Pacific Lamprey (*Lampetra tridentata*) Breeding and Rearing Methodologies- Recommendations for Chelan County P.U.D.

Prepared by:

Joy Wade, Fundy Aqua Services

and

R. J. Beamish

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Summary of Report and Seminar Discussions

The results of the rearing studies by Beamish's group and the results described at the Juvenile Pacific Lamprey Seminar in Wenatchee, Washington, in August 2012 were similar. In the Beamish studies, ripe females were stripped of eggs and fertilized with sperm from at least two males. Eggs and sperm must flow readily from the adults with only gentle pressure. Beamish's group mixed fine sand with the fertilized eggs to prevent adhesion to the walls of the container or other eggs. In the Beamish studies, small batches of eggs were reared, but in the studies described during the seminar, larger numbers of eggs were successfully reared. It appears therefore, that the fertilizing and rearing of eggs from Pacific lamprey is not a significant obstacle. If very large numbers of eggs are incubated, typical of large Pacific salmon hatcheries, it will be necessary to develop protocols similar to those used in large production hatcheries.

From the seminar it was determined that acquiring mature broodstock is a problem. Although catching maturing adults is not a problem, only a percentage of the upstream migrating adults can be induced into the running ripe maturing state. The results of the Beamish studies indicate that the upstream migrating Pacific lamprey need to be held over winter in tanks where they are provided an environment that is similar to the deep pools in which they can remain undisturbed and are generally able to hide. It is during this period that males and females use their body reserves to undergo gametogenesis. Migration timing varies depending on the freshwater entry time. In some areas such as the Skeena River, migration can occur up to 12 months before the onset of spawning. Pacific lamprey from other, more coastal rivers, begin entering freshwater up to 2 months before the onset of spawning. Pacific lamprey from the Fraser and Somass rivers have a freshwater entry time of June and can extend to September for spawning the following April/May. The work by Beamish indicated that Pacific lamprey were ready to spawn when they would leave the refuge where they were able to hide,

and cling to the sides of the tank during the day. The loss of the avoidance of daylight seemed to be an indication that the individual was preparing to spawn. These individuals were put in an artificial stream that had strong flows at one end. The exact length of time it took from their introduction to the tank until spawning was not recorded. However, Beamish recalls that it was about one to two weeks. Nest building started first, followed by spawning, which initially started at night. It is possible that this type of simulated environment in the hatchery will be required to induce maturation at a production level. Certainly, it appears that additional experimentation is needed to acquire large numbers of running ripe males and females. It is also possible that hormone injections could be used to induce different lamprey species into spawning condition.

Once eggs have hatched and larval ammocoetes have commenced exogenous feeding it is necessary to decide the size and time that they should be released into the natural environment. We think that they will survive in the wild. It might be expected that the first winter in the natural environment be marked by significant ammocoete mortality, indicating that the young-of-the-year ammocoetes need to grow at a rate similar to the naturally spawned individuals. The suggestion to compare lengths of Pacific lamprey with young *Lampetra richardsoni* seems to be a reasonable substitution. Some comparisons are needed and because it may be difficult to identify the species of naturally spawned young-of-the-year ammocoetes, we suggest not worrying too much about the "wild" species being used for comparisons.

Some of the cultured lamprey could be held for a year under experimental conditions, but it may not be possible to raise large numbers through to metamorphosis in captivity due to the time required and potential for significant mortality during that time. Accelerated growth may shorten the time to metamorphosis and this would be a useful study, recognizing that it is unlikely that this could be a production level approach in order to outplant metamorphosed animals.

In general, we think that it is possible to supplement the natural population with hatchery reared young-of-the-year ammocoetes. It is self evident however that the surviving metamorphosed juveniles need to enter the ocean, feed for two or three years and return to

the rearing area or outplanting efforts will be in vain. Tagging technologies, including perhaps genetic marking appear to be a necessary part of any evaluation of the supplementation effort.

Because considerable research is still required in both the artificial propagation and life history of Pacific lamprey and the need to move forward with what information is currently available, we recommend a two pronged approach.

1) Continue collaborative research to answer questions such as *what factors drive the return of Pacific lamprey to a river; what is the survival rate of Pacific lamprey survive after passing through dams (up and downstream migration); what is the best artificial diet for Pacific lamprey ammocoetes; at what developmental stage should Pacific lamprey be outplanted; can a tag be developed that is suitable for both upstream and downstream migrating Pacific lamprey*.

2) In tandem with this collaborative research, if culture is pursued, it is important to begin culturing and outplanting animals with the best available information. These initial trials will move efforts forward providing information to improve culture and outplant methods, as well as developing techniques to measure success. The importance of the enhancement of the species cannot wait until all possible and probable issues and scenarios are taken into account. Continued communication and collaboration between research teams and culture teams cannot be undervalued.

Introduction

Chelan County Public Utility District No. 1's request for proposals stated that projects were to address either or both of the following objectives:

1) Evaluate specific growth rates, health, and survival of Pacific lamprey reared at various densities to determine space requirements and vessel designs for culture of various life history stages, particularly ammocoetes.
2) Identify and develop foods, rations, and feeding methods for optimal juvenile Pacific lamprey growth and nutrition.

Our response and subsequent agreement was that we would provide any and all information available on various aspects of Items 1 and 2. Information from previous work by Dr. Richard Beamish would be compiled and summarized and; in addition, a literature search would be performed to enable decision makers to determine the best course of action in the capture and culture of Pacific lamprey in order to fulfill section 4.2.3 of the Rocky Reach Pacific Lamprey Management Plan.

It was made clear that Dr. Beamish's experience with the breeding and culture of lamprey was varied, but in no way was it a commercial scale breeding program; it was for experimental purposes and focused on providing accurate identification of ammocoetes.

In the 1970s and 1980s, Dr. Richard Beamish conducted a number of field and laboratory studies on many different species of lamprey throughout British Columbia and northwestern United States at the Pacific Biological Station in Nanaimo, BC. Dr. Beamish and his research team developed methods to identify ammocoetes as well as discovered and described new species and varieties such as the Morrison Creek lamprey *(Lampetra richardsoni* variety *marifuga*) and the Cowichan Lake lamprey (*Lampetra macrostoma*). It is likely that several more new species remain to be described.

During his research, techniques to successfully transport, breed and maintain many species of lamprey in captivity were developed. In this report, we focus on information derived from log and record books as well as personal accounts from Dr. Beamish relating to *Lampetra tridentata* in the 1970s and 1980s. As was outlined in the proposal provided to Chelan County P.U.D., the information presented is a summary of his research of which little of this information was published.

Note that the species name is changing from *Lampetra tridentata* to *Entosphenus tridentatus*. We use the former in this report.

In addition to the summary of the historical work, a systematic literature review of primary and grey literature was performed with no restrictions placed on publication date. Literature search criteria are included in Appendix 2.

Capture

Capturing Pacific lamprey for breeding purposes will be most successful if capturing upstream migrating lamprey in the summer. Although, it should be noted that even if capture is easier during this time, these animals may not successfully condition to spawn in captivity if they are not provided appropriate environmental conditions in which to do so. A description of different capture methods for this life stage follows. For additional capture methods suitable for non-returning lamprey or ammocoetes see Appendix 3.

Obstructions

Obstructions, be they are manmade or natural obstructions are good locations to capture upstream migrating lamprey beginning in the fall. Depending on the body of water, capture may be possible throughout the winter. Obstructions to their migration such as dams, fish ladders and fences concentrate the lamprey for relatively easy capture. A manmade "wire trap" was proven effective in the capture of *Lampetra macrostoma* in Mesachie Lake, BC (Figure 1). These types of traps are most effective in smaller rivers and streams or those areas of larger bodies of water which have low water flow. A weir is constructed from wire mesh and installed perpendicular to the shore and held in place with rebar. The shore-most end is placed at the high water mark to prohibit fish from swimming around the weir. The diameter of the wire mesh used for the weir should prohibit lamprey from swimming through it. A diameter is not recommended here

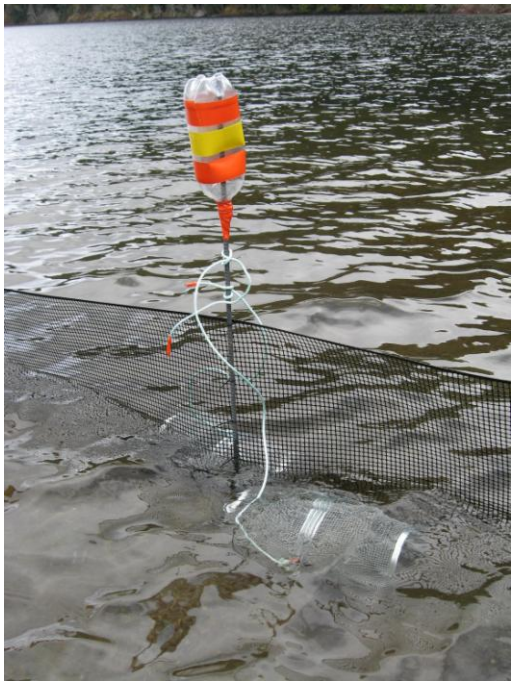

Figure 1: Photograph of lamprey weir with wire mesh trap.

as there are significant differences in lamprey sizes depending on the water body to which they return. The diameter would need to be determined for each trapping situation. Wire traps are placed every 2 ft along the weir fence. Minnow traps were used to capture *L. macrostoma*. A trap net is placed at the end of the weir. Traps are burrowed into the sediment with the opening touching the side of the fence. Lamprey will then swim along the shore, come across the obstruction (weir), be re-directed along the weir and swim into the wire traps. Traps should be checked daily.

Culture Methods and Rearing Conditions

Transport

During any capture activity, it is recommended to use knotless, wet nets when catching lamprey. It is important to avoid handling adult lamprey. They are particularly susceptible to fungal infections and once infected they are very difficult to treat successfully. Any equipment used in the capture and transport of lamprey should undergo effective cleaning and disinfecting (C & D) before being used. It is advisable that the smaller equipment, such as dip nets and buckets, be only used for this purpose. When transporting lamprey captured from freshwater to a holding facility, experience has shown that transport in approximately 20 ppt saltwater will aid in the inhibition of fungal infections, particularly if the fish are transported at low densities. Optimal salinities could be studied, but probably range from 10 ppt to 20 ppt. Transport at this salinity significantly reduces the incidence of transport mortality. It was common to transport fish at densities of approximately 24/m^3. Fish should be transported in disease-free transport water at the same temperature as the ambient water from which the fish re captured; water quality such as dissolved oxygen should be monitored throughout transport. It is recommended that the transport tanks be insulated if possible; although, insulated tanks were not used in Beamish's studies. Lampreys are sensitive to motion and tanks should be filled completely to reduce sloshing. As with any fish transfer, appropriate biosecurity measures should be in place to minimize the potential for disease transfer during transport into and out of a facility. Any fish that appear to have fungus should be euthanized and disposed of immediately.

Broodstock

In late fall, upstream migrating adult *L. tridentata* were captured in freshwater from areas of confinement such as fish ladders, salmon weirs, counting fences or deep pools where they may aggregate. In some instances, *L. tridentata* were captured in the spring and brought back to the laboratory for spawning studies. Animals captured in the fall were held over the winter before experiments began the following spring.

Fish were captured either on their own or in association with returning salmon and transported to the Pacific Biological Station as described previously. All fish (both sexes together) were placed in holding tanks at low density (no specific density recorded) and supplied with de-chlorinated ambient freshwater and aeration throughout the winter. Tanks were approximately 2 m wide and 1 m deep; however, larger tanks may help reduce fungal development. It is most important whenever holding or culturing lamprey to not allow water to flow into the tank by running down the side of the tank, otherwise lamprey will climb up the side of the tank and escape. Photoperiod was similar to ambient. Rocks, ABS pipe and other structures were placed in the tank to allow the fish to take refuge. Over this holding period, lamprey were not fed and not handled to check for fungal development. Visual inspections were difficult as they were most often under refugia. If tanks needed cleaning it was done carefully to avoid touching the lamprey. When culturing lamprey or any fish species, it is recommended that the water be treated in such a way that it is pathogen free and temperatures can be controlled. At this stage, Pacific lamprey are nocturnal and are rarely seen; the onset of spawning season is evident when they cease to be nocturnal and cling to the sides of the tanks. It is at this stage that they will attempt to “climb” out of the tank.

Literature search results

Literature search results pertaining to the capture and transport of broodstock (or maturing) lamprey regardless of the species were similar to those used by Dr. Beamish and his research team. However, there was greater variation found in the holding conditions of the animals once in the laboratory.

Smith et al. (1968) collected five species of lamprey from various freshwater locations around the Great Lakes and brought them to the lab to determine the appropriate temperatures for culture. Lampreys at or nearing sexual maturity were placed in wooden troughs outfitted with sand and rocks similar to those used by Dr. Beamish and his research team.

Langille and Hall (1988) captured upstream migrating *Petromyzon marinus* from Nova Scotia fish ladders in May and June and transported them to the laboratory (approximately 1.5 hour drive) in aerated, plastic pails with river water cooled with ice to water temperatures of 7-10^{o}C. Throughout the multi-year project, broodfish were placed in different types of tanks and provided chlorinated as well as de-chlorinated tap water depending on the study. The different types of tanks included, a fiberglass tank provided with flow through water; recirculating fiberglass tank outfitted with filters located in a temperature controlled room and; Living Stream tank with aeration. Living Stream tanks are insulated fiberglass tanks with refrigeration, aeration and filtration capabilities. These tanks are able to exchange water every 1.5 minutes in 520 L tanks (Cochran, 1985). Some tanks were held in complete darkness, while others were placed in 16hL:8hD photoperiods. It was found that those animals kept in dim light at 10^{o}C in dechlorinated water in the recirculation tank had a lower mortality rate than any other treatment regime.

Fredricks and Seelye (1995) describe tank designs for spawning *P. marinus* in a recirculation system. These will not be described here because a recirculation system is not recommended for any large scale production of *L. tridentata* due to the difficulties in effectively prohibiting the growth of fungus once it has established in the system. Water treatment to ensure culture in water that is pathogen free is recommended regardless of the system type.

Spawning

In captivity, Dr. Beamish found that Pacific lamprey can and will spawn naturally by placing ripe males and females together in a spawning trough; or they can be manually stripped to fertilize the eggs and produce viable offspring. Both methods were employed by Dr. Beamish and his research team.

Spawning in a trough

At the onset of spawning, when eggs were visible through the body wall and males were ripe and running, animals were removed from their communal holding tanks and transferred to spawning troughs at a ratio of 2 females:2-3 males. It was known that animals were getting close to spawning conditions when they ceased to be nocturnal and attached to the walls of the tank. The spawning troughs were rectangular tanks approximately 3 m in length 0.5 m in width and 0.5 m deep. These troughs mimicked the conditions present in a natural spawning stream. Fast flowing inflow water was delivered at one end of the trough, creating a downstream current (flow was not measured). This water was ambient and de-chlorinated. Because of the desire of lamprey to build spawning nests and our desire to reduce stress, pea gravel, rocks of about 2-3 cm in diameter, and coarse sand were placed at the bottom of the troughs approximately 6 cm deep. The sand was taken from nearby freshwater streams or abandoned sand pits. It is recommended that the sediment be washed thoroughly with hot water and allowed to dry in the sun for at least 24 hours before being added to the tanks to reduce the potential for the introduction of pathogens.

In addition to this sediment, small rocks, approximately the size of the oral disc, were placed near the water inflow. These small rocks would be used by the lamprey to build their nests, in the area immediately downstream of the larger rocks. Downstream of the nest building site, sand was placed followed by large rocks and the water outflow. The drain consisted of a double stand pipe with mesh screen over the top to stop lamprey from entering the drain. When lamprey were allowed to spawn naturally in the trough, fertilized eggs were retrieved by sifting through the sediment.

Males and females were placed in each tank at a ratio of 2 females to 2-3 males. Before spawning commenced, lamprey congregated near the outflow of the tank. As spawning began they “migrated” up the artificial river and built a nest. Spawning often occurred at night. On some occasions, eggs were left in the trough to develop. Ammocoetes were removed once they hatched.

Artificial fertilization

Dr. Beamish's research team used dry rather than wet fertilization techniques when spawning lamprey. Animals in spawning condition were removed one at a time from their holding tanks and anesthetized in an anesthetic bath of TMS (Tricaine methanesulphonate). Water was gently removed from the outside of the fish by dabbing the gonadopore with a paper towel, being careful not to damage the skin. Eggs were expressed from the female with a slight downward motion on the ventral surface beginning near the head and terminating at the vent. Expressed eggs were collected in a small dish that had been cleaned and disinfected. Milt was removed from male lamprey in a similar manner as eggs are removed from females. After ensuring that no water from the body of the fish will contaminate the milt, milt was expressed directly onto the eggs with a light downward motion on the ventral surface. It was common practice to use milt from several males to fertilize a batch of eggs from one female. Eggs and milt were gently mixed together by hand and a few milliliters of freshwater were`added to activate the sperm and harden eggs. Alternately, water can be added to the eggs and milt before swirling. Eggs, milt and water were allowed to stand, undisturbed for 10-15 minutes to allow for fertilization. After fertilization, the eggs were placed in a dish with sand to de-anneal as Pacific lamprey eggs are adhesive. Eggs were gently swirled in the sand and then transferred to incubators.

Literature search results

Literature search results found that either a wet fertilization or dry fertilization method was used when spawning Pacific lamprey or other lamprey species. Meeuwig et al. (2005) describe the use of wet fertilization for both Pacific lamprey and western brook lamprey. Here, eggs were expressed from anesthetized females into a container of water and milt was similarly expressed into the same container. The two were mixed for 5 minutes followed by a 30 minute rest period before being placed in an incubator. Langille and Hall (1987) also employed the wet fertilization method for the artificial fertilization of *P. marinus*. After the eggs were fertilized, they were allowed to rest for one hour at 16-21^{o}C. The solution was then decanted and replaced with fresh dechlorinated water or 10% Holtfreter's solution.

Egg incubation

Several variations in lamprey egg incubation techniques were used during Beamish's experiments, all however used modified Heath Trays. The most successful incubation tray was a modified Heath Tray basket fitted with a plexiglass frame and a 200 micron mesh covering the bottom. These modified Heath Trays were then floated in a large nursery tank provided with a moderate flow of freshwater at ambient temperature to ensure the eggs were well oxygenated.

Water circulation and low density are critical to maximizing survival. Experimentally, it was found that if the egg density exceeded 8 eggs/cm^2 fungus development would always occur; keeping in mind that water was only dechlorinated and not further treated. Density was therefore kept at a maximum of 8 eggs/cm^2 regardless of the lamprey species or cross being cultured. Survival rates were not calculated.

Depending on the water temperature and species, it may take several weeks for eggs to hatch. In the lab it was found that hatching would consistently occur around 24 days at approximately 13.5^{o}C. Because of the time required to hatch, the water quality must be maintained at a high standard to help inhibit fungal development of the eggs; this includes the routine removal and disposal of eggs with fungus. Prevention of fungal development through the maintenance of high water quality was the key to egg survival. Ideally, eggs should be incubated in water which has been treated sufficiently to be free of pathogens and provided in such quantities as to ensure a culture environment which does not promote pathogen development.

During Dr. Beamish's experiments, lamprey eggs were not routinely disinfected. In some cases, eggs developed fungus that was very difficult to stop. It was best to remove eggs with fungus as soon as they were observed. However, products do exist which can inhibit the formation of fungus on developing freshwater eggs such as those used for salmonids. These products are not specifically designed for lamprey eggs but there is no reason they cannot be used with success. It will be necessary to experiment with dosage, timing and frequency of these chemotherapeutants. Chemical disinfectants which are used on freshwater eggs include hydrogen peroxide and formaldehyde. It is essential to consult local, state and federal regulations regarding the use of and discharge of any chemical products used for egg

disinfection. In addition to chemical disinfection, ensuring that incoming water is free of potential pathogens and the prompt removal of dead eggs will both decrease the likelihood and incidence of fungal infection. Depending on the system, water treatment may include de-chlorination (is using potable water), cartridge filtration, UV sterilization or ozonation.

Literature search results

The literature search results pertaining to egg incubation techniques were limited to laboratory settings and small scale culture activities. Because the techniques used by Japanese researchers are well known by the Forum and have been implemented with success, they will not be discussed here.

After wet fertilization, Meeuwig et al. (2005) used McDonald hatching jars (6.86 L) for egg incubation of both Pacific lamprey and western brook lamprey (*Lampetra richardsoni*) at various temperatures. Free embryo survival appeared optimal between 10-18^{o}C with survival significantly reduced when temperatures exceeded 22^{o}C.

Yamazaki et al. (2003) reported that time to hatch for *L. tridentata* was shorter than previously reported; 11 days at 18^{o}C (198 dd) vs. 19 days at 15^{o}C (285 dd). Hanson et al. (1974) collected mature *Petromyzon marinus* from the wild and spawned them artificially in a laboratory. The fertilized eggs were incubated in standing aerated water in small jars and aquaria at a constant water temperature of 18.3^{o}C. Dead embryos were removed from the incubating jars and aquaria. After wet fertilization, Langille and Hall (1987) incubated *P. marinus* eggs in static containers filled with dechlorinated tap water at 15^{o}C, 18^{o}C, and 21^{o}C. Development was increased with increasing temperature; mortality was not reported. Frequent water changes or treatments with 10% Holtfreter's solution every 3-4 days aided in the prevention of fungal development. Darkness or dim light increased embryonic survival. Rodriguez-Munoz et al. (2001) found that incubation temperature significantly affected the development and survival of *P. marinus* embryos. The time from fertilization to 50% hatch and, the time from hatch to 50% burrowing was inversely related to the temperatures tested (7 ^{o}C, 11 ^{o}C,15 ^{o}C, 19 ^{o}C and 23 ^{o}C); fertilization survival was poorest at 7 ^{o}C. Smith et al. (2009) examined the factors affecting sea lamprey (*Petromyzon marinus*) egg survival in the wild as well as some laboratory

experiments. Of importance to the culture of lamprey, egg survival from laboratory settings were greatest when incubated on silt (69.2%) followed by sand (50.8%); the poorest survival was on gravel (19.1%).

Smith et al. (1968) collected five species of lamprey from various freshwater locations around the Great Lakes and brought them to the lab to determine the appropriate temperatures for culture. Successfully spawned and fertilized eggs were incubated in Pyrex jars in aerated, static water baths to maintain a constant temperature. This is in contrast to the incubation set-up used by Beamish in the 1970s and 1980s, where water flow was key to maintaining good water quality to developing embryos. The optimal temperature range for the development of each of the five species varied considerably. *P. marinus* developed viable ammocoetes between 15.5^{o}C and 21.1^{o}C.

Rearing ammocoetes

As eggs began to hatch, ammocoetes were transferred to separate nursery tanks outfitted with the same modified Heath Trays, densities were not recorded. Here, ammocoetes absorb their yolk for up to 10 days (depending on the species). Ammocoetes must be removed as the eggs hatch in order to ensure that no fungus develops on the newly hatched ammocoetes.

A newly hatched ammocoete is usually a pale cream colour. As the ammocoete absorbs the yolk and begins exogenous feeding, they change from pale cream to grey in colour. These yolk-sac ammocoetes remained in these new Heath Trays at the surface of a separate nursery tank, unmoving until they absorbed all their yolk. After they absorbed their yolk, they began moving and were removed from the nursery trays and transferred to rearing tanks. Ammocoetes then descended to the bottom of the rearing tanks and burrowed into the sediment. Rearing tanks were approximately 1 m in diameter and filled 0.75 m deep with water. The same sand provided to the broodstock was placed at the bottom of rearing tanks. The sand came from either natural sand deposits or from nearby streams. Tanks were provided water (approximately 3.75 L/min) at the surface and the outflow was a centre standpipe outfitted with a screen to prevent ammocoetes from escaping down the drain. Screen size was not recorded.

Ammocoetes are suspension feeders. As such, they were fed several combinations of the following: green algae, diatoms/algae scrubbed from rocks, Brewer's yeast and yeast extract. The most common diet (90% of the time) was Brewer's yeast fed at approximately 10 mg/L at 14-20^{o}C (1 mg contains approximately 5 x10^{7} yeast cells) with the addition of some "wild" diatoms and algae scraped from rocks from freshwater streams. Diatoms and algae were scrubbed from rocks into buckets of freshwater and added twice a week to the tanks. Keeping in mind that these studies were undertaken for experimental purposes only, not all methods are suitable for large scale production.

During feeding, water was shut off to allow the food to settle on the bottom and ammocoetes to consume the feed. For the first two months of ammocoete feeding, 200 g of Brewer's yeast was provided twice a week, after which 100 g of food was provided once a week to each tank. The larger ration and increased frequency of feeding during the first two months is provided under the belief that they are consuming more food during this time of rapid growth. The yeast was dissolved in a bucket of water and slowly poured into each tank. Water was turned back on after approximately 3 hours. It is important that air be provided to the tanks at all times, but particularly during feeding. All feeding occurred in the morning.

Ammocoete growth was not routinely monitored as there was a tradeoff between rearing the animals and monitoring their survival and growth; the more handling stress, the greater chance of fungal development.

On occasion, ammocoetes were captured in the wild and brought into the laboratory to help develop methods for identification. Larger ammocoetes were selected and held in captivity for up to 2 years at which point they metamorphosed and could be positively identified. If there is a desire to collect and rear ammocoetes in captivity for stocking, larger ammocoetes should be chosen as they most likely are closer to metamorphosis than smaller ammocoetes. In Dr. Beamish's experiments, most ammocoetes derived from the hatching of eggs were held in the laboratory for about 3 to 6 months until they were about 2 cm. The specimens were used to develop keys for identification of *L. richardsoni* and *L. ayresii*. In addition, crosses among species were made to determine if the eggs and ammocoetes were viable, which they were.

Literature search results

Sawyer (1957) wanted to hold *Petromyzon marinus* for extended periods of time (up to 6 months). He developed a system whereby larvae were placed in 57 L tanks at densities of 100 larvae/tank. Tanks were set up in a cascading fashion with ambient de-chlorinated tap water supplied to the uppermost tank at a rate of 1.9 L/minute; each tank had its own aeration. Coarse sand was placed at the bottom of each larval tank for burrowing. Larvae were held in this experimental size set up for 6 months. Above each tank were suspended 20 L carboys of algae ("green water"), 3.7 L of which was delivered to the tank each day. Each carboy was lit with flood lights and provided inorganic fertilizer to encourage growth. Each afternoon, the water was turned off to the cascade of tanks and each tank drained by approximately 5 cm. 3.7 L of algae was then supplied to each tank. The water remained turned off over night and turned back on in the morning. This allowed enough time for the larvae to access the algae, whatever remained would be flushed out with the incoming water when the water supply was resumed.

Hanson et al. (1974) spawned, incubated fertilized eggs and reared larval *Petromyzon marinus* in captivity. Several different sizes of tanks were used in the experiments to determine feeding and growth rates at various stocking densities. Results of these studies found that lamprey can be cultured at >600 larvae/m^2 however, the higher densities did result in a decrease in growth rate. A density of 600 *P. marinus* larvae/m^2 equated to an average length of 50 mm after 1 year. A moist yeast diet was determined superior to a dry yeast diet. A ration of 105 g moist yeast cake/m^2 was determined to be optimum, with particular note that bottom area was more important than water depth when determining feeding rates. A feeding period of twice a week (Monday and Thursday) for 24 hours resulted in the best growth and survival. During feeding (24 hours) water was turned off to the tanks; tanks remained aerated.

Sutphin and Hueth (2010) determined in the lab that ammocoetes (72-143 mm total length) of Pacific lamprey were able to sustain swimming in a swim chamber for 43 minutes at 10 cm/s velocity and 0.4 min at 50 cm/s velocity. Burst swim speed increased with an increase in length from 33.3 cm/s (107 mm TL) to 75.0 cm/s (150 mm TL).

Smith et al. (1968) collected five species of lamprey from various freshwater locations around the Great Lakes and brought them to the lab to compare their development at a constant temperature of 18.4 ^{o}C. Not surprisingly, development varied considerably between species. Ammocoetes were placed in concrete tanks outfitted with sand and rocks similar to those used by Beamish in the 1980s.

Murdoch et al. (1991) describe in general terms the larval tank set-up for *P. marinus* and *L. aepyptera*. Ammocoetes of these two species were collected in the wild by electroshocking and held in 60 L (0.3 m^2) tanks filled to a depth of 7.5 cm with sand and silt in which the ammocoetes could burrow. Tanks were supplied with non-chlorinated water and water was aerated. Water temperature was maintained at 16^{o}C. After a period of time they were transferred to experimental tanks that were the same as the holding tanks however the water temperature was not controlled. In the experimental tanks they were fed brewer's yeast (14 g/tank) 3 times/week for 1 year, the number of animals/experimental tank was difficult to determine. A total of 254 *P. marinus* and 202 *L. aepyptera* were used in the experiment. Growth measurements were taken every three months for one year. Growth between and within species varied with the treatment group. The experiment was to determine the effects of the presence of other lamprey species on the growth of *P. marinus*. The presence of other lamprey had no effect on growth.

McGree et al. (2008) collected Pacific lamprey ammocoetes from the wild in Washington State using electrofishing in order to determine the physical characteristics associated with metamorphosis of *L. tridentata* and *L. richardsoni* in captivity. No information was provided on transport techniques or transport survival for ammocoetes of either species. The entire experiment occurred between June and early December. Plastic tanks (12 cm x 61 cm x 20 cm deep) held ammocoetes during the experiment. Animals were stocked at a density of 215 ammocetes/m^3. River sand was placed at the bottom of each tank approximately 5-7 cm deep. Four tanks were placed in a trough that was supplied with unfiltered creek water at a flow rate of 1.5 L/min at ambient water temperatures between 18.8^{o}C in July to 6.3^{o}C in November 2004. The water would flow from the trough and exchange through small holes in each tank covered

in mesh. Ambient photoperiod was provided. Once lamprey began metamorphosing, rocks were provided for non-burrowing animals. Three different feeding regimes were tested: ration one was no feed at all; ration two was baker's yeast at a concentration of 1 g/ammocoete (high); and ration three was 0.27 g baker's yeast plus 0.03 g Biokyowa/ammocoete (low). Biokyowa is a commercial fish diet. Ammocoetes were fed the prescribed ration once a week. For the first three weeks of the experiment, water was turned off during feeding for 24 hours; thereafter, the water was left on during feeding. Mortality was the highest for ammocoetes fed the high ration diet (30% between late June and late July). Mortality was 100% attributed to anoxic conditions experienced in tanks that were fed this high ration and had the water shut off for 24 hours. This prompted a change in methods and water was left on during feeding thereafter. It should be noted however, that at no point did the authors describe the use of oxygen or aeration in the tanks even though they attributed the mortalities to anoxic events. Mortalities dropped from 30% to 3.6% when the water was left on during feeding with no significant variation in survival between low ration and no ration treatments. Tanks were carefully cleaned and fungus removed every two weeks. Because every lamprey was examined every two weeks, all the substrate was removed from the tanks at this time and cleaned before the lamprey were put back. It was determined that supplemental feeding increased ammocoete growth. Ammocoetes in the no-feed treatment however did experience growth. This can be attributed to the unfiltered water which was provided. Supplemental feeding did not influence the incidence of metamorphosis in this study; however, because ammocoetes were collected close to the onset of metamorphosis, this cannot be a definitive statement.

Mallat (1983) performed lab experiments with *L. tridentata* and determined that the greatest growth was found in ammocoetes fed between 4-13 mg/L daily yeast concentrations at 14^{o}C and densities of <0.05 g ammocoete/L. Holding conditions were similar to those described by other authors with dechlorinated tap water being supplied to different size aquaria with a silica sand substrate (100-600 micron diameter). The one exception was that the system appears to be a recirculating system with a series of filters. Ammocoetes were fed each day during the day and the water was filtered at night. The photoperiod was a simulated natural photoperiod of 16HL: 8HD. The water was changed every 7 days.

Richards and Beamish (1981) found that the coarse substrate in which transforming *L. tridentata* were found in the wild was high in oxygen, whereas, the fine silt in which ammocoetes burrow was low in dissolved oxygen. This requirement is attributed to the significant physiological changes to the respiratory apparatus which are occurring in a metamorphosing lamprey. *L. tridentata* in Phase 7 of metamorphosis in salt water exhibited feeding behaviour when presented with live Pacific herring and Chinook salmon. They attached, fed and killed some of the prey animals. Animals in the same stage of metamorphosis in freshwater were not observed feeding on any introduced species. Both water temperatures were 13^{o}C.

Holmes and Youson (1994, 1997) determined that the temperature requirements for the onset of metamorphosis were critical to the initiation of metamorphosis of *P. marinus*. Metamorphosis would not occur if the water temperature did not change. Pre-metamorphic *P. marinus* exposed to ambient water temperatures began metamorphosis when predicted. This emphasizes the importance of temperature in regulating the onset of metamorphosis.

Culture Considerations for the Artificial Propagation of Lamprey

When moving from a research or trial sized culture operation of any fish species to a large production hatchery, many different factors should be considered, some with greater importance than others. A short description of some of the more important factors is provided, many of which are inter-related.

1) **Water quality and treatment**

   The source of water entering the facility, how it is treated within the facility and wastewater are important considerations for both long term and short term success of a culture operation. Water should be treated in such a way as to ensure it is pathogen free before being supplied to individual tanks and; should ideally be pathogen free when exiting the facility if it is being discharged directly into the environment. Being able to manipulate water temperature and adjust dissolved gasses such as oxygen will increase

successful spawning and rearing. Water should be supplied to each tank individually and not flow from one to another.

2) **Holding conditions and techniques**

Holding conditions will vary and should be appropriate for the life stage. For example, the use of a raceway will be very useful in encouraging upstream migrating fish to spawn when they are provided with refuge and a strong to moderate flow of water. Techniques such as manipulating water temperature, water flow or photoperiod can be used to condition broodstock, increase ammocoete growth and potentially reduce stress.

3) **Biosecurity**

Good biosecurity in any hatchery or broodstock facility regardless of the size will contribute significantly to successful production. Biosecurity is comprised of three parts: bioexclusion, biomanagement and biocontainment; all of which are aimed at creating a healthy environment in which to rear animals. A good on-site biosecurity plan will consist of methods and procedures to prevent the introduction and/or within facility spread of pathogens of concern, while at the same time protecting both the wild and cultured animals.

4) **Pathogens**

It is important to know to which pathogens Pacific lamprey are susceptible. Currently, little is known about disease susceptibility. However, a survey of wild Pacific lamprey of varying ages can provide some insight. Health screening should include viruses, bacteria and parasites. These data can inform decisions such as levels of water treatment in hatcheries; requirements for surface egg disinfectants and; pre-release screening tests.

5) **Record keeping**

Good record keeping, although seemingly simple, can provide a lot of information when facing a challenge. Corporate memory is useful but in order to build on successes and learn from failures, good record keeping is required. Some examples of types of records include: fish health records; broodstock capture records; fish transfer records; cleaning

and disinfecting records; water quality records; feed and feeding records and; systems maintenance records to name a few.

Recommendations

A list of recommendations is provided for the culture of Pacific lamprey.

Recommendation 1

Ensure that the culture facility has enhanced biosecurity including bioexclusion, biomanagement and biocontainment; particularly if collecting wild broodstock and holding them in captivity for spawning or using a facility which is multi-purpose or multi-species. A biosecurity audit should be performed for both the physical facility and practices to identify areas of improvement.

Recommendation 2

Collect upstream migrating Pacific lamprey for broodstock. Transport fish in pathogen-free water adjusted to 20 ppt. It is not necessary to acclimate to freshwater before transferring to tanks in the laboratory.

Recommendation 3

Hold wild broodstock at low densities with moderate flow and aeration. Provide rocks for refuge and do not provide food. Remove mortalities frequently and expediently.

Recommendation 4

Culture and transport water should be treated to be pathogen-free. Source water should not be used.

Recommendation 5

Because of the extended period of time a Pacific lamprey may spend as an ammocoete, it is advisable to outplant cultured ammocoetes in a secure area rather than hold them for several years and release as metamorphosed animals. This area should provide protection from predators and allow for routine monitoring of animals. It is important to note that the age of metamorphosis of *L. tridentata* is unknown. Estimates in the

literature are educated guesses. Outplanting of cultured ammocoetes would provide the first reliable estimates of age at metamorphosis.

Recommendation 6

Designated ammocoete outplant areas should be secure. That is, barriers should be in place to prohibit disturbance by humans or predation by animals via land or water.

Recommendation 7

Experimental work should be undertaken to determine effective disinfection regimes for eggs. Various chemical treatments can be used (e.g. ozonated water, formaldehyde etc.), but the dose required as well as the frequency and duration specific to the destruction and/or inhibition of bacterial, fungal and viral pathogens of Pacific lamprey and their eggs should be determined.

Recommendation 8

The key to successful culture will be in maintaining low rearing densities, water temperature control, pathogen-free water and minimal handling of animals. Standard operating procedures should be created to reflect these requirements.

Recommendation 9

Protocols should be developed and in place to determine the impact and effectiveness of the program to both wild and cultured lamprey in both the short term and long term.

Recommendation 10

A complete analysis of factors contributing to the fluctuation in returning adults should be conducted in a systematic fashion in order to determine gaps in information, prioritize mitigation measures (if required) and inform funding decisions. The analysis should be ecosystem based and not focused solely on the physical structure of the hydroelectric dams.

Appendix 1: Life History of *L. tridentata*

Pacific lamprey *Lampetra tridentata* are an anadromous parasitic lamprey species found in the Pacific Ocean and freshwater tributaries extending from California to the Aleutian Islands and west to Japan (Hart, 1973). Within its range in North America, the Pacific lamprey is of significant cultural importance to many First Nations and non-first nation communities. Nevertheless, this species is still harvested for subsistence by First Nations (Close et al., 2002).

The life history of the species is complicated and many of the intricacies remain unknown or poorly understood. For example, the amount of time a Pacific lamprey spends as an ammocoete is for the most part based on an educated guess of between 3-7 years. Attempts have been made to estimate ages using statoliths (Meeuwig and Bayer, 2005), with more confirmatory work required. The time pre-adults spend in the ocean is also uncertain. Adults in the 25-35 cm range may spend 2 years in the ocean, returning in their second year and spawning in their third year. However, larger lamprey in the 50-70 cm range as occur in the Skeena River, BC, may spend many more years at sea. The question as to whether or not a lamprey "homes" to a particular stream is hotly debated. The genetic work performed to date has shown no genetic differences between lamprey from different streams. This does however not mean that there are no differences, only that they have not been detected. The argument that *P. marinus* in the Great Lakes do not home therefore lamprey do not home is not valid as the Great Lakes lamprey are a recent invader and are not necessarily behaving "normally". It is believed by the authors that lamprey do home. For example, *L. tridentata* travel hundreds of kilometers up the Skeena River in BC to reach Babine River and Babine Lake to spawn. These *L. tridentata* are significantly larger than other members of the species found elsewhere in BC and are comparable in size to those found in larger rivers such as the Columbia River and Fraser River. Why would these animals be consistently larger and migrate such long distances if they could spawn in other rivers with less expenditure of energy if they did not home?

Pacific lamprey eggs are deposited and subsequently fertilized in freshwater nests prepared by both the males and females between April and July. Nests are made in freshwater rivers and

streams where the substrate is coarse enough to allow the lamprey to move small gravel with their mouth to form the nest. Additionally, nests are also constructed through the rapid vibrations of the tail (Hart, 1973). The eggs are adhesive and adhere to the nest substrate. Laboratory work by Dr. Beamish's research team report that Pacific lamprey eggs incubate for 24 days at 13.5^{o}C before they hatch and larval ammocoetes emerge. After they emerge, young ammocoetes do not begin exogenous feeding immediately but are consuming yolk. After 2 or 3 weeks, larvae leave the nests and are carried downstream where they borrow in mud, sand and silty substrate. At this time, exogenous feeding begins and they consume diatoms, detritus and algae (Moore and Mallatt, 1980) for up to 7 years (Beamish and Northcote, 1989; Beamish and Levings, 1991). After emergence, larval ammocoetes may be preyed upon by other fish species such as coho salmon (*Oncorhynchus kisutch*) (Pfeiffer and Pletcher, 1964), or may be used as fishing bait by humans (Close et al., 2002).

Ammocoetes begin metamorphosis while burrowed in coarse substrate. This change in water flow and substrate from ammocoete (slower moving water over mud, silt and sand) to that occupied by metamorphosing lamprey (moderate current over coarse substrate) has been attributed to the increase in oxygen demand; ammocoete substrate is less oxygenated than metamorphosing lamprey substrate (Richards and Beamish, 1981; Beamish, 1980). Metamorphosis begins in July and is completed by October (Beamish, 1980); throughout which they do not feed (Whyte et al., 1993). It is through metamorphosis that the toothless larvae transform into the pre-adult form. This is a true metamorphosis as most or all of the organ systems undergo reorganization (Potter, 1980). Physical changes include: asymmetric growth; increase in snout depth; changes to the mouth, eye and branchial region (McGree et al., 2008). One of the greatest physiological changes is in the ability to begin tolerating saline water. Clarke and Beamish (1988) report that recently metamorphosed *L. tridentata* are not able to tolerate being held over in freshwater. The amount of time they can survive in freshwater varies between populations. Babine River, BC, *L. tridentata* were able to survive until February, whereas animals from the Chemainus River, BC, population was able to survive until July (Clarke and Beamish, 1988). Beamish and Northcote (1989) reported that the construction

of a dam on the outlet of Elsie Lake, BC, did not create a landlocked population of *L. tridentata*. To the contrary, lamprey were only found upstream of the dam for approximately 7 years, which coincides with the approximate developmental time for *L. tridentata* ammocoetes. The production of a landlocked population is therefore not as rapid as reported previously (Beamish and Northcote, 1989).

Once metamorphosis is complete, the juvenile lamprey begin downstream migration. This normally beings in mid-November and is often associated with an increase in freshwater discharge (Richards and Beamish, 1981). Feeding may begin in freshwater during downstream migration and has been reported to occur beginning in October. There are two migration peaks, one in the fall, usually after a major rainfall and one in the spring also usually after a major rainfall. Downstream migrants are subject to predation by many species of fish and water fowl (Close et al., 2002). Because traditionally there was a high abundance of downstream migrants, it is proposed that predation on downstream migrating lamprey relieved the predation pressure on downstream migrating salmonids (Close et al., 2002). Juveniles enter saltwater between December and June (Beamish, 1980). Studies by Richards and Beamish (1981) demonstrated that juvenile Pacific lamprey have the ability to osmoregulate in the marine environment well in advance of their downstream migration.

Parasitic juvenile Pacific lamprey may spend up to 3.5 years in the marine environment (Beamish, 1980) parasitizing many different species of teleosts as well as whales (Beamish, 1980). They have been observed in the laboratory to feed throughout the year and remain attached to an individual, feeding for several days at a time (Beamish, 1980). Whyte et al. (1993) showed that *L. tridentata* adults and recently metamorphosed lamprey can survive extended period of time on endogenous reserves under laboratory conditions. Those animals held without food for 6 months had similar body compositions as those held without food for two years. This may be of significant importance for extended upstream migrations.

The importance of parasitic lamprey to the ecosystem was demonstrated by Beamish and Neville (1995) when estimates of mortality attributable to river lamprey (*Lampetra ayresi*) were made for 1990 and 1991. It was estimated that the river lamprey killed a minimum of 20 million and 18 million Chinook salmon, in 1990 and 1991 respectively; in addition to killing 2 million coho salmon in 1990 and 10 million coho salmon in 1991. This equates to killing approximately 65% of total Canadian hatchery and wild production of coho salmon and 25% of the same Chinook salmon production in 1991.

The timing of return to freshwater varies with the stream or river. Beamish (1980) reported that *L. tridentata* were caught in upstream traps in Chemainus, BC, between April and June, with the majority during May; migration in the Somass River, BC, at Stamp Falls occur in the summer. Once this species begins its upstream migration they no longer feed. The spawning migration is complete by the end of September, depending on the length of migration (Beamish, 1980).

Spawning occurs between April and July in areas of coarse gravel substrate where nests can be constructed with the use of their oral disc as well as tail movements. Pacific lamprey die shortly after spawning (Beamish, 1980). Adult and spawned Pacific lamprey are important prey species for many birds, mammals and fishes (Close et al. 2002) including white sturgeon (*Acipenser transmontanus*) (Semakula and Larkin 1968; Galbreath 1979), mink (*Mustela vison*) (Beamish 1980) and blue heron (*Ardea herodias*) (Wolf and Jones 1989).

Appendix 2: Literature Search Criteria

Geographic location was also not restricted because of the potential to apply techniques from other lamprey species to that of *L. tridentata*. The following search terms were used: lamprey, lamprey and culture; lamprey and rearing; lamprey and breeding; lamprey and spawning; lamprey and captivity; lamprey and egg incubation; lamprey and feeding; lamprey and laboratories. In addition, species names *Lampetra tridentata* as well as *Entosphenus tridentatus* were substituted for lamprey in the above terms. The following databases were searched: American Fisheries Society (AFS); Biological Sciences; Canadian Business and Current Affairs Complete (CBCA); SpringerLINK; ScienceDirect; BioOne; GreenFILE and ; Biological and Agricultural Index Plus. Additionally, Google Scholar and Google were also used to retrieve journal articles.

While these search terms retrieved numerous results, more often than not the focus of the retrieved articles was control or elimination of lamprey populations, not propagation or enhancement. The majority of results were specific to sea lamprey (*Petromyzon marinus*) with few results specific to Pacific lamprey. One of the most effective strategies for locating relevant literature on Pacific lamprey breeding and rearing was to refer to cited references.

Appendix 3: Additional Capture Methods

Milk crate traps

Standard milk crates filled with straw and a plywood lid strapped to the top can serve as effective traps for ammocoetes and small adults (Figure 2). The trap is installed in a stream or river with firm sediment composed of loose gravel or sand. The trap may be completely submerged. A rock is placed on top of the plywood lid to help keep the straw inside the trap and a line tied from the trap to a tree or another suitable anchor onshore. Lamprey burrow inside the trap and use the straw as refuge. To retrieve the lamprey, the rock is removed from the lid; the trap is lifted from the water body and placed directly into a tub or tote. The lamprey will fall out of the trap into the tote. The straw can be removed to ensure that there are no lampreys remaining. The advantage of this trap is also its disadvantage depending on the application. The advantage being that the trap does not have to be checked every day; the disadvantage being that the lamprey are free to move into and out of the trap at any time. A similar type of "trap" has been used in Europe. Here, straw bales were placed directly in the body of water to capture upstream migrating *L. fluviatilis*.

Figure 2: Photograph of milk crate lamprey trap.

Live downstream traps

Live downstream traps are effective in capturing metamorphosing lamprey, ammocoetes, as well as recently spawned lamprey and any other species of small animal found in the water body. This type of trap must be checked at least once a day to ensure the fish remain in good condition and by-catch can be released without harm. It is not uncommon in streams in British Columbia to have salmon fry in the traps; they are removed and released back into the stream as quickly as possible. Crayfish are also routinely captured.

Depending on what is available, and the depth of the stream or river, variations in the size (and depth) of tank can be adjusted accordingly (Figure 3). What has been used in the past are re-purposed oblong tanks approximately 110 cm long, 50 cm wide and 40 cm deep. Three rectangular holes are cut in the sides and rear of the tank and screens are siliconed over the holes using aquarium-safe silicone. These “windows” are the outflow of the tank. Depending on the depth of the intake, the height of these “windows” will vary. The screening can be made of any firm material as long as the mesh diameter will not allow lamprey (of any stage) to escape. This screening will need to be cleaned daily and material should be chosen with that in mind. The inflow to the tank is a 4” PVC pipe approximately 60 cm in length. The actual length need not necessarily be that long, but it must allow for enough pipe to stick out of the tank to attach the funnel and enough pipe inside to provide a rigid surface for a screening cone. A hole slightly larger than the diameter of the pipe is cut approximately 2/3 of the way up the side of the tank; the pipe is inserted and siliconed in place.

Figure 3: Photograph of live downstream lamprey trap.

A funnel is constructed of wire coated mesh or old fishing mesh and affixed to the exterior portion of the 4” pipe with a hose clamp. It is essential that the sizing of the mesh be appropriate for the species of lamprey. The holes must not allow lamprey to pass through. A rigid wing is attached to each side of the funnel and secured with cable ties to form a seamless transition between the wings and the funnel. Wings are commonly constructed of wire coated mesh or plastic mesh of an size appropriate to prohibit lamprey from passing through. This mesh should not be too small as it will foul easily and maintenance will become onerous. Wings are held in place in the stream by affixing them with cable ties to rebar sledge hammered into the sediment. To ensure lamprey do not wriggle under the wings, the bottom of the mesh should be bent to a 90^{o} angle and sit firmly in the sediment with sandbags or rocks placed on top. Inside the trap, a wire mesh funnel was placed around the 4” pipe, as it was found that

lamprey could wriggle out the 4” pipe if this obstruction was not in place. The funnel is merely a piece of mesh placed over the pipe tapering to an opening of approximately 5 cm; it is enough of a deterrent to stop the lamprey from trying to escape.

Traps should be located in areas of moderate running water to ensure that the 4” inflow pipe is submerged at all times. It is our practice to not barricade the entire width of the stream to allow for the free movement of fish of all species upstream and downstream of the trap if they wish.

Traps should be checked daily and any animals removed from the trap should be released downstream from the trap. Rocks are placed inside the tank to ensure that the tank does not move in the current, a plywood lid is affixed to the top with clips.

Electroshocking

Electroshocking is an effective way to capture ammocoetes, recently metamorphosed and adult lamprey in shallow waters (Figure 4). As with other electroshocking activities, the life stage of the species, water temperature, depth and time of year should be a consideration when choosing settings for an electroshocker. Slade et al. (2003) describe a common method of lamprey capture using electroshocking. They describe a two-stage sampling method. The initial step involves a 90-125 V direct current with a 10-25% duty cycle and a slow 3 pulse/second pulse rate which encourages emergence of juvenile lamprey. A 3:1 pulse ratio at the same rate in which the fourth pulse is skipped can also encourage emergence.

Figure 4: Photograph of electroshocking for lamprey in British Columbia.

The second step, which takes place immediately after the lamprey emerge

involves a much faster, 30 pulse/second rate which immobilizes the juvenile lamprey (Slade et al., 2003). Following immobilization, the lamprey can be captured with dip nets or seines. Prolonged exposure to electroshocking can result in narcosis of buried ammocoetes and failure to emerge, therefore, it is important that personnel and electrofishing devices be tested under a range of field conditions prior to the development of sampling protocols (Moser et al., 2007).

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