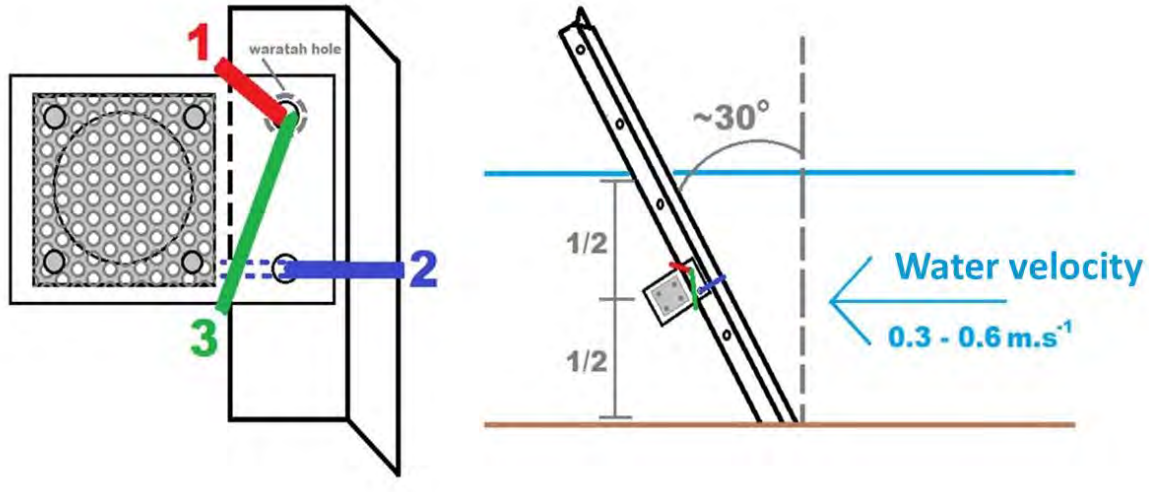
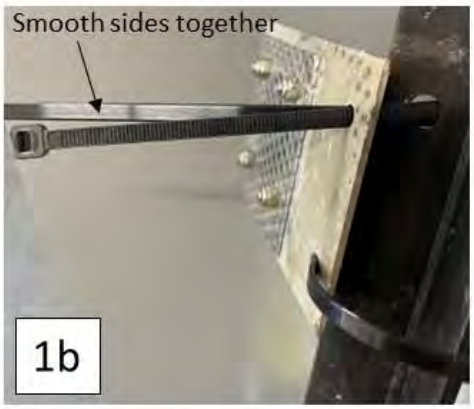
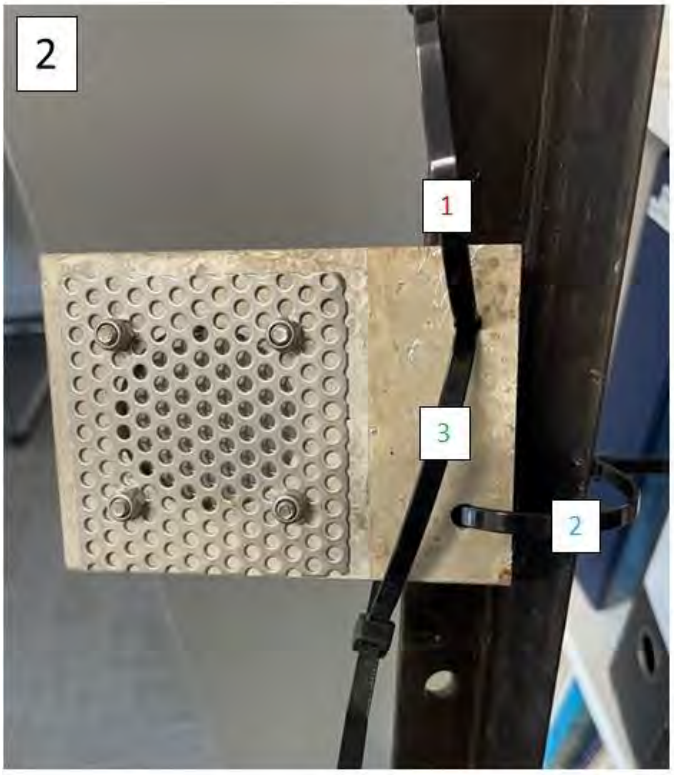


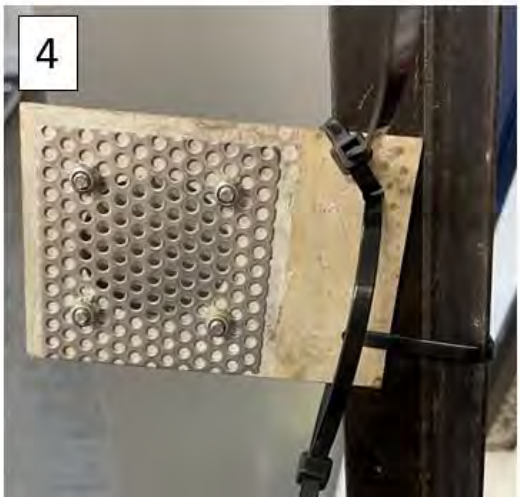
Figure B-3: Schematic of POCIS sampling configuration within a stream. The order of cable tie attachment to ensure a tight, secure fit is shown.

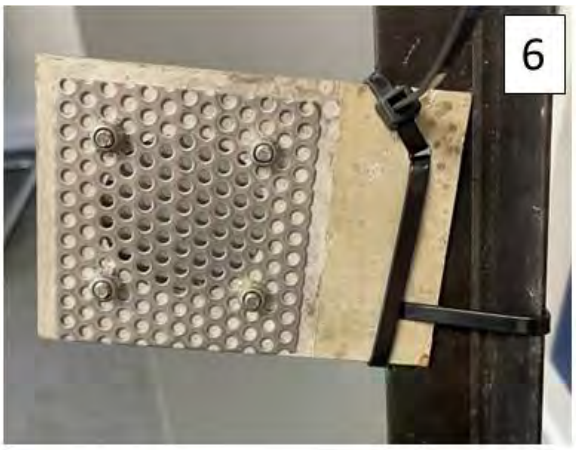
B.2 Fastening POCIS samplers



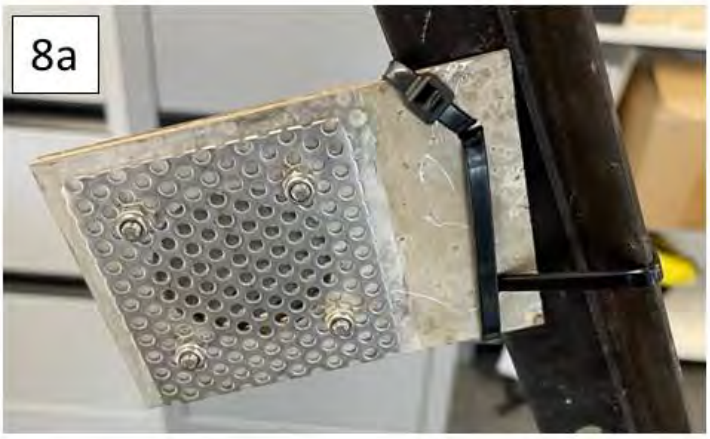


- Thread all three cable ties through the sampler (1a & b). Ensure the two ties put back-to-back through the top hole have the smooth sides facing each other. Cable ties labelled by number and colour as per Figure B-3.
- Hold the sampler underwater and thread the two back-to-back ties through a hole in the waratah approximately mid-water column (see Figure B-3).
- Loosely tie cable tie 2 as pictured in plate 1a and b. Note this doesn't thread through a hole in the waratah but ties around the waratah.
- Loosely tie cable tie 1 and 3 as pictured in plate 2.
- Holding the sampler firmly in place as shown in plate 3, tighten cable tie 1.
- Keeping holding the sampler firmly in place while tightening cable tie 2 around the waratah (plate 4).





- After tightening cable tie 1 and 2 the sampler should sit directly in line with the waratah flange it is tied against (plate 5).
- Tighten cable tie 3 (plate 6) and ensure the sampler is tight against the waratah (plate 7). There should be no up and down or side to side movement of the sampler with moderate hand pressure.
- Snip the ends of all three cable ties to reduce debris attachment points.
- Plate 8a & b show the fully secured sampler.



B.3 Purchasing POCIS samplers and analyses

NIWA prepare and breakdown the POCIS samplers, providing a time weighted average concentration of PS for each sampler. POCIS can be ordered from Cindy Baker (cindy.baker@niwa.co.nz), with instruction for use provided in Appendix B.1. Presently, **each POCIS sampler is $500 + GST**. Results are provided in an Excel spreadsheet.

As the POCIS samplers are deployed for in-situ, they account for spatial and temporal variability in the pheromone signal and only one POCIS is required at each site. If larger mainstems of rivers are being sampled as check points, deployment of two POCIS (one on each side of the river) is recommended to provide a back-up in case one is lost or damaged during the deployment period.

POCIS are stable at room temperature but are best stored in the fridge until use. They are activated in water and once submerged they cannot be removed from the water until retrieval. Once purchased it is recommended the POCIS are deployed either that summer or the following summer, i.e. POCIS are usually sent October/November and if received in 2024 they should be deployed by March 2026.

The POCIS casings are purpose built and expensive to manufacture. For any POCIS ordered, please return the casings even if the sampler isn't set within the recommended timeframe or the sampler is damaged/unable to be used.

B.4 Measuring water velocity

If you do not have a velocity meter then the float method of estimating the average water velocity at a site can be used. The time it takes for a float to pass from an upstream point to a downstream point is measured using a stopwatch (Figure B-4). Mandarins or oranges make good floats as they sit in the water column with only a small portion protruding above the water surface and so are less influenced by friction at the air-water interface. The water velocity at the site is then calculated as follows: Water velocity (metres per second) = distance travelled (metres) ÷ time taken for float to travel the set distance (seconds). Repeat the exercise three times and calculate the average water velocity.

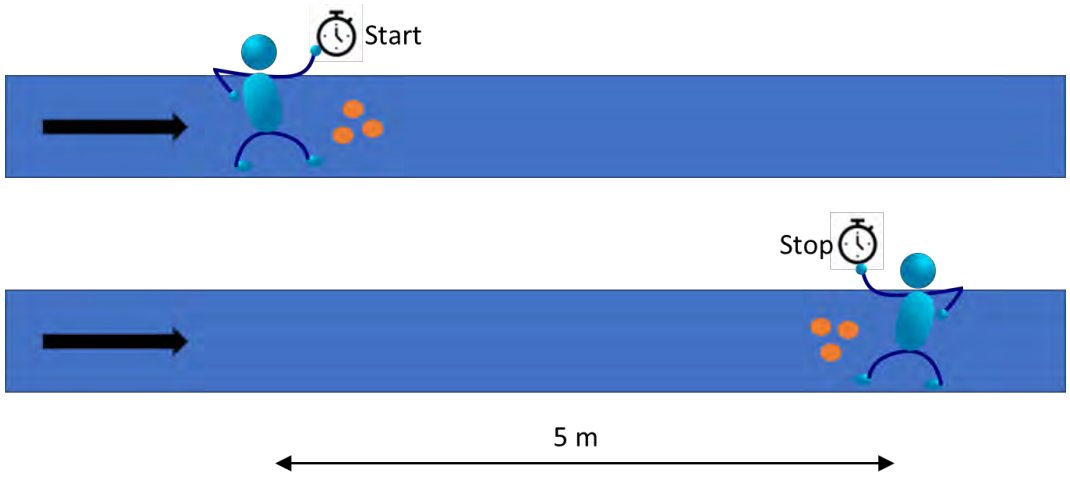
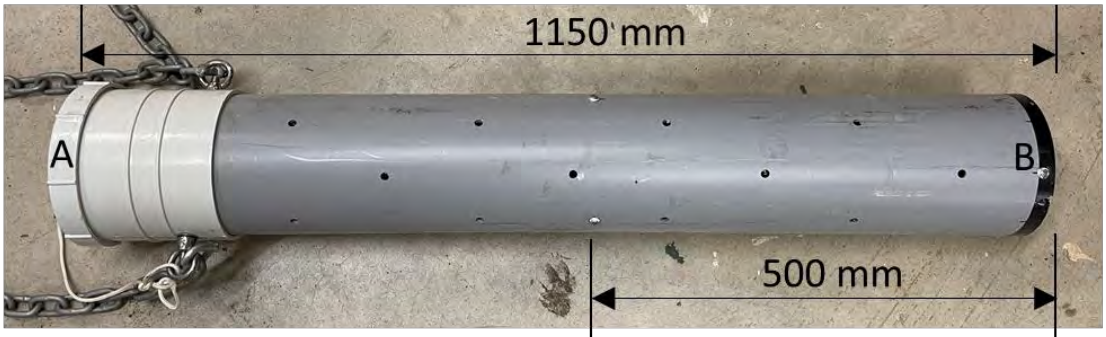


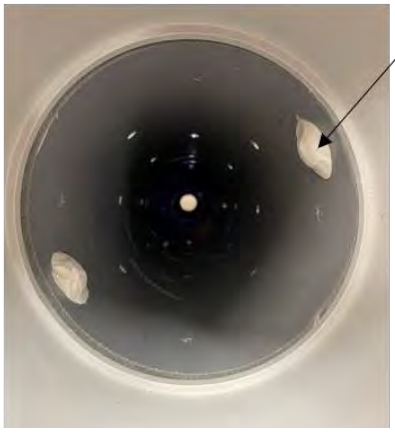
Figure B-4: Illustration of how to use the float method of measuring water velocity. The floats are released individually by the upstream person who starts the stopwatch simultaneously as a float is released. The time taken for each float to reach the other person is recorded. This is used to calculate water velocity.

Appendix C Lamprey pipe trap design and dimensions



- Screw cap pressure pipe end glued (PVC cement) to 150 mm diameter PVC pipe
- Use rope to attach the lid to the trap to prevent loss during emptying
- Eyelets bolted on each side of the trap to secure chain for attachment in the stream
- Ensure all metal is stainless steel as lamprey are very sensitive to elemental metals in the water
- Funnels with 40 mm openings bolted into trap entrance and at 500 mm up inside the pipe
- Holes drilled throughout trap and lid to provide continuous water flow
- Cover all nut and bolt ends in silicon to prevent lamprey damaging themselves inside the trap

Inside trap at capped end "A"



Inside trap entrance "B"

Appendix D NIWA Portable PIT antenna design

We retained the wand casing from the Oregon RFID product as it is well balanced, rugged and easy to use. The read range of the antenna based on tag orientation is outlined in Figure D-1. In general, the larger the oval antenna, the large the read range will be for the tag in both orientations. To improve the detection efficacy of the 12 mm tag, NIWA constructed the antenna from four loops of 12 AWG high power cable. Two different sized antennae are used, an oval 65 cm x 54 cm for smaller streams (Figure D-2A), and a 75 cm x 60 cm for larger streams.

The reader and logger unit are also custom made to enable the GPS location of each tag to be automatically recorded and the tag number to be displayed on a screen during tracking.

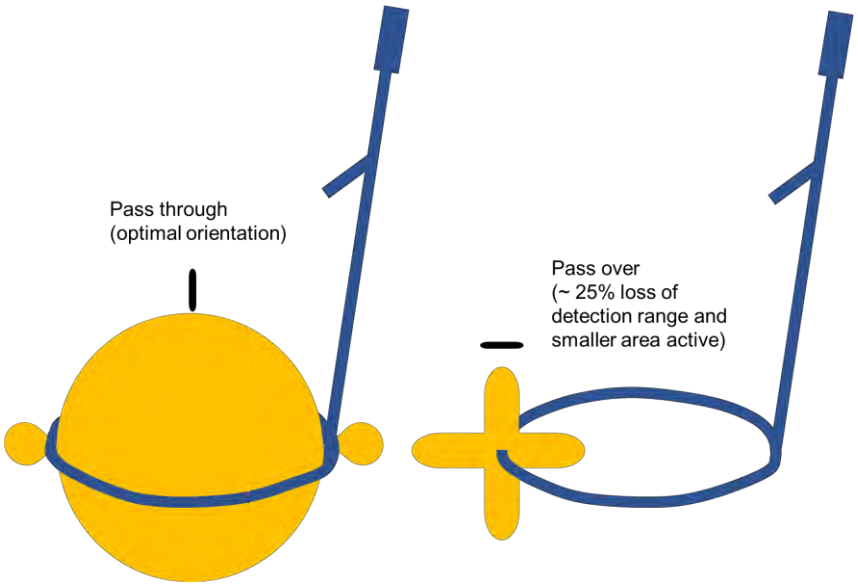


Figure D-1: Detection field for the HDX PIT tag in optimal and sub-optimal orientations.

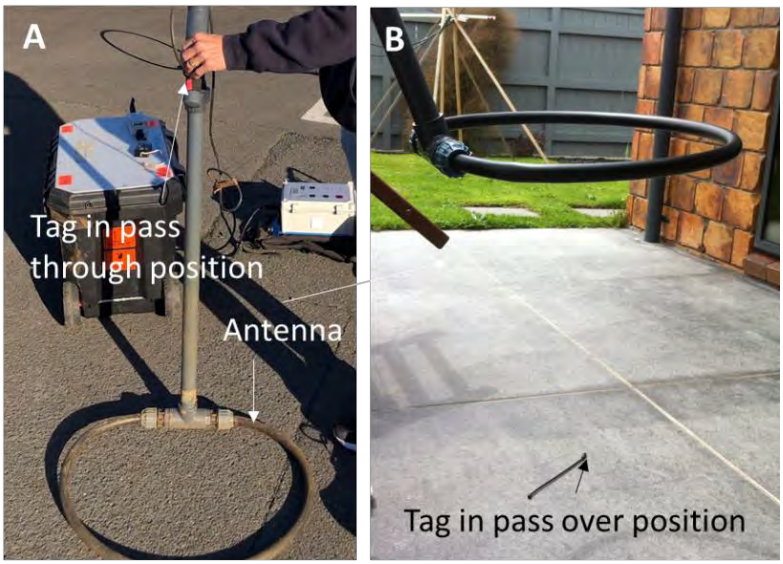


Figure D-2: NIWA's portable PIT system. A, small antenna (65 cm x 54 cm) showing the 12 mm PIT tag read range when held in a vertical position perpendicular to the antenna (termed pass through and it is the optimal direction). B, 12 mm PIT tag read range when in a horizontal position parallel to the antenna (termed pass over and is a sub-optimal direction).

Appendix E Habitat selection for larval electric fishing surveys

A random selection of survey sites would provide the ability to determine what habitat is present versus what habitat larval lamprey select, but it would also increase the number of sites needed to monitor lamprey. Consequently, based on habitat suitability studies, international approaches target preferred larval lamprey (Type I) habitat within the reach of interest (Reid & Goodman 2015). A targeted approach optimises larval lamprey capture, is efficient in evaluating lamprey distributions, and can be used to monitor relative abundance over time (Lumley et al. 2020).

Following international approaches, habitat is first visually categorised into Type I, Type II and Type III (Figure E-1 and Figure E-2) based on the criteria of Slade et al. (2003):

- Type I habitat is regarded as “optimal” and is located primarily in the depositional zones preferred by larvae, consisting primarily of a mixture of sand and fine organic matter.
- Type II habitat is considered “acceptable” and may contain some gravel, is utilised by some larvae for burrowing, but is inhabited at much lower densities. It also often consists of shifting sand, which can be fine sediment without "coarse" substrate (i.e., similar to Type I), but is typically not stable (moves and shifts around every year) and the density of larvae tends to be lower than true Type I. Type II is also any habitat that has a mix of Type I and III. Consequently, it is further broken into A and B categories:
  - Type IIA has a mix of fine and coarse sediment, but the % of fine is higher than the % of coarse (surface area).
  - Type IIB has a mix of fine and coarse sediment, but the % of coarse is higher than the % of fine (surface area).
- Type III habitat is considered “unacceptable” because larvae are unable to burrow into it and includes substrates such as hard packed clay, gravel, cobble, boulder and bedrock.

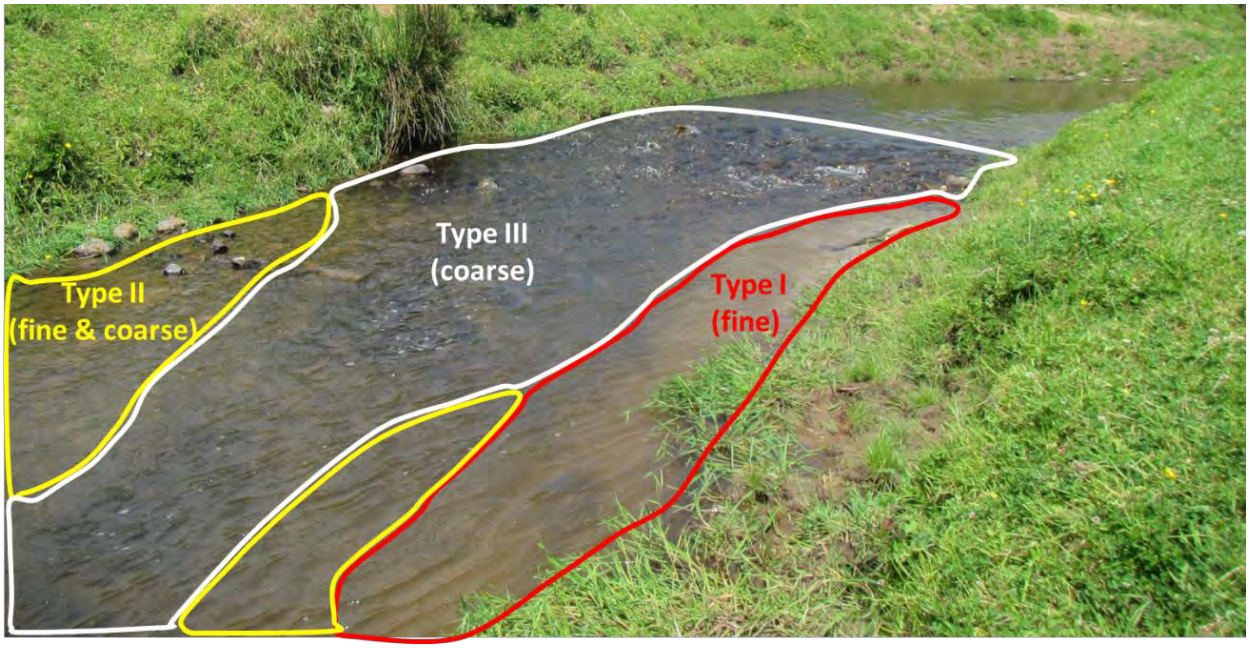


Figure E-1: Type I, II and III habitat along a length of stream.

Figure E-2: Examples of Type I, II and III habitats. The Type II displayed is IIA as fine sediment is dominant over coarse sediment.

Appendix F Electric fishing datasheets

NIWA Larval/Juvenile Lamprey Monitoring Survey

Date:	**Start Time:**	**End Time:**	**Crew:**
Stream:		**Reach length (if not 50m):**	
Location Description (incl. land owner contacts): ____			
Measurements	**Type I Habitat**	**Type I Habitat**	**Type IIA Habitat**
Area, Time (s), Location	_____m², _____, TL / M / TR	_____m², _____, TL / M / TR	_____m², _____, TL / M / TR
# Missed, # Captured	_____ missed, _____ capt.	_____ missed, _____ capt.	_____ missed, _____ capt.
Detritus %F %C, % Aq. Veg.	F____ %, C____%,_____%	F____ %, C____%,_____%	F____ %, C____%,_____%
Sed. Type (1=◯, 2=X)	Clay / Silt / Sand	Clay / Silt / Sand	Clay / Silt / Sand / Coarse
Max Depth: Sed.(m), Water(m)	________m,_________m	________m,_________m	________m,_________m
Area, Time (s), Location	_____m², _____, TL / M / TR	_____m², _____, TL / M / TR	_____m², _____, TL / M / TR
# Missed, # Captured	_____ missed, _____ capt.	_____ missed, _____ capt.	_____ missed, _____ capt.
Detritus %F %C, % Aq. Veg.	F____ %, C____%,_____%	F____ %, C____%,_____%	F____ %, C____%,_____%
Sed. Type (1=◯, 2=X)	Clay / Silt / Sand	Clay / Silt / Sand	Clay / Silt / Sand / Coarse
Max Depth: Sed.(m), Water(m)	________m,_________m	________m,_________m	________m,_________m
Area, Time (s), Location	_____m², _____, TL / M / TR	_____m², _____, TL / M / TR	_____m², _____, TL / M / TR
# Missed, # Captured	_____ missed, _____ capt.	_____ missed, _____ capt.	_____ missed, _____ capt.
Detritus %F %C, % Aq. Veg.	F____ %, C____%,_____%	F____ %, C____%,_____%	F____ %, C____%,_____%
Sed. Type (1=◯, 2=X)	Clay / Silt / Sand	Clay / Silt / Sand	Clay / Silt / Sand / Coarse
Max Depth: Sed.(m), Water(m)	________m,_________m	________m,_________m	________m,_________m
Area, Time (s), Location	_____m², _____, TL / M / TR	_____m², _____, TL / M / TR	_____m², _____, TL / M / TR
# Missed, # Captured	_____ missed, _____ capt.	_____ missed, _____ capt.	_____ missed, _____ capt.
Detritus %F %C, % Aq. Veg.	F____ %, C____%,_____%	F____ %, C____%,_____%	F____ %, C____%,_____%
Sed. Type (1=◯, 2=X)	Clay / Silt / Sand	Clay / Silt / Sand	Clay / Silt / Sand / Coarse
Max Depth: Sed.(m), Water(m)	________m,_________m	________m,_________m	________m,_________m
Area, Time (s), Location	_____m², _____, TL / M / TR	_____m², _____, TL / M / TR	_____m², _____, TL / M / TR
# Missed, # Captured	_____ missed, _____ capt.	_____ missed, _____ capt.	_____ missed, _____ capt.
Detritus %F %C, % Aq. Veg.	F____ %, C____%,_____%	F____ %, C____%,_____%	F____ %, C____%,_____%
Sed. Type (1=◯, 2=X)	Clay / Silt / Sand	Clay / Silt / Sand	Clay / Silt / Sand / Coarse
Max Depth: Sed.(m), Water(m)	________m,_________m	________m,_________m	________m,_________m
GPS (reach centre)	Lat: ____________________	Long: ____________________	
Wetted width (m)	1	2	3
Bankfull width (m)	1	2	3
Flow: EL / L / M / H	50 m reach: %Type I_________%Type IIA_________IIB__________%Type III__________		
Photos: Upstream____________, Downstream____________, Lamprey____________, Other (__________)___________			
Water Quality / E-Fishing Parameters			
Cond.________µS/cm, Temp________, Voltage ________, Pulse Rate (pps / Hz) _________, Pulse width (ms) ____________			

Comments:

Number of larvae per 60 seconds

Number of larvae :					
Plot	**60 sec**	**120 sec**	**180 sec**	**240 sec**	**300 sec**
Type I 1	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss
Type I 2	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss
Type I 3	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss
Type I 4	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss
Type I 5	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss
Type I 6	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss
Type I 7	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss
Type I 8	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss
Type I 9	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss
Type I 10	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss
Type IIA 1	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss
Type IIA 2	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss
Type IIA 3	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss
Type IIA 4	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss
Type IIA 5	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss	_____capt._____miss

Size and weight measurements of larvae and macrophthalmia

Larval Lamprey Biological Data					Stream _____, Date _____									
Total # of lamprey _____														
#	**Length (mm)**	**Weight (g)**	**Life Stage (L/M)**	**Type I/II habitat**	**#**	**Length (mm)**	**Weight (g)**	**Life Stage (L/M)**	**Type I/II habitat**	**#**	**Length (mm)**	**Weight (g)**	**Life Stage (L/M)**	**Type I/II habitat**
1					26					51				
2					27					52				
3					28					53				
4					29					54				
5					30					55				
6					31					56				
7					32					57				
8					33					58				
9					34					59				
10					35					60				
11					36					61				
12					37					62				
13					38					63				
14					39					64				
15					40					65				
16					41					66				
17					42					67				
18					43					68				
19					44					69				
20					45					70				
21					46					71				
22					47					72				
23					48					73				
24					49					74				
25					50					75				

Protocols

1. Count total # of lampreys (separate totals for each plot).
2. Across all plots, measure length of all or a subsample of 50 (if > 50) larval lamprey (L) (including max & min).
3. Of those, measure 25 weights (including max & min).
4. If some lamprey are counted and not measured then categorise those by size and habiat type below.
5. Measure up to 10 representative lengths & weights of macrophthalmia or those in the process of transforming (M).

Counts:	<50mm	50-80mm	80-120mm	Total
Type I				
Type IIA				

Description of variables measured

General & overall reach data collected	
Data	Description
Date	Date of survey
Start Time	NZST that you arrive at stream survey area (50 m reach)
End Time	NZST that you leave the stream survey area (50 m reach)
Crew	Crew members initials
Stream	Stream name
Location Description	Description to identify the reach and access to site with land marks, etc.
Reach length	Channel length investigated for habitat availability (only if diff. from 50 m)
Centre Point GPS	Latitude, longitude in decimal degrees at centre point of reach
Wetted Width	3 x wetted width (m) based on representative habitats within survey reach
Bankfull Width	3 x measurements of bankfull width (m) based on representative habitats within survey reach
Flow	Flow extremely low (EL), low (L), moderate (M), or high (H) (based on bankfull flow conditions)
50 m Habitat Availability	Total sum of Type I, Type IIA and B and Type III habitat (m^2) within the identified reach. Minimum of a 50 m reach surveyed in each stream
Data collected from each plot fished	
Area (m^2)	Total area electric fished (m^2), aiming for 1 - 1.5 m^2 plots. A minimum of 5 plots in Type I and 3 plots in Type IIA in each reach, aiming for 10 x Type I and 5 x Type IIA plots
Shock Time	Total electrofishing time, 300 seconds per plot, in 60 second allotments
Location	Where site is located, true left (TL), true right (TR) side of stream, or mid-channel
# Caught / Missed [Total]	# captured, # observed but failed to capture (left the survey patches), and grand total. Need a count of total lamprey numbers for each area (plot) fished
% detritus	% fine (powdery brown organic matter) and % coarse (like twigs, leaves) organic detritus within electrofishing area of each plot
% Aquatic Veg.	% area covered by aquatic vegetation within electrofishing area of each plot
Sed. Type (1=◯, 2=X)	Primary (circle) and secondary (x) sediment type at each habitat fished (clay, silt, sand, coarse)
Max Sed. Depth (each plot fished)	Maximum fine sediment depth at the habitat fished
Max Water Depth (each plot fished)	Maximum water depth at each habitat fished
EFM300 settings	Voltage will depend on conductivity but generally between 100-300v, 60 pps, 3 ms pulse width
Lamprey Size Classes (in Data sheet)	Number observed in each size class; Small <50 mm, Medium 50-80 mm, Large 80-120 mm.

Appendix G Equipment list

The following list is not exhaustive but instead highlights key equipment required for each method outlined in the manual. It is expected that each organisation will have different PPE and equipment that is standard for field trips.

G.1 Passive sampling

Desktop assessments

- Access to all records from the NZFFDB (https://nzffdms.niwa.co.nz/search).
- The NZ species DB (Jowett Consulting - NZ Species DB) (if unable to access/use R or ArcGIS platforms)
- The freshwater fish probability of capture model (NZ River Maps)

Field deployments of samplers

POCIS and eDNA samplers

- Chilly bin and ice packs for POCIS deployment and retrieval
- Sledgehammer or waratah driver
- Leather palmed gloves
- 1.5 or 1.8 m waratahs with end caps
- Waratah removal tool for retrieval
- Cable ties, must be 5 mm wide and UV resistant
- Cutting pliers
- Velocity meter (if possible)
- Oranges/mandarins
- Stopwatch
- Measuring tape
- Adjustable spanner (if manifold eDNA passive sampler used)
- Marker pen
- GPS recording unit (e.g. cell phone)
- Zip lock plastic bags and a waterproof marker pen
- Waterproof paper and pencil or cell phone for recording date, time and deployment information

G.2 Determining spawning sites

Capturing adult lamprey

Fyke nets or pipe traps

- Sledgehammer and/or waratah driver
- Leather palmed gloves
- Wooden stakes and/or 1.5 or 1.8 m waratahs
- Waratah removal tool (if waratahs are used)
- 1.5-2 kg lead weights with clips
- Cable ties (UV resistant)
- Cutting pliers
- Rope or chain
- D shackles
- GPS recording unit (e.g. cell phone)

Hand capture at night

- Life jacket
- Head torch
- Cotton gloves (to hold lamprey securely)
- Buckets (20 litre)
- Closable mesh bags for containing lamprey within the bucket
- Portable battery powered aerator for lamprey held in buckets
- Throw rope
- Cell phone (for communication)

Lamprey are best held within the river of capture until tagging. The pipe traps outlined in Appendix C can be easily made into holding flumes by removing the funnels and gluing an end cap to the base.

Tracking and spawning surveys

- Portable PIT system
- GPS recording unit (e.g. cell phone)
- Hand-held proximity PIT reader with stick antenna
- Crowbar (>1.2 m long)
- Spade
- Two endoscope cameras (recommended models are Aerpro Bullant G5000 inspection camera (https://aerpro.com/products/inspection-cameras) and the Teslong NTS300 series endoscopes (https://teslong.com/products/nts300-industrial-endoscope)
- Leather or neoprene gloves

- Clipboard, waterproof paper and pencil or cell phone for note taking
- Flagging tape and waterproof marker pen for marking nest locations

G.3 Electric fishing surveys

- Kainga EFM300 or Smith Root backpack electric fishing machine
- Polarised sunglasses (optional but recommended to reduce glare)
- Water quality meter for measuring conductivity, water temperature and dissolved oxygen
- Batteries
- 2 x Scoop nets (1 mm x 1 mm mesh)
- 3 m long weighted rope with articulated lengths of 500 mm
- 4 x 20 litre buckets
- Measuring tape, 50 m minimum
- GPS recording device (e.g. cell phone)
- Stop watch
- Scales accurate to 0.01 g
- Pottle for holding ammocoete on scales
- Photarium for measuring ammocoete. If a photarium is not used then a ruler and anaesthetic will be needed.
- Solid 1 m ruler for measuring water and sediment depth
- Clipboard, data sheets printed on waterproof paper and pencils
- Flagging tape and waterproof marker pen for labelling buckets with ammocoetes from Type I and IIA habitats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