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Research Report No. 1040

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RESEARCH REPORT

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RESEARCH REPORT

Summary

The collection of larval sea lamprey (*Petromyzon marinus*) by drift netting, suction pumping, and airlift sampling: a trial to inform the development of methods suitable for Site Condition Monitoring

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Key words

Lamprey; larval lamprey sampling; drift netting; suction pumping; airlift sampling; Site Condition Monitoring.

Background

Site Condition Monitoring (SCM) surveys of rivers notified for sea lamprey have, to date, used conventional electrofishing equipment to survey larval habitat in shallow water. The low numbers of sea lamprey larvae caught using this approach have been a cause for concern. This report describes trials in the rivers Forth and Teith of alternative methods aimed at improving the results of SCM surveys.

Main findings

- Trials using a scaled-down portable suction pump to survey larval lamprey habitat in water < 1 m deep suggested that similar estimates of larval density could be established to those obtained by conventional electrofishing. The method under-sampled the larger individuals and was associated with a 10% larval mortality rate.

- Trials using a modified Yorkshire-pattern airlift sampler showed that it could collect larval lampreys from sediment in water 0.8–3.0 m deep and in channel reaches where conventional electrofishing methods are impractical. *Lampetra* and *Petromyzon* larvae were collected by airlift sampling and larvae were collected from 34 of the 53 sampling sites. Larval length ranged 8–98 mm, and there was no significantly higher likelihood of encountering larval *Petromyzon* in deeper water habitat using this method than by using conventional shallow water electrofishing methods along the channel margins.

- The results suggested that airlift sampling could be used as an alternative way to survey larval lampreys in locations where electrofishing in marginal habitat might not be possible, e.g. where access from a bank was restricted or channel profiles were steep.

- The results of a nocturnal drift netting trial failed to provide evidence that the method could be used to establish the status of *Petromyzon* but did provide an insight of the drifting behaviour of larval lampreys. The paucity of *Petromyzon* larvae caught in drift nets during the summer emergence period suggested that they do not comprise a higher percentage of the larval community at the dispersal stage in the trial river system.

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1. INTRODUCTION

1.1 Sea lamprey ecology

Populations of sea lamprey *Petromyzon marinus* (L.) have declined throughout Europe over the last hundred years (Maitland, 2003). The causes of decline are many but are thought primarily to be pollution, barriers to migration, damage to river channels, and land management practices leading to the degradation or loss of spawning areas. All three UK species (brook lamprey *Lampetra planeri* (Bloch), river lamprey *Lampetra fluviatilis* (L.), and sea lamprey) are listed on Annex IIa of the Habitats Directive and Appendix III of the Bern Convention. The river lamprey is also listed on Annex Va of the Habitats Directive. The Habitats Directive requires EU member states to designate Special Areas of Conservation (SAC) for listed species, and regular population monitoring of Scottish riverine lamprey SACs is undertaken according to Joint Nature Conservation Committee protocols (JNCC, 2005).

Sea lamprey biology has been comprehensively reviewed by Hansen *et al.* (2016). In Scottish rivers sea lamprey spawning takes place in late spring and early summer. Female fecundity is high, and Maitland (2003) suggests an average of 172,000 eggs per female. Applegate (1950) recorded a maximum egg number of 108,000 for a female of 530 mm total length and a mean number of 61,500 eggs for a female of 442 mm total length. Hardisty (2006) suggests a maximum of 147,000 eggs per female. Based on fecundity estimates proposed by Applegate (1950), Manion (1968) suggests that adult female sea lampreys of around 40 cm in total length in a Lake Superior stream would produce around 55,000 eggs. Unlike the smaller *Lampetra* species where spawning is communal (Hardisty 2006), sea lamprey are monogamous spawners, unless the sex ratio of females to males is unusually high (Li *et al.*, 2003). Applegate (1950) noted only single pairs of sea lamprey occupying spawning pits, but Manion (1968) reported nests being used by two pairs of spawning adults. Investigations on moribund adult female sea lampreys following spawning indicated that most available eggs were released, with on average only 5% of eggs remaining in the body cavity (Applegate, 1950).

Egg survival to hatching is markedly influenced by water temperature, with no survival to the burrowing stage recorded in laboratory rearing trials under 11 °C (Rodríguez-Muñcoz *et al.*, 2001). These authors also showed that survival from egg to the burrowing larval stage was increased at warmer water temperatures, recording a maximum of 70% survival, and suggested that survival rate was very steady between 15 and 23 °C. This estimate of survival to larval stage contrasts markedly with those recorded during field investigations. Applegate (1950) reported hatching success of 0.4–1.1% for single sea lamprey nests in the Ocqueoc River, Lake Huron. Following the manual excavation of nests and the collection of larvae in nets placed immediately downstream, Manion (1968) reported the collection of a mean of 5,047 larvae and suggested that this result, when combined with estimated fecundity of spawning females of known size, related to an average hatching success of 6.3%. It is clear that survival rates from egg to hatching are variable and will be influenced by a combination of both abiotic and biotic factors that may be difficult to account for in the field.

The emergence and drifting of larvae occurs at the cleft-gill prolarval stage 16 and burrowing at prolarval stage 17 (Piavis, 1961) with individuals measuring 6–9 mm in length. The transition to larval stage 18 (with all systems differentiated except genital) occurs when larvae are burrowed in substrates after reaching a size 9–10 mm (Piavis, 1961); more recently Rodríguez-Muñcoz *et al.* (2001) reported sea lamprey larvae burrowing behaviour commencing at a size of 9–10 mm.

The emergence and subsequent downstream drifting of larval sea lamprey occurs mainly at night (Potter, 1980), presumably as an adaptation to minimise predation. In studies of North

American sea lamprey populations, Bennet & Ross (1995) recorded that the highest densities of drifting larvae occurred between 23.14 and 00.23 hrs, and Derosier (2001) reported that the incidence of drifting larvae declined sharply with the approach of dawn. Brumo (2006) found that the maximum abundance of Pacific lamprey larvae (*L. tridentate*) occurred during the darkest part of the night. Larvae are thought to drift passively with the current (Bennet & Ross, 1995) and to settle on suitable streambed substrates into which they burrow and continue their development.

The exact time at which prolarvae emerge from a nest is determined by the water temperature control over the larval development rate (Rodríguez-Muñcoz *et al.*, 2001; Meeuwig *et al.*, 2005), and this will vary both locally and annually. Piavis (1961) reported this stage being reached 15–17 days after spawning. Derosier (2001) reported that natural temperature fluctuations increased the length of time to emergence over those reared at constant temperature. The same author found an emergence period of 3–14 days from known spawning locations, indicating that adult spawning activity at specific locations may have been occurring over a more protracted period than thought. Rodríguez-Muñcoz *et al.* (2001) reported that 50% of eggs had hatched at 7.5 days at 23 °C and 27 days at 11 °C.

1.2 Monitoring sea lamprey populations in river habitats

Whilst several methods have been proposed for collecting data on lamprey populations, it must be recognised that the collection of data for any monitoring programme should only be considered as a first step in the conservation process, guiding the implementation of a suitable conservation management plan and specific actions to protect the species (Cowx *et al.*, 2009).

The recognised methods for establishing the presence of sea lamprey and reporting on the status of the population, is to sample the larval habitat using electrofishing equipment (e.g. Harvey & Cowx, 2003) and combine the results with any visual records of adult migration or spawning activity. The Common Standards Monitoring criteria (JNCC, 2015) use data generated mainly from electrofishing surveys of larval populations to provide a condition assessment of a riverine site's sea lamprey population.

The methods used to monitor sea lamprey populations are subject to considerable limitations as a result of the life history of the species and its overall scarcity. Relying upon the identification of adults during migration and spawning has several drawbacks and limitations for sea lamprey monitoring in large gravel-bed rivers, as adults largely travel at night (Potter, 1980; Hardisty, 2006) and trapping is not always a viable proposition on wide channels. Spawning habitat is often inaccessible and unsafe to examine visually at the time when spawning may be taking place, and the evidence of spawning activity (i.e. the excavation nests) may be quickly masked by substrate movements or the scour of benthic algae associated with high flow events (Batchelor, 2009). Larval investigations are often limited as habitats are often widely dispersed and consist of small patches of habitat that would be categorised as sub-optimal quality using the standard definitions. Many of these are likely to be located in water depths that prevent efficient electrofishing, or are too small to be identified during visual surveys and are subsequently overlooked. Watt *et al.* (2012) and Bull & Watt (2012) provide further discussion of the issues surrounding conventional electrofishing for larval sea lamprey.

Even in suitable habitat, it would appear that the probability of capture for larval sea lamprey using conventional electrofishing techniques is very low. Sea lamprey larvae appear to be widely dispersed in habitats downstream of spawning locations, and in Scottish rivers they regularly make up < 2% of larval lamprey samples (Watt *et al.*, 2008; Bull & Watt, 2012; Watt *et al.*, 2012 a, b). The low probability of capture leads to an increased risk of incorrectly recording local extinctions during population monitoring surveys based purely on this

methodology. This is clearly undesirable and necessitates greater electrofishing effort in order to maximise the opportunity of capturing larval sea lamprey, often at increased cost.

Deep water sampling using combined electroshocker and suction removal methods have been attempted for larval lampreys (Bergstedt & Genovese, 1994), with sampling capture efficiency being found to be relatively good for small larvae, but negatively related to larvae length, presumably as a result of difficulties in consistently retrieving the larger specimens at depth. Modified Surber Sampler methods (Lasne *et al.*, 2010) have been used to provide quantitative larval density estimates. Despite being rather labour intensive as it requires a lot of substrate sorting, the method was shown to provide higher abundances of the smallest larvae than electrofishing. Sieving sediment directly downstream from lamprey spawning sites on the River Shannon produced larval densities around 20 m^{-2} (Igoe *et al.*, 2004). Taverny *et al.* (2012) carried out suction pumping in deep water habitats of the Gironde-Dordogne river complex and found a higher incidence of sea lamprey larvae in deeper water areas than the shallower stream margin habitats. In the UK, trials using a modified airlift sampler (Mackey, 1972) have been carried out on the Welsh Dee (APEM, unpublished data) with results indicating that sea lamprey larvae were relatively more abundant in samples from deeper water habitats than in samples from marginal electrofishing sites.

1.3 Sea lampreys in the River Teith SAC

The sea lamprey population dynamics of the River Teith SAC have been studied more than others in Scotland in recent years (Gardiner *et al.*, 1995; Maitland & Lyle, 2000; Bull, 2004; Batchelor, 2009; Henry, 2009; Bull & Watt, 2012), but information on some basic aspects of the geographical distribution and abundance remains limited. Sea lamprey larvae have been recorded as far upstream as Callander (approximately 22 km upstream of the normal tidal limit) (Maitland & Lyle, 2000) and anecdotal evidence of sea lamprey spawning has been reported in this area, but a more recent juvenile survey reported a reduced geographic distribution of larvae (Bull & Watt, 2012) indicating that the sea lamprey spawning activity is largely confined to the lower reaches of the River Teith. Larval sea lamprey densities encountered in suitable habitats in routine surveys are extremely low, even those in samples taken in close proximity to known spawning locations (Bull & Watt, 2012), but it is clear that larvae are settling in the lower River Teith and in the River Forth upstream of the lower boundary of the River Teith SAC.

Surveys of sea lamprey spawning activity on the River Teith SAC in 2008, 2009, and 2010 recorded the locations of spawning nests in the lower reaches of the River Teith downstream from Deanston Bridge (Batchelor, 2009; Bull & Watt *unpublished data*) with a mean of 15 nests per year being observed. Nest building and spawning activity has in recent years been observed in both the same and new areas within a 5 km stretch of the River Teith during the first days of June (Batchelor 2009; Bull & Watt *unpublished data*) with a mean water temperature of 16.4 °C recorded during the spawning period in 2009 (Batchelor, 2009). Nest sizes encountered have ranged 0.35–4.86 m in diameter. The fate of the sea lamprey eggs laid in the spawning pits that have been recorded in the lower reaches of the River Teith remains largely unknown. It appears that the potential for conventional electrofishing surveys in downstream shallow marginal habitat to detect numbers of surviving larvae that accurately reflect the level of spawning effort is limited.

When considering the population monitoring of rare or endangered species such as the sea lamprey, it may be prudent to attempt to target sampling during periods of life cycles when individuals are at their most abundant (McDonald, 2004) and when the individuals are dispersed over wide areas. This would maximise the opportunity to encounter them during routine sampling activities, and increase sample sizes and the power to detect temporal changes in the population. The life cycle of the sea lamprey appears to offer an opportunity

to conduct additional sampling to improve detection rates and better inform future management decisions for this rare species.

Despite the lack of information on the survival rates of sea lamprey eggs to the emergence stage, it appears that there is potential for relatively high densities of drifting larvae to be present in the water column at night in the lower reaches of the River Teith during the month of June. This larval dispersal stage of the sea lamprey life cycle may afford the opportunity to develop and test techniques to sample drifting larvae. These techniques could be used to improve detection rates in river systems where the number of spawning individuals is low (Brumo, 2006), or to provide better information on the distribution of spawning individuals where verification by other means is not possible.

Several other authors have undertaken drift sampling for larval lamprey in North America but the technique does not appear to have been used in the UK before. Bennett & Ross (1995) compared the drifting larval sea lamprey catch efficiency of four sets of gear: a towed bongo net; a benthic sled; a large (2.5 m diameter) static plankton net; and standard (0.25 m) diameter drift nets. Although no measure of the volume of water sampled by each type of sampling gear was provided, the results suggested that the standard sized drift nets set in shallow riffle areas captured the greatest number of larvae of all the methods employed. Brumo *et al.* (2009) reported the successful deployment of a large (0.70 x 1.50 m aperture) zooplankton drift net secured to the streambed to sample drifting larvae of the Pacific lamprey (*L. tridentate*). Using similar drift net sampling, Derosier (2001) reported the capture of drifting 0+ sea lamprey larvae up to 874 m downstream from known spawning locations on the South Fork Coquille River in Oregon, USA.

1.4 Sampling deep river habitats

The relationship between larval density and habitat features has received much attention in the literature. Larval density has been shown to increase with increasing organic material in the substrate (Potter *et al.*, 1986). Substrate organic material, chlorophyll *a*, macrophyte roots, and low angle shading were found to be the most consistent explanatory environmental variables for larval lamprey density in a south western Australian stream (Potter *et al.*, 1986). However, other authors have presented results that indicate organic content to be a poor predictor of larval density (Malmqvist, 1980; Moore & Mallatt, 1980; Beamish & Jebbink, 1994) and suggest that the grain structure of the substrate is of prime importance (Goodwin *et al.*, 2008). In surveys of lamprey populations in other major Scottish river systems (Bull *et al.*, 2014) eddy features and the presence of large woody features (fallen trees and branches) were most frequently associated with the presence of lamprey larvae. The same study suggested that sediment depth was generally a poor predictor of larval density.

In addition to the importance of substrate composition, the selection of habitat patches in deeper water by the larger larvae of certain lamprey species has also been reported in the literature. Torgensen & Close (2004) reported that water depth and shading were important variables in determining larval distribution at the catchment scale. Manion & McLain (1971) reported that larger larvae displayed a tendency to occupy deeper water areas when compared with the youngest, smallest larvae.

With conventional electrofishing sampling of larval lamprey populations being confined to marginal substrate patches in water depths that remain wadeable by surveyors (frequently < 0.50 m depth), there is the possibility that aggregations of sea lamprey larvae in substrates in deeper water are not being accounted for. This may well be the case in the lowermost reaches of large river systems where access to suitable areas to conduct electrofishing is often hampered by steep banks and deep water. Little is known about larval occupancy rates in these deeper water areas, but indications from research elsewhere suggests that they

provide suitable conditions for sea lamprey larvae. Therefore, an alternative method of sampling them effectively may provide a useful addition to the existing Site Condition Monitoring (SCM) methods and improve the efficacy of population monitoring.

1.5 Aim of the project

The aim of the research project was to compare the utility of a variety of equipment and strategies for obtaining samples of the River Teith SAC's larval sea lamprey population. The results from the various sampling methods were compared with those from traditional shallow water electrofishing to provide an assessment of the efficacy and feasibility of integrating novel methodologies into CSM protocols for the species.

2. METHODS

2.1 Trial of methods

In order to trial two methods for sampling larval lampreys in the River Teith SAC and to compare the samples with those collected using standard electrofishing, a suitably long marginal reach of larval habitat on the right bank of the River Forth (midpoint NGR 278577 695481) was selected for shore-based trials. These were conducted in wadeable depth water during July 2015. This enabled equipment to be trialled in water up to 1 m deep and allowed the rapid modification of the sampling technique and equipment without the need for resource intensive deployment of a boat crew. Quadrats covering 0.25 m^2 (50 cm x 50 cm) were used to delineate areas of substrate along the river margin. These were located randomly in blocks of three along the length of the habitat patch, and samples were collected using three different methods. Unsuitable flow conditions and equipment failures restricted the completion of the trial , but a total of 20 quadrats were sampled using standard three-run depletion electrofishing (Harvey & Cowx, 2003), 20 using a modified airlift sampler (Mackey, 1972) and 12 using a suction pump based on the design of Taverney *et al.*, (2012).

2.2 Suction pumping

A suction pump was constructed following the guidance provided by Taverney *et al.* (2012). The equipment was scaled down to enable it to be used with fewer operators and to make it more portable. The outlet from an air cooled, petrol driven, portable water pump (rating 35 m^3 hr^{-1}) was connected using a specially manufactured reducer piece to a 30 mm diameter flexible pipe. This pipe led to a specially manufactured Venturi valve with 60 mm diameter outlet vents. Sixty millimetre flexible pipes were then secured to this valve (piece) and provided the sampling nozzle inlet for the collection of lampreys, and the collection point where a mesh bag retained the sample—see Figure 1.

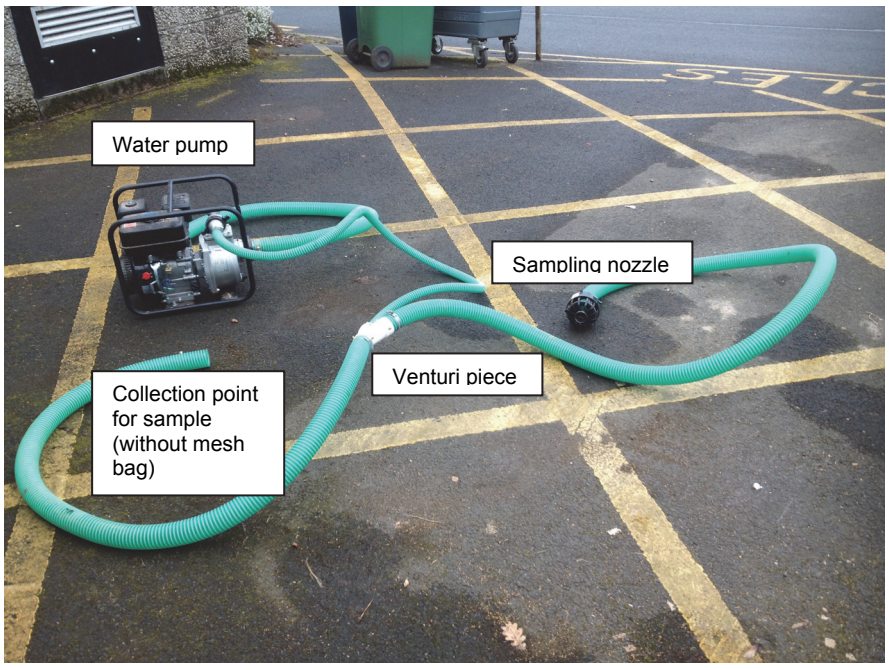

Figure 1. The suction pump equipment used in the 2015 trials.

When the pump was in operation and the sampling nozzle was held on the bed of the river, water and sediment were drawn up and passed through a series of sieves to a mesh bag which retained the solid matter of interest. The Venturi suction effect allowed the collection of a benthic sample without it having to pass through the pump mechanism—see Figure 2.

Three people were needed to operate the equipment effectively: one ensured that the pump was primed and working effectively; one guided the suction inlet on the river bed substrate; and at least one held the outlet, sieves, and mesh bag to collect the sample. Taverney *et al.* (2012) operated this system using two divers and a surface support crew on a boat. Sampling often required temporary removal of the suction nozzle from the sediment as the rate of material transfer from the output was too high for the collection sieves. The suction nozzle was moved across the entire area of the quadrat during a period two minutes. This was considered comparable with the sampling effort typically applied to an electrofishing run. All of the samples were sorted and examined for the presence of live or dead larval lampreys.

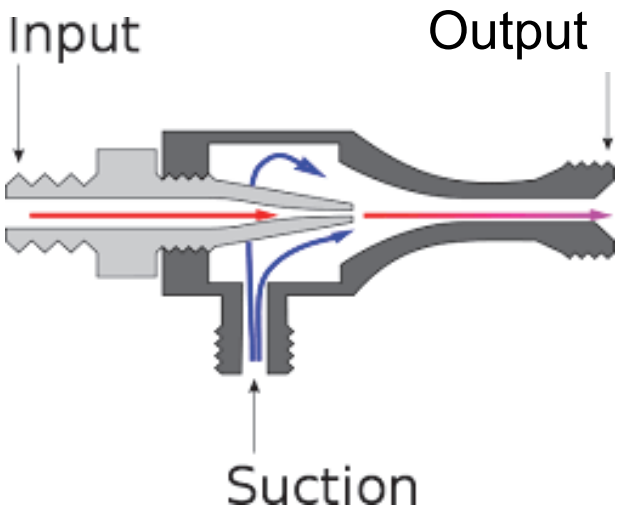

Figure 2. The Venturi suction effect. The pump forces water into the system at the input. Water, sediment, and entrained organisms are sucked into the system at the suction entrance, and are collected at the output. Source: http://physics.stackexchange.com/

2.3 Airlift sampling

The equipment used for airlift sampling (Figure 3) was constructed using the design provided in Mackey (1972) and Davy-Bowker *et al.* (2014). During the trials undertaken in 2015 the head of the airlift pipe was positioned on the substrate and the equipment was held as vertical and as submerged as the depth of water would allow; 10 kg of diver's weights were secured to the bottom as ballast. Samples were collected in a 1 mm mesh bag. Two second bursts of high pressure air were then blown into the surface of the substrate using the air release valve. This was repeated ten times per quadrat, with the operator moving the head to cover as much of the quadrat as possible.

It was recognised that the equipment and sampling method would be likely to lead to lower capture efficiencies than those for boat based sampling in deeper water using the method outlined in Davy-Bowker *et al.* (2014). This was due to the limited transfer of the substrate and organisms into the sample collection bag by air bubbles travelling up the collection pipe because the released air was under lower pressure than it would be when the equipment was operated in deeper water (Mackey, 1972). Nevertheless, the trials were undertaken to take advantage of a suitable period of low flows and to maximise the time devoted to equipment testing and troubleshooting for boat-based surveying.

To test if certain environmental variables could explain patterns of larval distribution and abundance and whether *Petromyzon* larvae were more abundant in deeper water habitats (Taverney *et al.*, 2012), a plan to deploy the equipment along transects across the river was made for 2016. The trials were undertaken in the summer using the equipment operated

from a small boat on the River Forth. Transects were set perpendicular to the flow. However, due to problems securing a transect rope across river channels > 60 m wide, the trials proved difficult. When transects were successfully established, water depths were often found to be < 1 m with unsuitable substrates, prohibiting the use of the sampling equipment. The considerable effort required to set up a transect across wide sections of river, and the likelihood that the equipment could be deployed effectively at only a very limited number of locations across each, resulted in the transect approach being abandoned and a more targeted sampling strategy being adopted.

Figure 3. The Yorkshire pattern airlift sampler used to collect larval lampreys.

The airlift sampling in 2016 was subsequently undertaken by targeting suitable habitat in much the same way that electrofishing surveys are conducted. Certain river features such as eddies and areas of low flow associated with obstructions or the inside of bends were investigated using a boat, and the suitability of the substrate checked using benthic grabs. Once it was established that potential lamprey habitat was present in the water beneath the boat, an anchor was deployed and the airlift sampling began.

The equipment was lowered onto the substrate and the air valve opened to start sampling. The operator then manoeuvred the equipment by bouncing it along the channel bed, positioning it at 10 locations. At each location the equipment was checked for readiness, and then a 5 second burst of high pressure (3–4 bar) air released. Sub-samples were rejected if no sediment cloud resulted from the air release to indicate that the sampler was working effectively on the substrate. When a sub-sample was rejected an additional sample was taken to ensure that 10 sub-samples were collected per location. A total of 50 seconds of air release were therefore used to collect each sample at a location. Using a 100 mm diameter collecting pipe, the minimum area of substrate sampled with 10 sub-samples was estimated to have been 0.08 m^2.

2.4 Drift net sampling

Drift net sampling was undertaken in 2016 as pilot studies on the River Teith during 2012 had shown that standard invertebrate drift nets (0.25 m x 0.40 m, 250 µm mesh, available at https://www.nhbs.com/), could be used to sample drifting larval lampreys (C. Bull unpublished data). Previous trials had indicated that replacing a small portion of the plastic wall of the collection bottle with a 500 µm mesh panel increased the retention of larvae (Figure 4 b). During pilot studies, nets were anchored using metal pegs in shallow gravel bed sections of the river where access to the water from the bank was safe and flow conditions were relatively stable (Figure 4c).

Figure 4a. The standard invertebrate drift net used in the study.

Figure 4b. The 500 µm mesh panel modification made to the collecting bottles to increase larval retention.

Figure 4c. The nets deployed from anchor points in the River Teith.

It was intended to evaluate the use of nocturnal drift netting as a means of providing additional data for sea lamprey SCM. Drift nets were to be deployed at known distances downstream from identified sea lamprey spawning sites, using methods similar to those described by Bennet & Ross (1995) and Derosier (2001). This would enable an evaluation of the utility of the methods for recording the presence and relative abundance of sea lamprey larvae in relation to the proximity of spawning sites. The investigation would, additionally, provide the opportunity to record information about the relationship between the size of the adult sea lamprey spawning population, its distribution, and the subsequent survival and pattern of emergence of larvae.

However, during walk-over surveys of the River Teith in 2015 and 2016, no sea lamprey spawning locations were observed. These surveys focussed on reaches in which activity had been recorded at the same time of year in successive years (see Bull *et al.*, 2011, and Figure 5). It is acknowledged that visual spawning surveys are limited in their ability to consistently identify spawning activity, but they are used as an integral part of site assessments for sea lamprey in Irish rivers (J. King, pers comm). It was possible that spawning was taking place in new areas of the River Teith during these years, or that the identification of spawning pits was hindered by poor survey conditions. Indeed, during the two week survey period in 2015, high flows and dull conditions prevented the identification of spawning activity, although low water conditions and bright, clear skies (i.e. optimum conditions for spotting submerged spawning pits in gravel bed rivers) predominated during the survey period in 2016. The use of drones to evaluate the distribution of spawning fish could be considered for future surveys.

Figure 5. Petromyzon *spawning pits (top) and adult lamprey in attendance at spawning sites (middle and bottom) in the River Teith, June 2012 and 2013.*

As a result of not locating any sea lampreys spawning pits in 2015 or 2016, the method of evaluating drift netting had to be adapted during the summer of 2016 as it was not possible to place drift nets at known distances from nest sites.

A location on the River Teith 200 m upstream of the 2016 airlift site 8G (Figure 9) at NGR NS 76188 96707 was chosen to sample the nocturnal patterns of larval lamprey drifting intensively, to investigate the relationship between drift and discharge further, and to determine if the method enabled the capture and identification of sea lamprey larvae. This location was 500 m downstream of the lowermost sea lamprey spawning nest identified during previous year's survey of the River Teith. It was chosen not only due to the proximity

of sea lamprey spawning areas but also because of a combination of suitable channel conditions for drift sampling and safe access for surveyors during the night.

Six identical drift nets were deployed from anchor points set perpendicular to the direction of flow. Each was fitted with a modified collecting bottle (Figure 4a). A 500 µm mesh panel covered a rectangular section that was cut out from each bottle (Figure 4b). The aim of this modification was to maintain a through flow of water at the end of the net and to maximise the retention of larval lampreys in the samples. The nets were deployed at dusk and emptied hourly until dawn over a total seven nights during July and August 2016. Water depth and velocity at the mouth of each net were measured hourly in order to calculate the volume that passed through between collection times; a water sample was collected at the same time for laboratory analysis.

Every hour, the contents of each drift net were placed into a sorting tray (Figure 6, inset) and any larval lampreys present were removed and retained in 100% ethanol for subsequent measurement of total length and to enable genetic identification. Clean nets were then re-deployed.

Figure 6. The summer 2016 nocturnal deployment of drift nets in the River Teith. Anchor points are shown protruding above the water surface. Inset: sorting trays showing the typical volume of drift material collected in one hour.

2.5 DNA methods for *Lampetra* vs. *Petromyzon* identification

A number of young-of-the-year larval lampreys captured in the drift nets were selected as representative of the cohort from across the range of sampling events and used for identification using DNA methods. The methods for distinguishing *Lampetra* vs. *Petromyzon* followed the protocol of Urdaci *et al.* (2014). A portion of the mitochondrial cytochrome *b* (cyt *b*) gene was amplified using polymerase chain reaction (PCR) and the primers cyt *b* F: 5'-CCTTCTCCTGCTAATATCTC-3' and cyt *b* R: 5'-GGGTTACTAGATCCTGTTTG-3' which resulted in a product of 560 base pairs (bp). PCR reactions were carried out in 25 µL volumes containing: 10X buffer with 1.5 mM $MgCl_2$; 0.6 U *Taq* DNA polymerase; 1.25 mM dNTP; and 5 µM of each primer. Reactions were run on a SureCycler 8800 (Agilent Technologies) with an initial denaturation at 94 °C for 5 minutes followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 50 °C for 1 minute, and extension at 72 °C for 1 minute, and a final 5 minute extension at 72 °C. PCR products were verified by separation on a Zero Agarose Gel (ZAG) electrophoresis system (VH Bio Ltd).

For samples that were successfully amplified, two restriction endonuclease enzymes (*Hinf*I and *Rsa*I) were used to generate different banding patterns for *Lampetra* vs. *Petromyzon*. PCR products were individually digested with each enzyme by overnight incubation at 37 °C and subsequently enzymes were deactivated by heating to 95 °C for 20 minutes. Restriction reactions were then run out on the ZAG electrophoresis system to visualise banding patterns. The expected *Hinf*I banding pattern is 285 bp & 275 bp for *Lampetra*, and 468 bp & 92 bp for *Petromyzon*. The expected *Rsa*I banding pattern for *Lampetra* is 333 bp, 164 bp & 63 bp, while for *Petromyzon* the enzyme does not cut, resulting in an intact 560 bp fragment.

3. RESULTS

3.1 2015 Results

Twenty 0.25 m^2 electrofishing samples were collected, 20 using the airlift sampler, and 12 using the suction pump. A total of 610 lamprey larvae were caught using these three methods (Table 1). The mortality rate of the larvae caught using the suction pump was 10%. No dead larvae were encountered using the other methods (Table 1).

Table 1. 2015 River Forth quadrat sampling details

Method	Total number of samples	Total area of substrate sampled (m^2)	Total number of lamprey larvae caught	Mean minimum density of larvae per sample (m^2) (SE)	Mortality rate of total larvae sample (%)	Proportion of total sample comprising *Petromyzon* larvae (%)
Electrofishing	20	5	305	61.00 (± 6.52)	0.0	1.97
Suction pumping	12	3	294	98.00 (± 8.59)	10.9	1.02
Airlift sampling	20	5	11	2.20 (± 0.73)	0.0	0.00

The density data was not normally distributed and transformations failed to normalise it. A non-parametric Kruskall-Wallis test of difference was therefore used to show that density significantly differed between methods (H = 40.08, df = 2, P < 0.001). Subsequently, a Wilcoxon signed-rank test suggested that the three groups were significantly different from each other. Suction pumping resulted in the highest density estimate for larval lampreys from the limited area of substrate sampled; airlift sampling produced a very low density estimate in comparison.

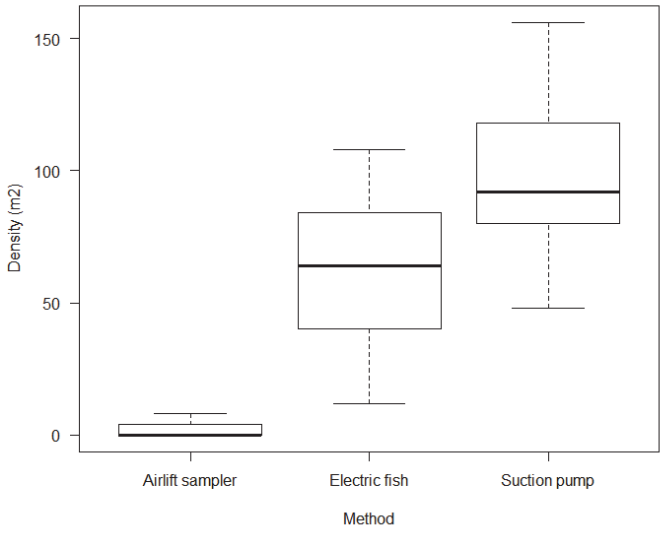

Figure 7. Box plot of mean estimated density of larval lampreys caught using the three sampling methods

Electrofishing provided the widest range of larval length classes (Figure 8, top). Larvae in the 36–60 mm length class (equivalent to the 1+ year class) dominated. The mean length of the larvae caught using this method was 43 mm (equivalent to the 1+ year class). More of the smaller larvae were caught by suction pumping (Figure 8, middle) than electrofishing, with individuals representing the 0+ year class dominating. The mean length of larvae caught using this method was 23.2 mm. The few larvae caught by airlift sampling were dominated by the 0+ year class. Their mean length was 19.1 mm. A single larger larva (42 mm long) was caught using this method.

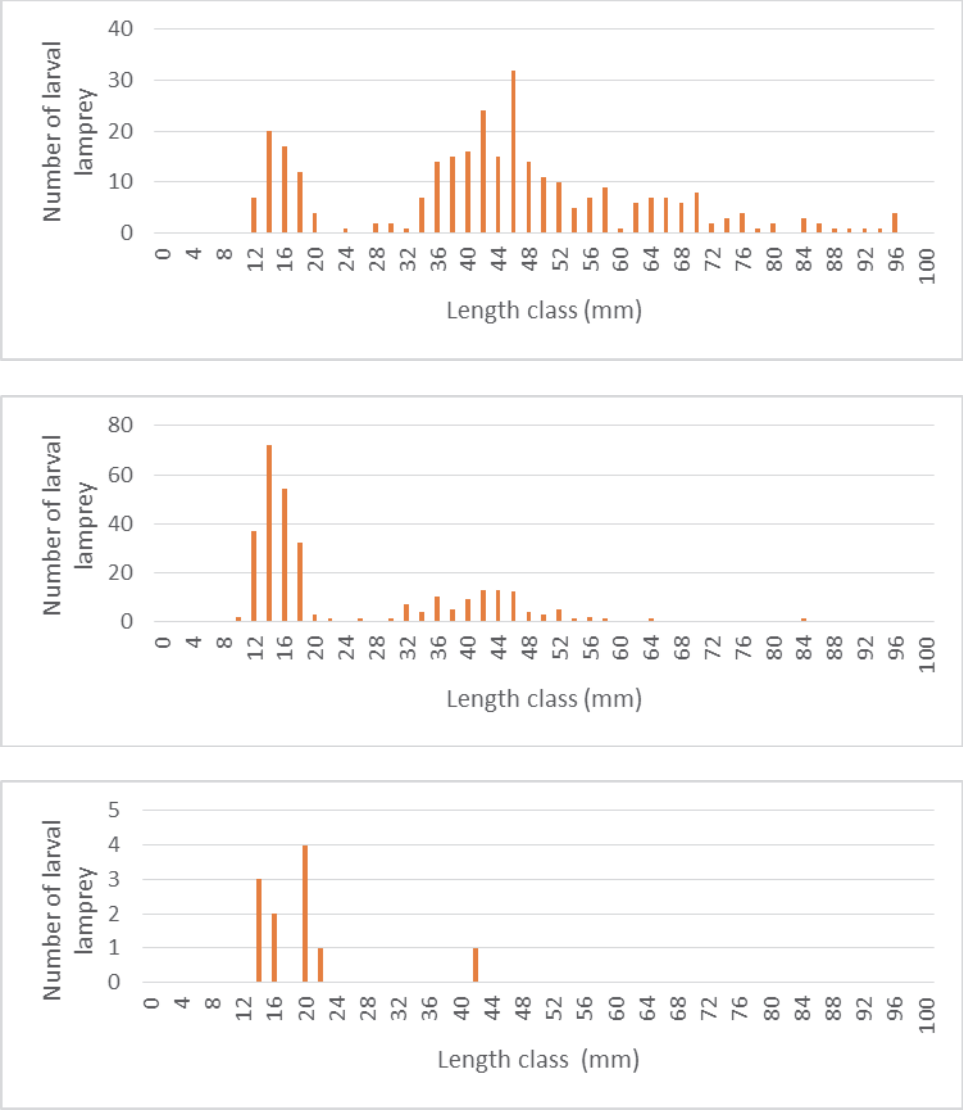

Figure 8. Length distributions of the larval lamprey caught using the three methods: top = electrofishing (N = 305); middle = suction pumping (N = 294); and bottom = airlift sampling (N = 11).

3.2 2015 results discussion

The trial results suggested that suction pumping was the best method for catching smaller larvae. It provided an estimate of density that was similar to that of electrofishing. But, the deployment of the suction pumping equipment required considerably more effort and resources including, as a minimum, a team of four surveyors to work effectively; effective electrofishing could be undertaken in the same location using backpack equipment and only two surveyors. The use of the suction pumping equipment in deeper water would require a

large and stable boat as a platform, with a rigid floor to allow the operation of a petrol-driven water pump. Additionally, considerable space would be needed in the boat to give the operators the space to safely use the water pump and to maintain the inlet and capture outlet at suitable locations around the gunwales without water entering the boat.

The 10% mortality rate associated with the suction pumping was concerning. Neither the electrofishing nor the airlift samples contained any dead larva. Whilst it was possible that the volume of sediment collected using the suction pump increased the chances of encountering dead larvae, the method itself could have been responsible for killing larvae. In combination, the high resource requirements and concerns that the method might be responsible for the mortality rate led to further suction pumping trials in 2016 being abandoned.

The airlift method was poor at collecting larval lamprey when the average water depth was only 0.61 m. When lampreys were caught they were the smallest individuals. This may have been due to the restricted water depth that the bank-based survey team could operate the equipment in, and the need to tilt the equipment to ensure that the collecting bag remained immersed. The successful use of the equipment relies upon the efficient vertical transfer of material in the pipe; tilting it may have decreased the capture efficiency considerably. As water depth increases so does the force generated by the rapidly expanding bubbles of air that dislodge material, including larvae, and transfer it to the collection bag. The 2015 trial in shallow water was considered to be unrepresentative of the potential use of air lift sampling equipment for lamprey surveys. Further trials were undertaken and modifications made to the apparatus in June 2016 in preparation for its use in a survey of the rivers Teith and Forth.

3.3 2016 Airlift sampling results

During the summer of 2016 a total of 53 sites were sampled on the rivers Forth and Teith using the airlift equipment. The sites were located downstream of previously identified sea lamprey spawning sites and were distributed largely in the River Forth just upstream of Stirling (Figure 10). Full details of the sites are given in Appendix. The selection of the sampling sites was dictated by the location of suitable access points for the boat and equipment, and then by suitable locations for sampling. The depth of water sampled was 0.80–3.00 m with a mean of 1.35 m (Table 2). Oxygen saturation at the river bed was approximately 100% and the water temperature was 14–24 °C (Table 2).

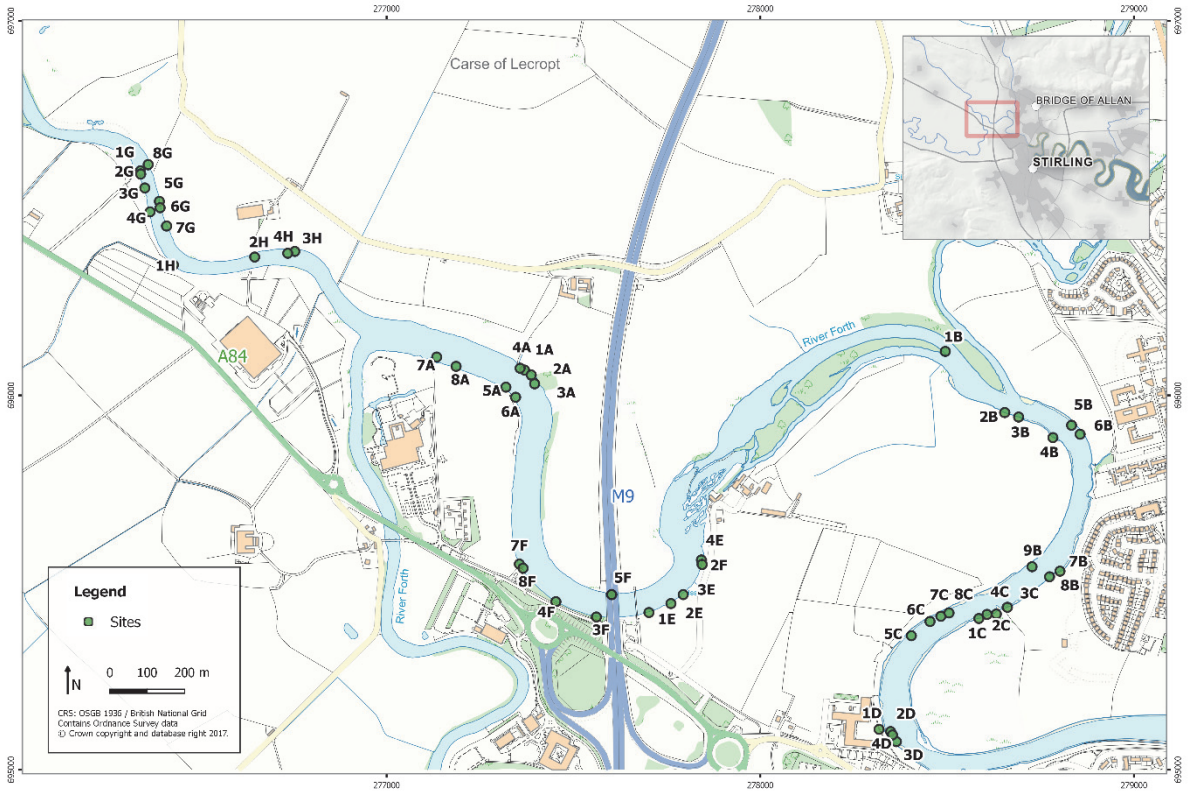

Figure 9. River Teith and River Forth airlift sampling site locations, August 2016.

Table 2. Physical and chemical water characteristics recorded at airlift sampling sites on the Rivers Forth and Teith, August 2016.

	Water Depth (m)	Dissolved oxygen at bed (%)	Temperature at bed (°C)
minimum	0.80	82.0	13.9
maximum	3.00	125.0	24.0
mean	1.37	103.9	19.3

A total of 537 lamprey larvae were caught using this method at the 53 sites. The number of larvae caught per site was highly variable. Larvae were frequently absent (Figure 10) but were caught at 34 sites. The maximum number of larvae caught at a site was 93 (site 7F, Figure 9). The mean number of larvae caught in the 53 sites was 10.8 (± 2.78 SE), but due to the number sites in which none was caught the median value was one larva.

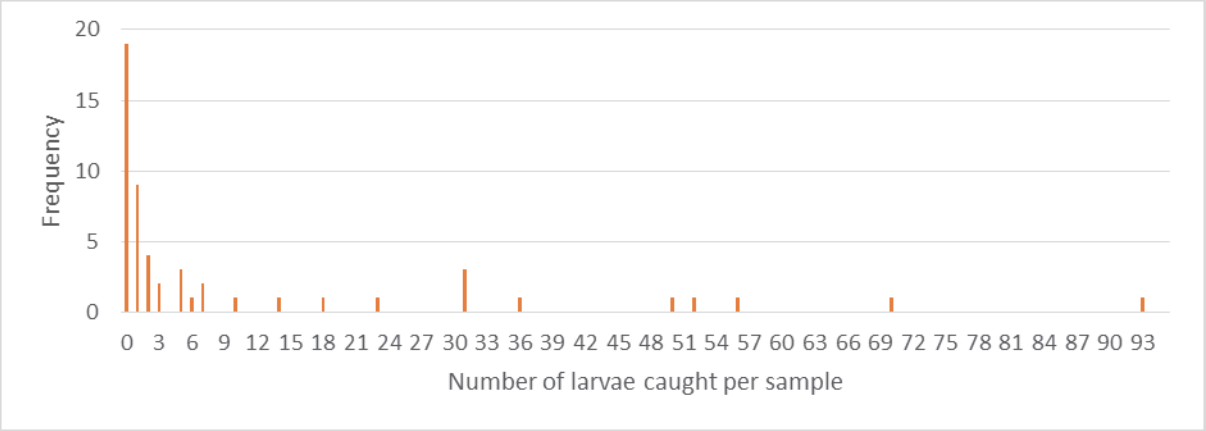

Figure 10. Frequency of larval lamprey catches by number of larvae caught.

An examination of the length distribution of the larvae caught by airlifting (Figure 11) suggested that at least three year classes were present. The mean length was 25 mm and the median 19 mm. These results suggested that the most frequently caught year class was representative of the 0+, i.e. larvae that had recently emerged from spawning locations upstream of the sampling sites and had either settled or were on their way to suitable habitat. The longest larvae caught was 98 mm which, together with the capture of several individuals 60–80 mm long, suggested that the method was capable of retaining some of the older, larger larvae and that the habitats in the deeper water areas had several year classes of larvae present.

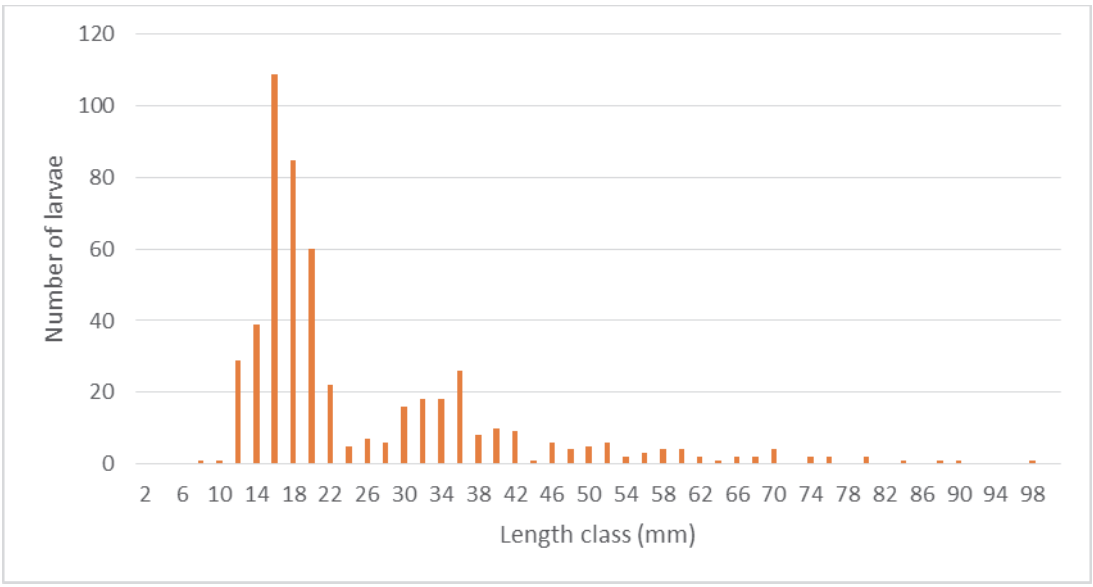

Figure 11. Length distribution of larval lamprey caught by airlifting in the Rivers Forth and Teith, August 2016.

In total 12 of the 537 larvae caught by airlifting were identified as *Petromyzon* (Table 3). This suggested a *Petromyzon* occurrence rate of around 2% in the deeper water habitat. The length distribution of *Petromyzon* larvae in the airlifted samples (Figure 12) suggested that two year classes were present, with a maximum individual length of 58 mm and a minimum of 18 mm.

Table 3. Distribution of Petromyzon *larvae caught by airlifting in the Rivers Forth and Teith, August 2016.*

Airlift sampling site	Number of *Petromyzon* larvae	Larvae length (mm)
1a	1	18
2a	1	27
2b	1	53
1g	2	58, 43
2g	1	47
1h	2	55, 35
2h	1	39
3h	1	20
4h	2	40, 41

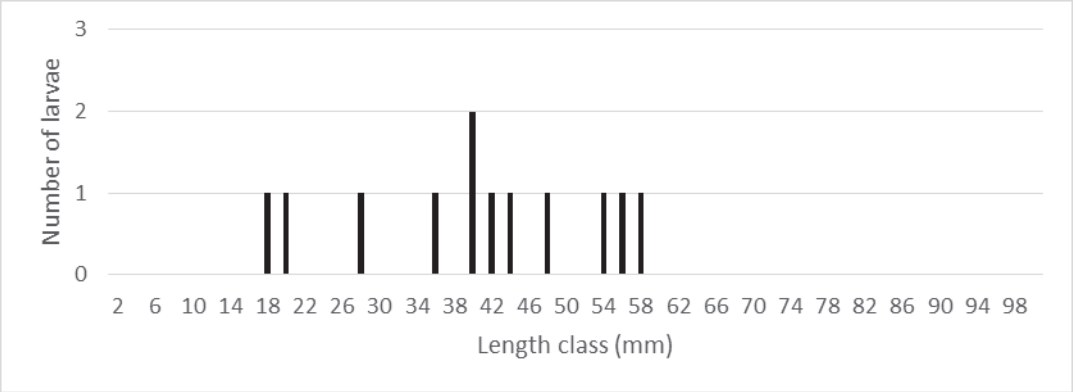

Figure 12. Length distribution of Petromyzon *larvae caught by airlifting in the Rivers Forth and Teith, August 2016 (N = 12).*

3.4 2016 drift netting results

Seven nights of drift net sampling were undertaken in July and August 2016. A total of 467 larval lampreys were caught (Table 4). Typically, larvae were only encountered during the darkest hours of each night (Figure 13); the highest numbers were encountered between 23.00 and 01.00 hrs. No larvae were caught before 22.00 or after 04.00.

Table 4. River Teith drift netting summary data, July and August 2016.

Drift net sampling night	Total volume of water passing through the six drift nets (m^3)	Total number of larval lamprey caught by the six nets	Mean number of larval lamprey caught per 100 m^3 of water
14 July	6087.6	13	0.2
20 July	5500.8	160	2.9
22 July	6364.8	18	0.3
28 July	3168.0	0	0.0
03 August	4147.2	66	1.6
08 August	5097.6	210	4.1
18 August	1670.4	0	0.0

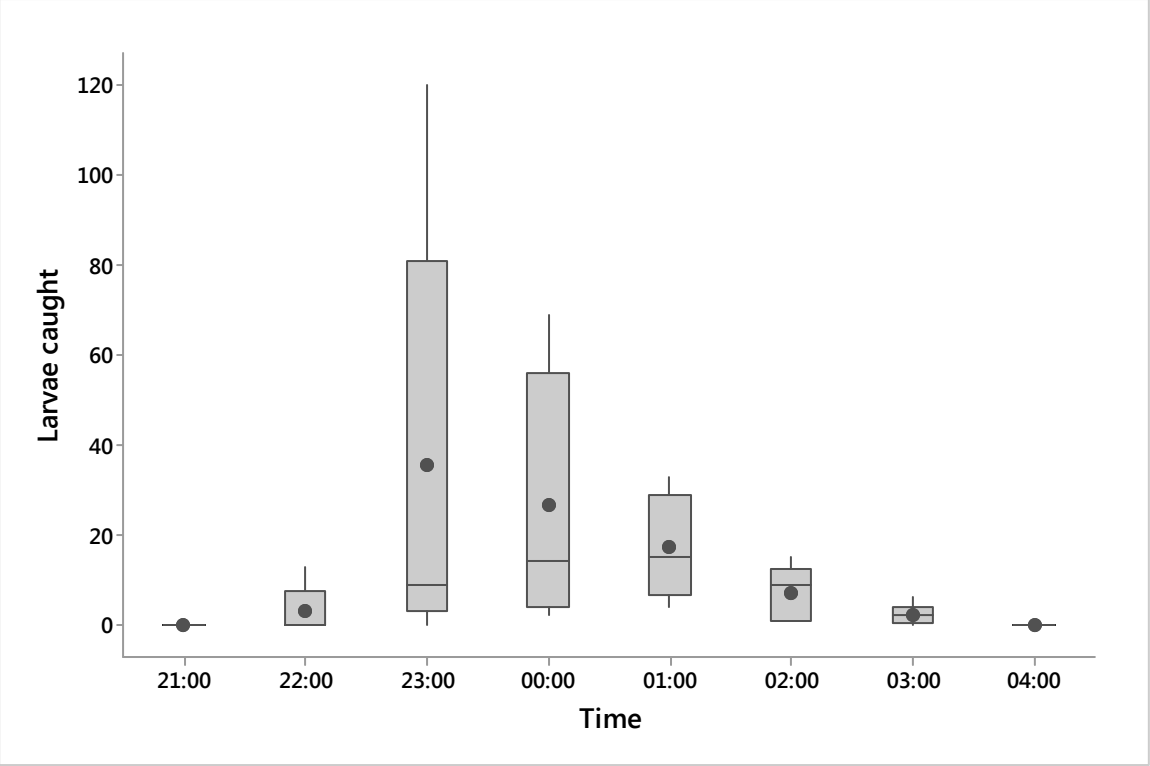

Figure 13. Box plot showing the distribution of total numbers of larvae caught for each sampling hour over the five nights when larvae were caught by drift nets. ● = mean number of lamprey caught per sampling hour.

There was considerable variation in the larval lamprey catch rates for drift netting (Table 4); no larvae were caught on two nights despite considerable sampling effort. Previous studies have indicated a link between the drift behaviour of larval lampreys and increased river discharge (Lucas *et al.*, 2007). When the Scottish Environment Protection Agency (SEPA) discharge records for the River Teith (Bridge of Teith gauging station) were compared with the total number of larva caught each night (Figure 14) it appeared that the three nights when the highest number of larvae were caught were associated with short-lived increases in discharge.

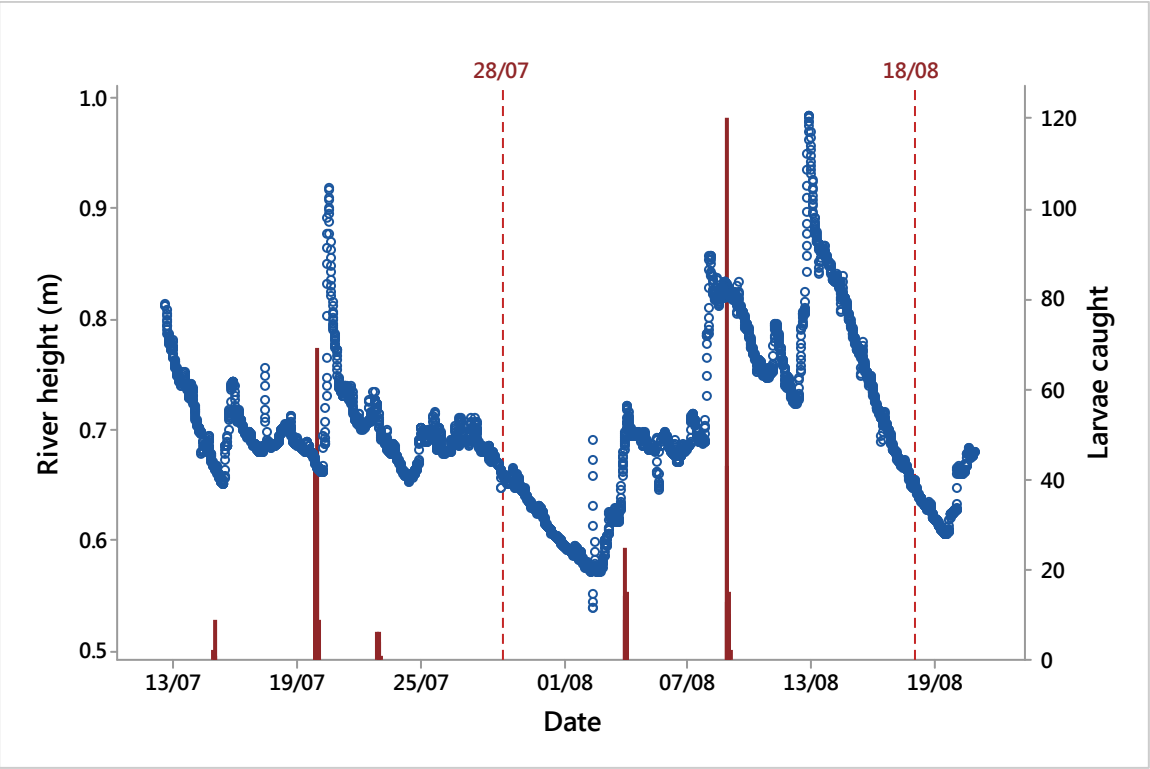

Figure 14. River stage (m) recorded at the SEPA Bridge of Teith gauging station (blue line) and the total number of larval lamprey caught each hour during each sampling night (red bars). Dashed red line = zero larvae caught.

The relationship between the river level and the total number of larval lampreys caught may have been due to an increase in the volume of water passing through the drift nets. Indeed, when the larval lamprey catch rate was corrected for the volume of water that passed through each net in an hour and was compared with the river stage recorded for the corresponding hour, no relationship was found.

In order to check whether the larval lamprey catch rates were influenced by changes in river stage, whether the catch of larval lamprey per m^3 of water was related to a falling, steady, or rising phase of the hydrograph was tested. The stage data was categorised according to whether discharge changed by 10 m^3 in an hour or not. If discharge increased by ≥ 10m^3 in an hour then it was categorised as *rising level*; if it fell by ≤ 10 m^3 it was categorised as *falling level*; and if it varied by < 10 m^3 then it was categorised as *steady level*. An ANOVA analysis of the total number of larval lampreys per m^3 of water for these three categories was undertaken and revealed that falling water levels increased the catch. No relationship was found for either the rising or steady level categories and the larval lamprey catch.

The length distribution of the larvae caught in the drift nets (Figure 15) suggested that two year classes dominated the samples. These corresponded to the 0+ and 1+ year groups (10–22 mm and 22–40 mm respectively). A few larger individuals were caught, but the occurrence of drifting larvae > 40 mm was rare. The mean length of larvae caught by drift netting was 18.5 mm; a median value of 16 mm suggesting that the most frequently sampled year class was 0+.

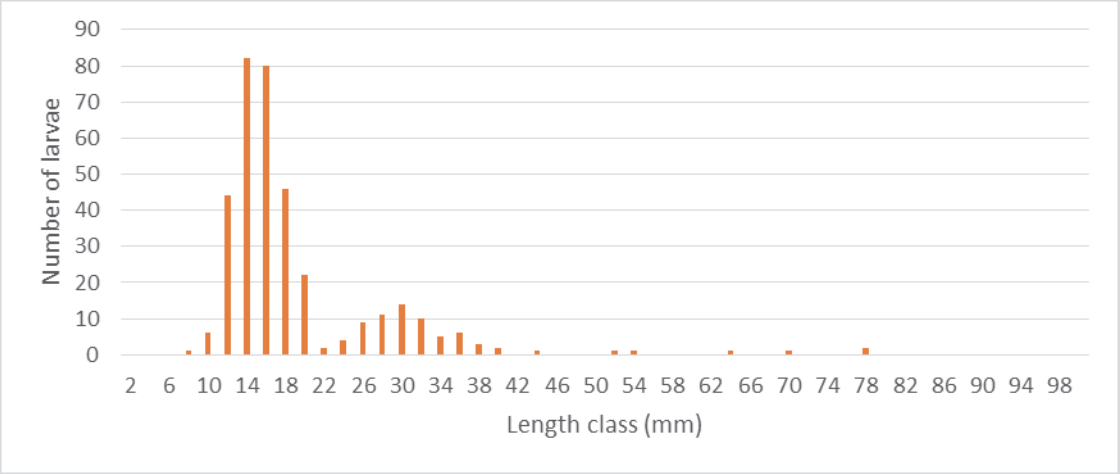

Figure 15. Length frequency plot of the larval lampreys caught by drift netting.

All of the preserved larval lampreys were photographed under 10X magnification to enable the examination of the head and tail pigmentation that is typically used to distinguish between *Lampetra* and *Petromyzon* larvae (Potter & Osborne, 1975; Gardiner, 2003; Renaud 2011). An examination of the external features was used to select a sample of 172 individuals to be identified using genetic techniques. These individuals were selected from the 10–22 mm long larvae (0+ year class).

A total of 172 samples were screened for restriction enzyme analysis. Of these, 156 individuals were screened for both enzymes while 15 were screened for only *R*saI and one individual was screened for only *Hinf*I. All 156 samples screened for both enzymes were consistently identified across both assays. Of the 172 samples, 171 were identified as *Lampetra* and one (larva code ICF 264, length 11 mm, caught 23.00, 22 July 2016) as *Petromyzon* (Figures 17 and 19). A total of 18 larvae were caught during this (22 July 2016) sampling night and, in addition to the *Petromyzon* larva, ten of those caught underwent DNA analysis and were identified as *Lampetra*. As these *Lampetra* larvae were 9–19 mm long, the presence of the single 11 mm long *Petromyzon* in the sample from 22 July 2016 was considered to be unlikely to indicate the appearance of a new cohort of recently emerged 0+ year class *Petromyzon* larvae (Figure 16).

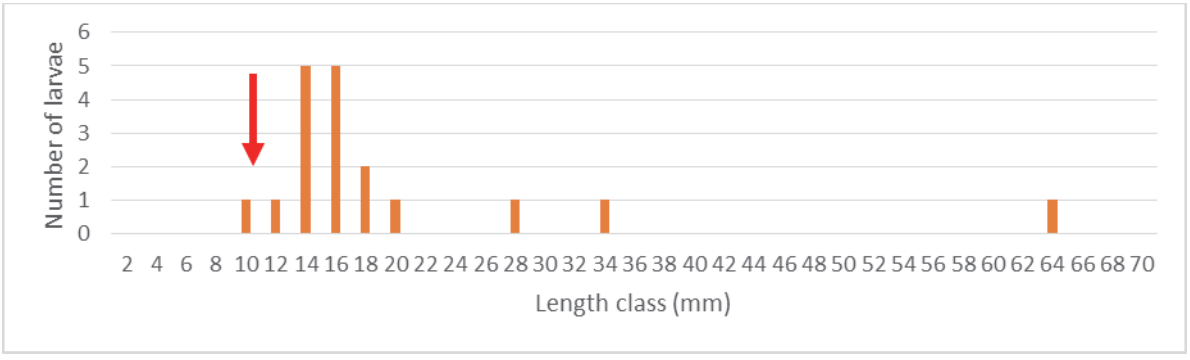

Figure 16. The length distribution of larval lamprey caught in drift nets in the River Teith during the night of 22 July 2016. The red arrow indicates the length of the single Petromyzon *larva.*

Differences between the pigmentation pattern in the caudal fin of the 11 mm long *Petromyzon* larva and the *Lampetra* larvae caught in the drift nets on the same sampling date were not readily distinguishable (Figure 17 and 18). Although the comparison was limited by being based on the examination of only a single drifting *Petromyzon* larva, it would appear that this external characteristic is not a reliable indicator of genus in very small drift-stage larvae. Indeed Renaud (2011) does not mention caudal fin pigmentation patterns in his list of features distinguishing *Petromyzon* from *Lampetra* but focuses more on the pigmentation in the head region. A key distinguishing feature of the *Petromyzon* genus is pigmentation in the upper lip region of (Renaud, 2011); whether this pigmentation was present in *Petromyzon* larva caught during the night of 22 July 2017 was unclear (Figure 19). The *Lampetra* larvae images (Figure 20) were clearer and suggested that there was no pigmentation in the upper lips of larvae 259, 262, and 265, although there appeared to be pigmentation in or close to the upper lip region of larvae 257. A problem associated with using the pigmentation patterns defined by Renaud (2011) is distinguishing the limit of the *upper lip region* as there is no real morphological differentiation between it and the cheek region. Perhaps the only clear way to judge whether pigmentation is present on the upper lip region is to establish whether pigmentation is present along the bottom edge of the upper lip. However, in the case of the *Petromyzon* larva referred to here, no pigmentation was present in this area.

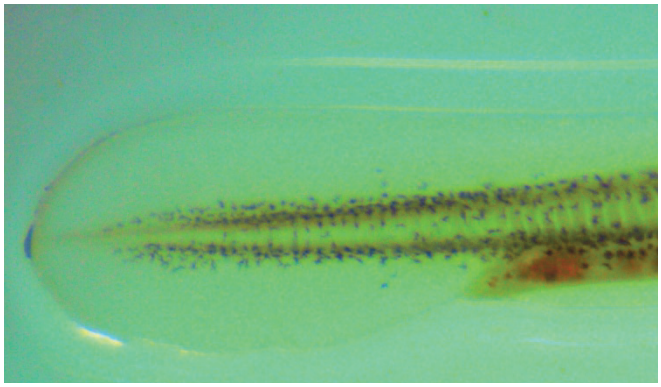

Figure 17. Caudal fin detail of the only Petromyzon *larva (larva code ICF 264, length 11 mm) caught in drift nets in the River Teith during the night of 22 July 2017.*

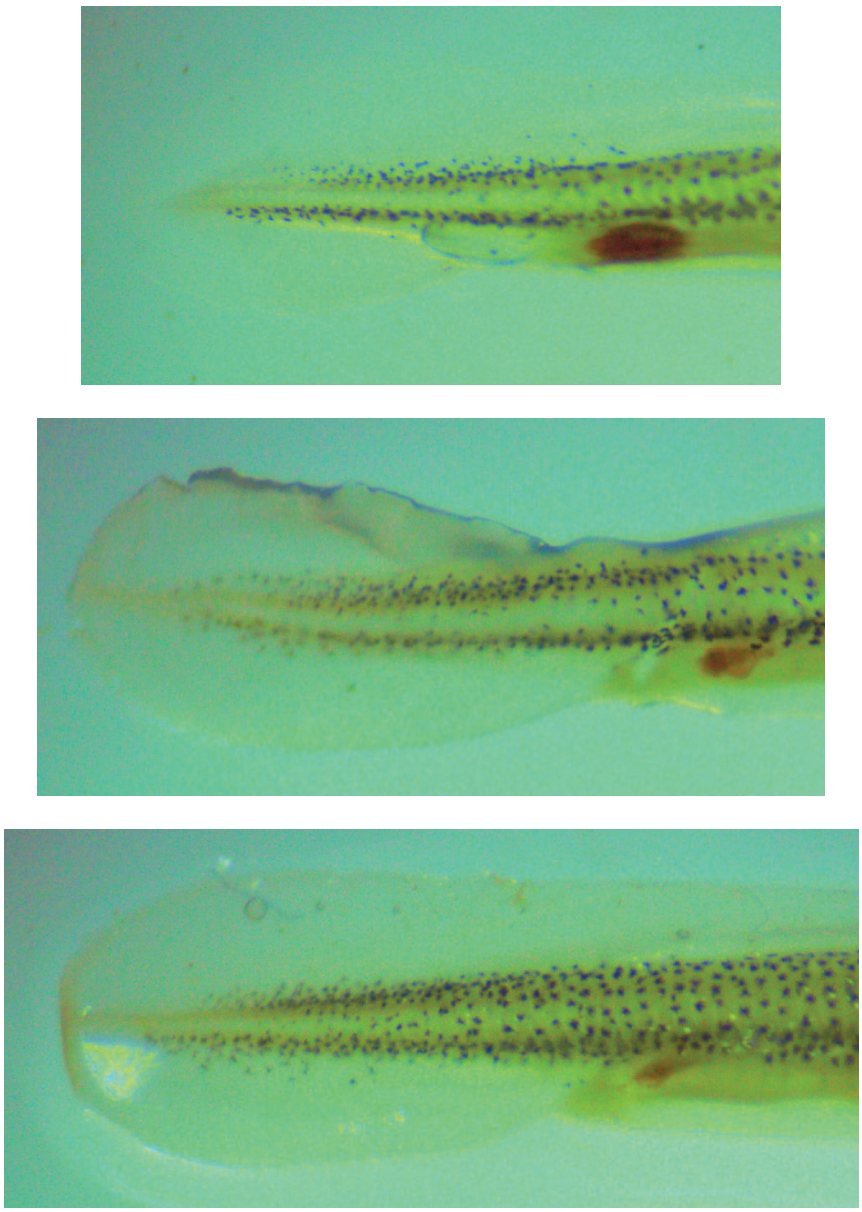

Figure 18. Caudal fin details of Lampetra *larvae caught in drift nets in the River Teith on the night of 22 July 2017. Top: larva code ICF 262, length 13 mm. Middle: larva code ICF 257, length 14 mm. Bottom: larva code ICF 259, length 15 mm. The tail from larva code ICF 265, 9 mm was damaged and not photographed.*

Figure 19. Head detail of the only Petromyzon *larva ICF 264, length 11 mm, caught in drift nets in the River Teith during the night of 22 July 2017.*

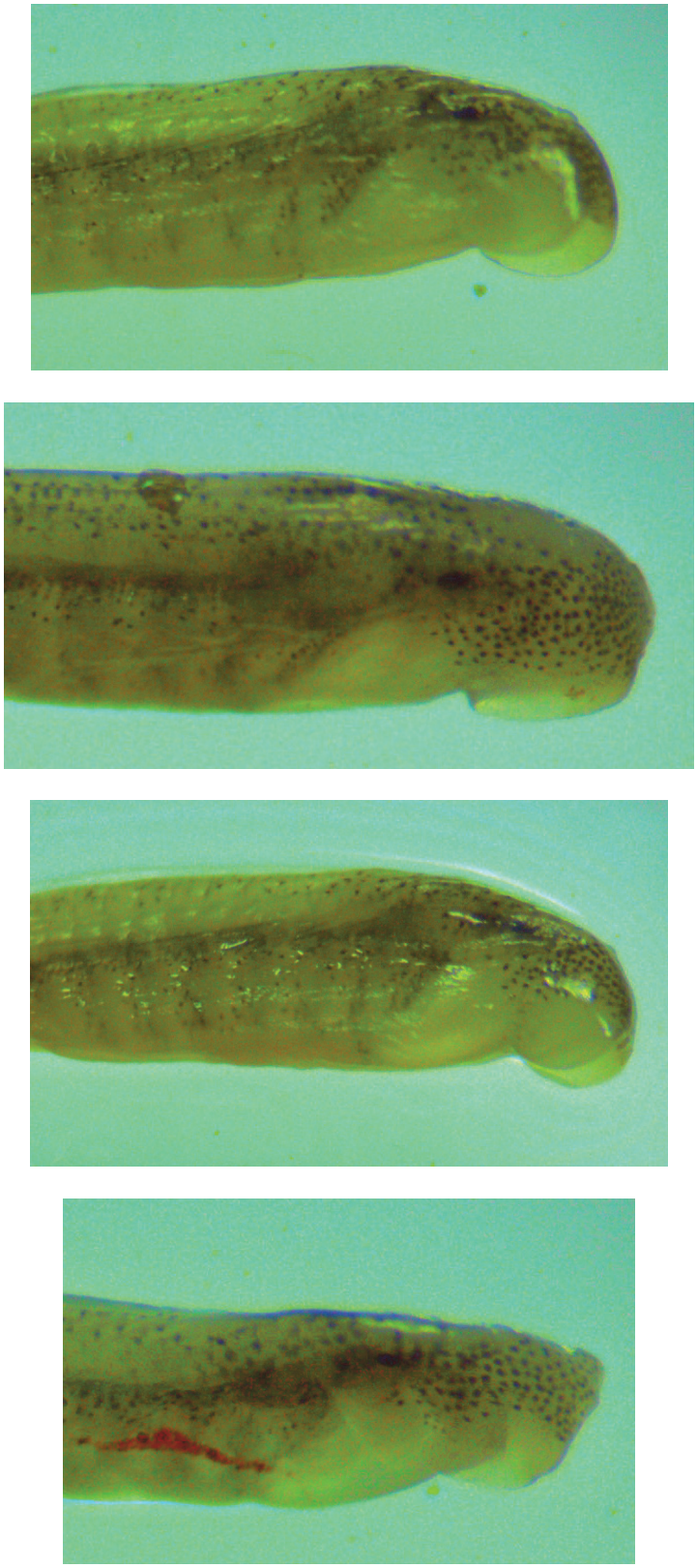

Figure 20. Head details of Lampetra *larvae caught in drift nets in the River Teith during the night of 22 July 2017. Top: larva code ICF 262, length 13 mm. Upper middle: larva code ICF 257, length 14 mm. Lower middle: larva code 259, length 15 mm. Bottom: larva code ICF 265, length 9 mm.*

3.5 Electrofishing validation

In order to provide some baseline information about localised larval *Petromyzon* densities in the lamprey community found in the shallow marginal habitat of the lowermost River Teith and River Forth, and to allow comparison with the results from the other survey methods, a number of sites in the same area were electrofished in October 2015 and August 2016. Each electrofishing site was selected by a visual survey of its flow and substrate characteristics. Standard electrofishing methods (Harvey & Cowx, 2003) were used over 3 or 4 m^2 at each site. The locations of the 2015 electrofishing sites are shown in Figure 21. The sites electrofished in 2016 constituted the lowermost seven sampling sites used for the River Teith SAC lamprey Site Condition Monitoring (see Figure 22).

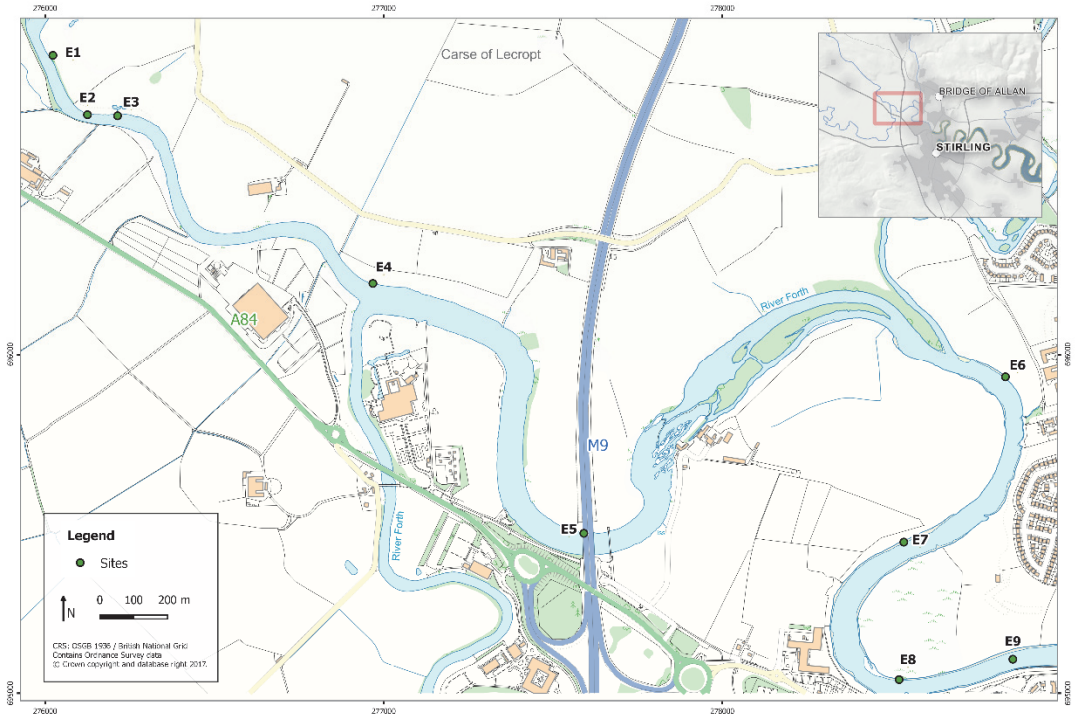

Figure 21. The locations of the nine electrofishing sites surveyed in October 2015 to provide baseline information about the larval lamprey community structure in the area where airlift sampling and drift netting was undertaken.

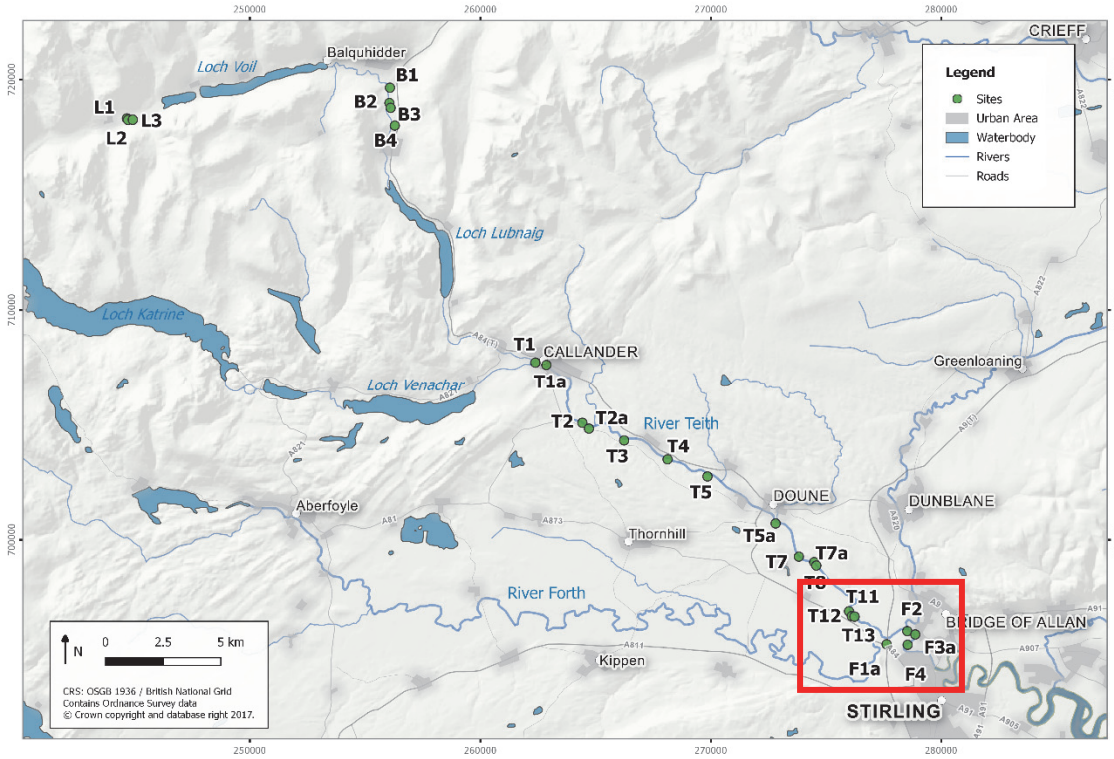

Figure 22. The locations of the seven lowermost electrofishing sites (red box) surveyed in August 2016 as part of the River Teith SAC lamprey Site Condition Monitoring to provide baseline information about the larval lamprey community structure in the area where airlift sampling and drift netting was undertaken.

A total of 817 *Lampetra* larvae and 15 *Petromyzon* larvae were caught in October 2015 at the nine electrofishing sites in the vicinity of the airlift sampling that was conducted in 2016 (Table 5). In August 2016 a total of seven sites were electrofished in the same area resulting in the capture of a total of 630 *Lampetra* larvae and seven *Petromyzon* larvae (Table 5). The results mean that in 2015 the typical occurrence rate of *Petromyzon* larvae in marginal habitats in the area was 1.8% of the larval lamprey community. In 2016 the occurrence rate was slightly lower at 1.1%.

Table 5. The total number of Lampetra *and* Petromyzon *larvae caught by electrofishing in 2015 and 2016 in marginal habitats in the vicinity of the airlift and drift net sampling.*

Electrofishing site	Survey date	Total *Lampetra* larvae	Total *Petromyzon* larvae
E1	October 2015	67	3
E2	October 2015	237	10
E3	October 2015	69	5
E4	October 2015	35	0
E5	October 2015	274	2
E6	October 2015	9	0
E7	October 2015	80	0
E8	October 2015	28	0
E9	October 2015	10	0
T11	August 2016	185	2
T12	August 2016	334	2
T13	August 2016	121	1
F1	August 2016	95	1
F2	August 2016	16	0
F3	August 2016	27	0
F4	August 2016	24	1

Figures 23 and 24 show that several year classes of *Lampetra* larvae were present in the shallow marginal habitat patches that were electrofished in 2015 and 2016. *Petromyzon* larvae were also present in both years. As the 2015 electrofishing was conducted later in the year, the larvae caught were correspondingly larger than those caught in 2015 and so the mean sizes of the 0+ and 1+ year classes of *Lampetra* were slightly higher.

Figure 23 suggests that in October 2015 at least two year classes of *Petromyzon* larvae were present in the electrofishing samples. The minimum length of the individuals caught suggests that they would have been representative of the 1+ and 2+ year classes.

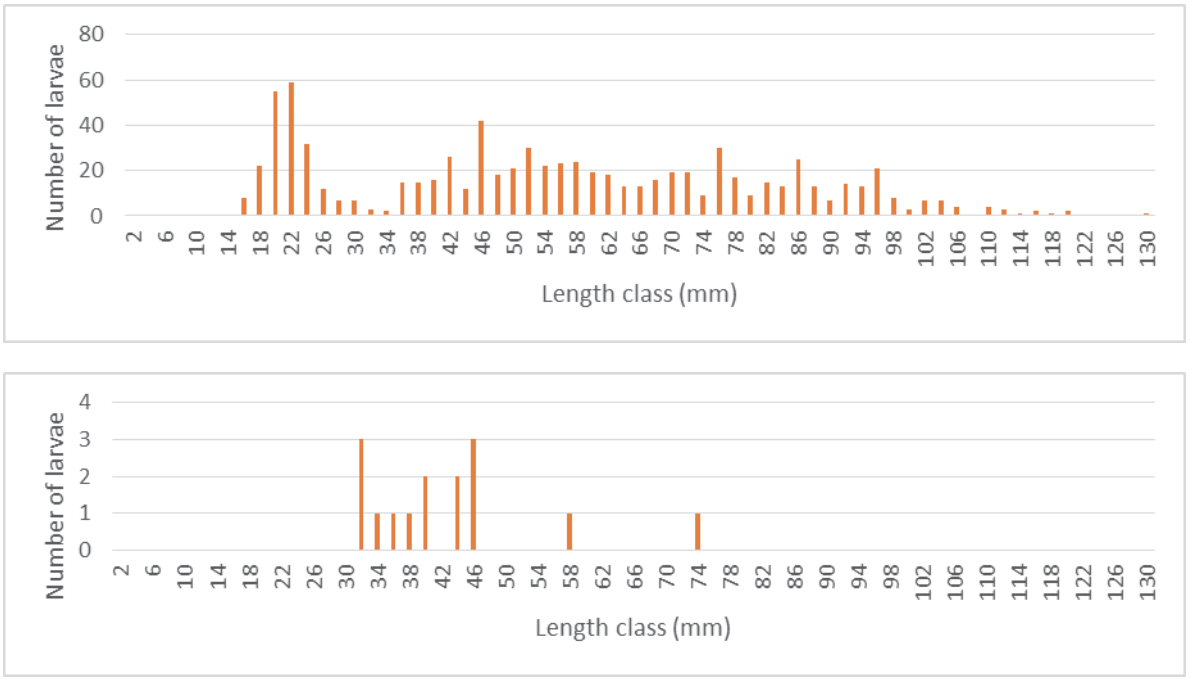

Figure 23. The length frequency of larvae caught by electrofishing at nine sites in October 2015. Top: Lampetra *larvae (N = 817). Bottom:* Petromyzon *larvae (N = 15).*

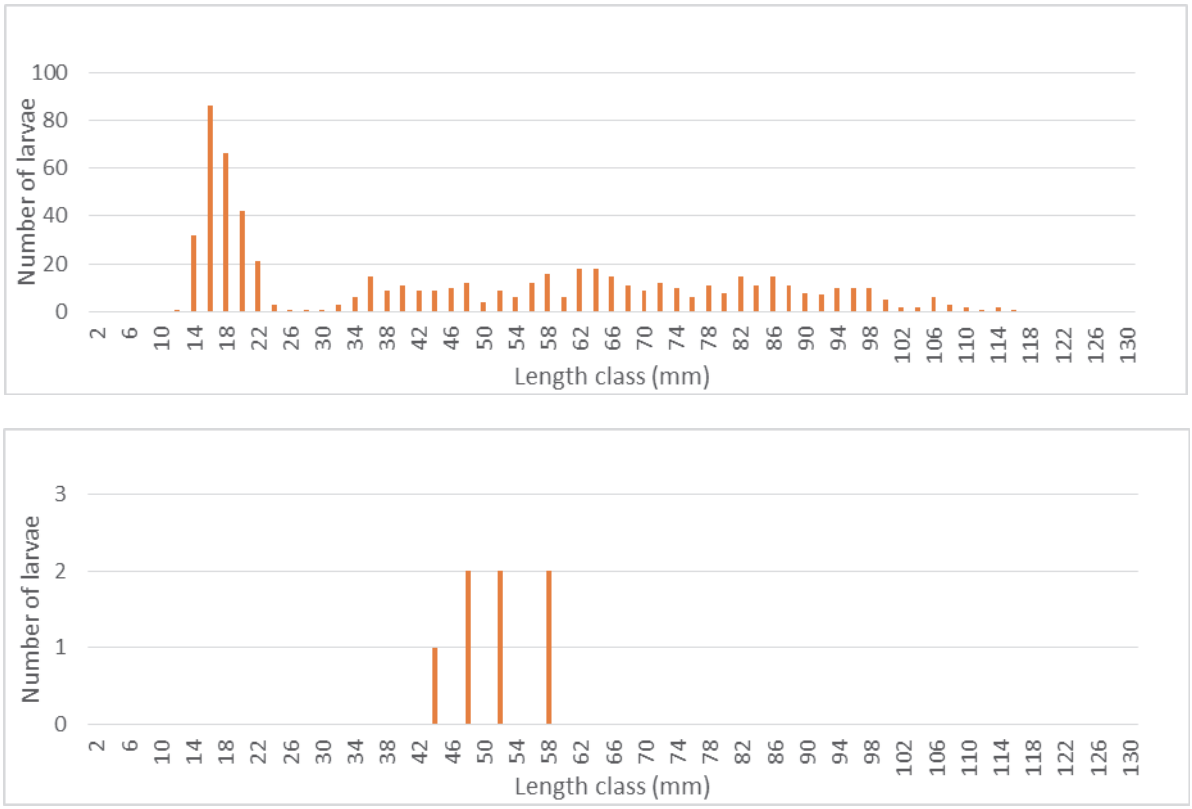

Figure 24. The length frequency of larvae caught by electrofishing at seven sites in August 2016. Top: Lampetra *larvae (N = 630). Bottom:* Petromyzon *larvae (N = 7).*

In August 2016 all seven of the *Petromyzon* larvae caught by electrofishing appeared to belong to the 1+ year class as the minimum individual length (43 mm) appeared to be well outwith the length distribution range for similar 0+ (young-of-the-year) *Lampetra* larvae at the time of sampling (Figure 24).

3.6 Comparison of results from the various sampling methods

A visual comparison of the length distributions of the larval lamprey caught by airlift sampling, electrofishing and drift netting (Figure 25) suggested that the patterns of capture were different. A box plot of the larval length data (Figure 26) further illustrates these differences.

The larval length data were non-normally distributed. They could not be normalised and so a non-parametric Kruskall-Wallis test was used to check if the lengths of the larvae captured differed according to the method used. The results (H = 368.74, df = 2, $P < 0.001$) and post-hoc Wilcoxon signed-rank test indicated that the lengths of all three sampling methods were significantly different ($P < 0.001$). Electrofishing therefore generally resulted in larger larval lamprey being caught than either airlift sampling or drift netting, whilst the airlift sampling tended to catch more of the 1+ year class than drift netting. Drift netting caught the smallest larvae and fewer individuals of the older year classes.

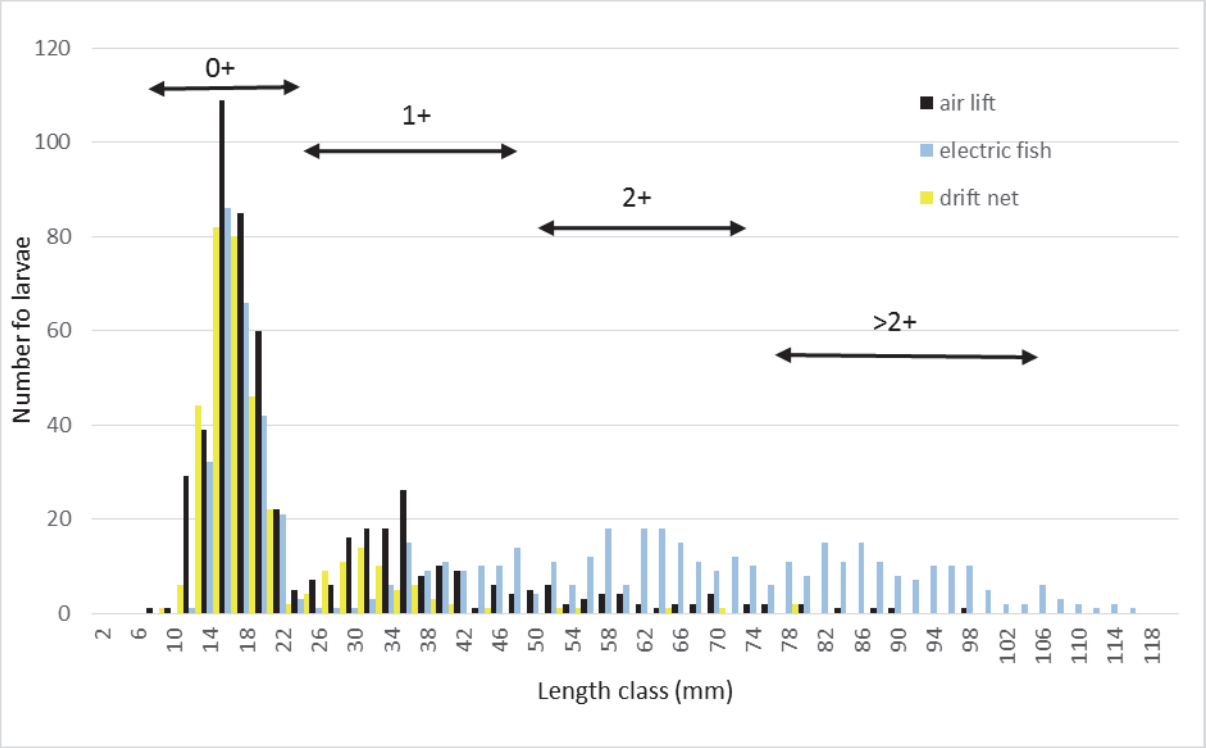

Figure 25. Length frequency distributions of the larval lamprey caught in the lower River Teith in 2016 by each of the survey methods: airlift sampling (n = 537); electrofishing (n = 637); and drift netting (n = 354). Black arrows indicate suggested year class groupings.

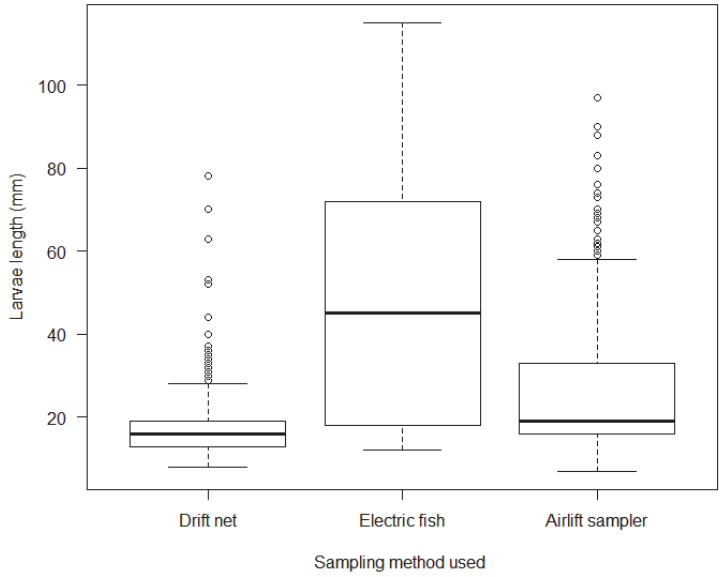

Figure 26. Box plot of the lengths of the larval lamprey caught in the lower River Teith in 2016 by each of the survey methods: airlift sampling (n = 537); electrofishing (n = 637); and drift netting (n = 354). Mean values are indicated by the thick black lines.

4. DISCUSSION

4.1 Airlift sampling

The collection of larval lampreys from habitats in deeper water provided evidence that: suitable larval habitat was present outwith the channel margin areas; and that larval lampreys were present in these deeper river habitats.

Using the airlifting equipment in deeper water presented challenges. The apparatus was difficult to deploy, capture efficiency was low, and samples that contained no larval lampreys were often collected. Although 65% of the airlift samples collected in 2016 did contain one or more larval lamprey, 100% of the 16 electrofishing samples collected in 2015 and 2016 contained larvae. This difference is unsurprising as it was not possible to fully assess the quality of the river bed habitat before deploying the airlift sampling equipment. The consistency of the substrate was checked with a benthic grab and the end of a ranging pole to ensure that soft substrate was present, and during sampling the surveyor moved the equipment away from areas where hard material was encountered. But, the measures used to guide the equipment into suitable substrate were inconsistent and not as thorough as those used in shallow water by surveyors preparing for electrofishing. As a result, the electrofishing gear was more likely to be used in suitable habitat for a greater proportion the survey time and so the capture efficiency was better. Many of the samples that were collected by airlifting may have initially been gathered from suitable habitat, but the sampling nozzle may have moved into less suitable habitat without the operator being aware.

The occurrence of *Petromyzon* larvae in the airlift samples was generally found to be low. Of the 34 airlift samples that contained any larval lamprey, 26% of them contained one or more *Petromyzon* larvae. This was approximately half that of the 56% occurrence of *Petromyzon* larvae for the 16 channel margin electrofishing samples collected in the same area during 2015 and 2016. The overall contribution made by *Petromyzon* larvae to the lamprey community sampled by airlifting was around 2%. This compares favourably with the 2015 and 2016 electrofishing survey contributions of 1.8% and 1.1% respectively. These results suggest that whilst *Petromyzon* larvae do not appear to be limited to the marginal benthic habitats of the River Teith and were encountered in depths of water exceeding 2 m, they do not appear to be more abundant or more widely distributed in the deeper water habitats. This is in disagreement with the results for the Dordogne River (Taverney *et al.*, 2012) and the Welsh River Dee (APEM, unpublished data).

The need for a boat to deploy the airlift equipment meant that the method required greater resources than electrofishing. Additionally, a considerable amount of time was required to sort the samples collected by airlifting, especially those from areas in which dense deposits of coarse particulate organic matter were encountered. Greater quantities of this material were contained in samples collected by airlifting than in those collected by electrofishing.

Whilst the results appear to suggest that the airlift sampling method favours the collection of smaller larval lampreys, the similar occurrence rates of *Petromyzon* larvae in the samples collected by airlifting and electrofishing suggest that the effectiveness of the airlift apparatus when sampling the benthic larval lamprey community was comparable. Where electrofishing in channel margins is made difficult by deep water, steep banks, or dense riparian vegetation, boat deployed airlifting could be an effective alternative method to provide information on the local status of the larval lamprey populations. Conditions where airlifting might be the favoured approach are particularly likely to occur in the lower portions of large SAC rivers where shallow marginal sampling sites are scarce, and knowledge on the spatial distribution of larvae is currently limited.

Airlift sampling was carried out at a mean water depth of 1.35 m and was effective in the River Forth at depths ≤ 3.00 m. This depth was far greater than the maximum (approximately 0.50 m) in which electrofishing apparatus could be used effectively.

In order to compare the larval lamprey densities established by airlift sampling with those by electrofishing, the capture efficiency of the airlift sampling equipment would need to be determined as well as some measure of the area that was sampled during each release of high pressure air. These calibrations would require specific, controlled laboratory test conditions and were outwith the scope of the investigation. However, as the current SCM guidance for *Petromyzon* (JNCC, 2015) does not include a density target because of the highly variable and low densities that are typically encountered, a method that could ease the collection of distribution data, even at the presence/absence level, would have value.

The limited larval *Petromyzon* capture rate results did little to improve the information about where in the Rivers Forth and Teith the *Petromyzon* larvae might be. Due to the downstream dispersal of emergent larvae and their subsequent passive (drifting) or active (swimming) movement between habitats, the spatial pattern of larval distribution across a river network will be largely determined by the past spawning and migratory behaviour of adults (Torgensen & Close, 2004; Neeson *et al.*, 2011) as well as the presence of suitable habitat patches resulting from fluvial processes (e.g. Gilvear *et al.*, 2008). White & Harvey (2003) reported that drifting larval lamprey limited their transport to estuaries by avoiding periods of high flow during the spring. However, other authors have reported that the drifting of larvae appears to be at its maximum under elevated flow conditions; this raises the question of how drifting is controlled so that larval lamprey avoid encountering lethal saline conditions.

Electrofishing and airlift sampling failed to record any sea lamprey larvae > 75 mm in length. The majority of larvae appeared to fall within either the 1+ or 2+ year class. As sea lamprey metamorphosis in European stocks occurs when the larvae are 3–5 years old and around 130–140 mm long (Dawson *et al.*, 2015), the whereabouts of the larger, older individuals in the rivers Forth and Teith remains unknown. The use of the airlift sampling equipment was restricted to specific reaches of the rivers Forth and Teith due to the size and power of the boat used and flow conditions. As a result, sampling was stopped 1 km upstream of Stirling Bridge. It was possible that the larger larval *Petromyzon* were most abundant in the lowermost reaches of the river system, in the habitat located in the 12 km of river between Stirling and Throsk. It was not until Throsk that the SEPA water quality records indicated that the salinity was > 10 ppt; concentrations greater than this have been found to be lethal to larval lampreys (Reis-Santos *et al.*, 2008).

4.2 Drift netting

The results of the drift netting study were disappointing. They failed to provide evidence that the method could be useful for surveys of the elusive larval *Petromyzon*. The results did however provide some insights into the distribution behaviour of larval *Petromyzon*. Of note was the positive association between falling river discharge and larval drift and the overwhelmingly nocturnal nature of this behaviour.

In keeping with other studies (e.g. Applegate, 1950) the drifting of larval lamprey was found to peak under the hours of darkness. Potter (1980) reported larvae moving downstream at night during elevated flow conditions. Bracken & Lucas (2013) reported that catches of drifting larvae were eight times greater during the night than day, and that larvae drifted passively with the flow. However, Quintella *et al.* (2005) provided evidence that larvae do change location voluntarily and that their movements are not entirely governed by displacement and transport in elevated flows. The peaking of drifting during the darkest part of the night lends weight to the idea that it is not entirely passive but is in fact voluntary

(White & Harvey, 2003) with larvae appearing to select certain light conditions. Other environmental cues may also to initiate drifting.

Following an incubation period that lasts approximately two weeks, *Petromyzon* larvae emerge from the nest at around 9 mm in length. Emergence may continue for several weeks (Applegate, 1950; Derosier *et al.*, 2007), with larvae settling close to the nest (Manion & McLain, 1971) or being carried downstream to depositional areas of silt, soft sand, and detritus. The rate and extent of larval dispersal from the nesting locations is likely to be highly variable and there are indications that it can be influenced by factors other than the flow variability.

Larval lamprey drift dispersal has been reported to cover hundreds of kilometres in river systems in North America (Moser & Close, 2003), but the settlement patterns of newly emerged larvae appear to be under behavioural as well as hydraulic control. Derosier *et al*. (2007) report sea lamprey larvae moving more than 150 m downstream from nest locations within three weeks of hatching with the behaviour being influenced by water temperature and the initial density of larvae. Although the results of Lucas *et al.* (2007) suggest that the drifting of very small larvae may mainly be the result of displacement by scour events, the presence of high densities of larvae in this study during only moderately high flows together with data from the River Ure (Lucas, unpublished data) suggest that there is more to drifting than passive displacement. In addition, the findings of this study suggest that there was a significant positive association between decreasing discharge and increasing larval drift. It is possible that this result was influenced by the passive displacement and drifting of larvae originating from upstream habitats during the peak discharge period, and their subsequent collection in nets further downstream several hours later when river levels were falling. However, when coupled with the obvious preference for nocturnal drifting, this result provides more evidence for lamprey larvae behaviour being directed not only by light levels but also by specific flow conditions, possibly as a means of limiting the distance that they drift. The precise triggers for larval lamprey drift remain unclear and Moser *et al.* (2015) suggest that *further study is needed to determine the ontogeny of dispersal in larval lampreys*.

Moser *et al.* (2015) suggest that larval lamprey may be under-represented in netting or trapping studies as they may be able to escape from even specialised traps. This may be due to their ability to pass through small mesh nets, their tendency to avoid light, and an association with debris and bottom structures that may not be effectively sampled by rigid-framed nets (Moser & Russon, 2009). Indeed, during this study trials in which drift nets were deployed for six hour sampling periods before retrieval resulted in no larval lampreys being caught; when the same nets were set in the same location but emptied hourly they contained larval lampreys. This provided further evidence that the sampling efficiency of nets, even when very small mesh size nets are used, can be reduced by larval behaviour.

Derosier *et al.* (2007) also suggest that the distribution of 0+ larval sea lamprey in Michigan rivers could be limited to within 200 m of the nest site during their first growing season. This tendency to limit the extent of drifting immediately after their emergence and during early settlement phases may help to explain the low occurrence of larval *Petromyzon* in the drift net samples collected during this study. The results of the DNA analysis suggested that drift netting resulted in a *Petromyzon* occurrence rate of just 0.5% of the 467 individuals caught. This is very low compared to the results from both electrofishing and airlift sampling raises doubts about the utility of the method as a way of collecting spatial distribution information about this species in large river systems. It is possible that recent developments in the use of lamprey eDNA (e.g. Gingera *et al.*, 2016) will result in the development of novel techniques that could advance our understanding of sea lamprey population distribution and dynamics in SAC rivers.

5. CONCLUSIONS

Trials using a scaled down portable suction pump to sample larval lamprey beds in water < 1 m deep suggested that similar estimates of larval density could be established as those by electrofishing. However, whilst the method provided an effective way to collect young-of-the-year larval lamprey it appeared to under-sample the larger individuals in the population, possibly due to higher rates of escape. It was also associated with a 10% larval mortality rate; the rate for electrofishing was negligible. The considerable additional resources required to undertake suction pumping safely and effectively were of considerable concern. It is suggested that, based on the findings of this study, suction pumping is not an efficient method for use in larval lamprey population assessments.

The results of the trials showed that airlifting can be used to sample larval lamprey populations situated in habitat patches in areas of deeper water and where access to larval lamprey habitats from a bank is impractical. Larval lamprey are not confined to shallow marginal habitats in the lower regions of the River Teith SAC, but their distribution and population density in deeper water habitats appear to be extremely patchy; this accords with the results obtained from electrofishing in shallow habitats. Importantly, there was no significantly higher likelihood of encountering larval *Petromyzon* in deeper water habitats using this method than using conventional shallow water electrofishing methods along the channel margins.

Larval *Petromyzon* were not found to be more abundant in deeper water habitats than in the more-easily sampled shallow marginal areas. This result suggests that electrofishing provides an adequate indication of the status of larval *Petromyzon* in the River Teith SAC and is not failing to record aggregations of larvae in deep water in the lower sections of the river. Whilst airlifting could be used to survey larval lampreys in locations where electrofishing in marginal habitat might not be possible (e.g. where access from a bank was restricted or channel profiles were steep) the higher resource implications of using the method would have to be taken into account when planning SCM.

The results of the nocturnal drift netting study were disappointing as they failed to provide evidence that the method could be useful for establishing the status of the rare larval *Petromyzon*. Methods that were originally intended to collect samples of drifting invertebrates can be successfully adapted to provide samples of drifting larval lampreys, and this may be of use for determining catchment distribution. However, the scarcity of larval *Petromyzon* caught by the nets during the summer emergence period suggested that they do not comprise a higher percentage of the larval community at the dispersal stage on this river system. Indications are that re-focussing sampling effort on larval drift would likely be of limited use in monitoring sea lamprey population variation.

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7. APPENDIX

Description of airlift sampling sites

Site	Sampling date	NGR	Site characteristics	Water depth (m)	Dissolved oxygen at bed (mg L^{-1})	Dissolved Oxygen at bed (%)	Conductivity of water at bed (µS)	Temperature of water at bed (°C)	pH of water at bed
1A	01/08/2016	NS 77369 96067	2 m from left bank, steep drop-off with reeds along the margin	1.35	10.8	121	92	22.6	7.46
2A	01/08/2016	NS 77387 96054	2 m from left bank, steep drop-off with reeds along the margin	1.69	9.0	94	79	22.9	7.40
3A	01/08/2016	NS 77396 96031	5 m from left bank	1.60	10.7	120	78	21.7	7.19
4A	01/08/2016	NS 77357 96072	2 m from left bank with extensive macrophyte cover	1.30	11.0	125	70	21.3	7.12
5A	01/08/2016	NS 77319 96022	Within 1.5 m of edge of right bank	1.20	9.9	125	117	22.4	7.01
6A	01/08/2016	NS 77345 95995	Behind promontory, next to rocks	0.80	9.4	109	111	22.3	7.13
7A	01/08/2016	NS 77134 96102	Marginal, cattle drink area behind promontory	1.00	6.3	86	124	24.0	6.79
8A	01/08/2016	NS 77186 96077	Right bank, marginal location amongst macrophytes	1.00	7.5	105	121	23.4	6.98
1B	02/08/2016	NS 78495 96117	Right bank, marginal location, steep, substrate mainly sand with LWD present	1.30	8.0	99	106	21.9	7.44
2B	02/08/2016	NS 78654 95954	Right bank, behind woody debris, steep mud bank	1.10	8.7	102	111	21.8	7.30
3B	02/08/2016	NS 78691 95942	Right bank, behind wooden pier foundations	1.30	8.8	101	99	21.9	7.22
4B	02/08/2016	NS 78783 95887	Right bank, 50 m downstream of wooden pier foundations	1.40	8.1	93	101	20.5	7.23

5B	02/08/2016	NS 78833 95920	Left bank, across from wooden pier foundations	1.10	9.0	101	150	20.5	7.41
6B	02/08/2016	NS 78856 95896	Left bank, clay with sediment on top, gravel bar at mouth of Allan Water	1.30	9.1	102	130	20.3	7.52
7B	02/08/2016	NS 78802 95530	3 m from left bank, in eddies behind pier structures	1.80	9.2	105	118	20.5	7.50
8B	02/08/2016	NS 78774 95515	5 m from left bank, in eddies behind pier structure, substrate possibly clay dominated	1.20	8.7	98	116	20.0	7.49
9B	02/08/2016	NS 78727 95542	2 m from left bank at edge of LWD deposit	1.20	8.4	95	113	19.7	7.44
1C	03/08/2016	NS 78585 95404	3 m from left bank, next to tree stump/clay, silt, LWD	3.00	9.7	102	139	17.3	7.31
2C	03/08/2016	NS 78607 95415	Left bank behind stump, substrate of clay and silt	2.50	9.7	101	133	17.2	7.32
3C	03/08/2016	NS 78661 95434	4 m from left bank, noticeable flow, clay substrate	1.20	7.5	98	124	18.9	7.30
4C	03/08/2016	NS 78632 95416	Left bank, eddy behind pier, gravel/mud substrate	1.20	7.2	92	134	18.7	7.10
5C	03/08/2016	NS 78404 95358	3 m from right bank, soft silt substrate	1.30	8.9	99	127	21.1	7.28
6C	03/08/2016	NS 78454 95396	3 m from right bank, soft silt substrate	1.40	7.5	82	128	20.0	7.40
7C	03/08/2016	NS 78483 95410	3 m from right bank	1.10	9.6	99	116	17.6	7.41
8C	03/08/2016	NS 78505 95419	3 m from right bank	1.20	9.6	100	116	17.6	7.41
1D	05/08/2016	NS 78318 95108	5 m from left bank, much gravel and cobble substrate	1.10	7.8	98	114	24.0	6.70
2D	05/08/2016	NS 78349 95102	5 m from left bank, eddy, gravel/clay substrate	1.10	8.2	98	99	21.0	6.90

3D	05/08/2016	NS 78364 95075	Middle of river, clay substrate	2.20	8.5	100	89	21.0	7.00
4D	05/08/2016	NS 78354 95093	6 m from left bank, clay substrate	1.30	8.8	100	88	20.0	6.90
1E	05/08/2016	NS 77702 95420	15 m from right bank, in main pool and 20 m downstream from pipe bridge	1.40	94	109	32	20.0	6.73
2E	05/08/2016	NS 77761 95444	1 m from right bank, clay and little gravel substrate	1.10	97	107	34	19.3	6.71
3E	05/08/2016	NS 77794 95468	By right bank, mixed stones/clay/sandy silt substrate	1.80	9.9	107	80	19.8	6.63
4E	05/08/2016	NS 77842 95560	1 m from right bank, emergent macrophyte growth	1.40	1.01	107	83	19.0	6.66
1F	08/08/2016	NS 77844 95548	2 m from left bank, macrophyte cover clay/mud substrate	1.20	12	98	130	18.7	7.23
2F	08/08/2016	NS 77844 95548	2 m from left bank, macrophyte cover, clay/mud substrate	1.20	12	98	130	18.7	7.23
3F	08/08/2016	NS77561 95408	1 m from left bank, patchy sediment and stone substrate, emergent vegetation	1.30	11	110	88	16.4	7.13
4F	08/08/2016	NS77453 95449	1 m from right bank, in eddy created by croy, mud bank, extensive reeds	1.00	12.8	103	99	16.0	7.16
5F	08/08/2016	NS 77602 95468	Within 2 m of left bank underneath motorway bridge, within patch of LWD	0.85	11.1	111	68	20.3	7.14
6F	08/08/2016	NS 77602 95468	3 m downstream of 5F, in LWD	0.85	11.1	111	68	20.3	7.14

7F		NS77355 95549	2 m from right bank alongside horsetail	1.30	11.7	124	104	17.3	6.56
8F		NS 77365 95538	3 m from right bank alongside horsetail	1.30	11.7	124	104	17.3	6.56
1G	10/08/2016	NS 76341 96599	5 m from right bank, by two logs, LWD, sand/silt substrate	1.20	9.3	97	51	17.6	7.52
2G	10/08/2016	NS 76342 96591	On right bank, LWD, riparian willow	1.20	8.7	90	54	16.9	7.40
3G	10/08/2016	NS 76353 96554	On right bank, LWD, is sandy/silt substrate, organic detritus	1.20	9.2	98	56	16.9	7.29
4G	10/08/2016	NS 76367 96490	On right bank, LWD, sandy/silt substrate, organic detritus	1.20	9.9	100	52	16.7	7.26
5G	10/08/2016	NS 76392 96518	1 m from left, steep, vertical bank, sand/mud substrate	1.50	9.5	95	53	14.1	6.57
6G	10/08/2016	NS 76394 96501	On left bank, silty/clay substrate	1.60	10.6	103	51	14.1	6.7
7G	10/08/2016	NS 76411 96452	1 m from left bank	1.90	10.2	98	53	14.1	6.73
8G	10/08/2016	NS 76362 96616	On left bank in eddy feature behind debris deposit	1.80	9.5	91	51	13.9	6.8
1H	11/08/2016	NS 76429 96348	On right bank, beneath riparian willow, sandy/muddy substrate, organic debris	1.10	11.7	120	11.7	17.9	7.48
2H	11/08/2016	NS 76647 96369	On left bank, beneath riparian willow, sandy silt substrate	1.10	11.5	120	42	18.0	7.50
3H	11/08/2016	NS 76754 96384	2 m out from left bank	1.20	12.2	114	62	20.1	7.49
4H	11/08/2016	NS 76735 96379	1 m from left bank, eddy behind fallen willow, sandy silt substrate	1.40	11.2	120	70	19.6	7.47

LWD: large woody debris

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