CHAPTER TWENTY TWO

DEVELOPING TECHNIQUES FOR ARTIFICIAL PROPAGATION AND EARLY REARING OF PACIFIC LAMPREY (*ENTOSPHENUS TRIDENTATUS*) FOR SPECIES RECOVERY AND RESTORATION

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Introduction

Of highest importance to the lower Columbia Basin Native American tribes is the focus on protection and enhancement of "First Foods" such as water, salmon (*Onchorynchus* species), Pacific lamprey (*Entosphenus tridentatus*), deer (*Odocoileus* species), cous root (*Sagittaria latifolia*), and huckleberry (*Vaccinium parvifolium*). These foods are central to the perpetual cultural, economic and sovereign benefit of the tribes. Lamprey or "eels" are served at tribal longhouse ceremonies and traditional funerals when they are available. The First Food serving ritual in the longhouse reminds people of the promise the foods made to the people and the people's reciprocal responsibility to respectfully use and take care of the foods. Humans have failed to carry out this reciprocal responsibility as demonstrated by the declining Columbia Basin Pacific lamprey population. Hence the tribes' culture and way of life has been greatly affected. The tribes adamantly desire to restore healthy abundant lamprey fisheries at all of their usual and accustomed fishing areas, which are recognized by treaties with the United States. Unfortunately, many of the local populations in the upper tributaries have been extirpated, or are functionally extirpated (Close et al. 2002).

Problems associated with adult passage to tributary spawning areas are likely one of the most significant threats to sustaining lamprey populations (Columbia River Inter-Tribal Fish Commission 2011; Luzier et al. 2011). Lack of passage is evident in fishways at the large, hydroelectric dams on the Columbia River. Adult passage rates at these dams are estimated to be between 50 – 75%. Even with a tribally recommended 80% *interim* passage standard, only 41% of the adults destined to ascend the four lower Columbia River dams will successfully pass. And there are more dams upstream, including numerous irrigation diversion dams, road crossings and thermal barriers that threaten successful passage to suitable spawning areas. Unfortunately, little is understood about the success of both adult and juvenile passage through many of these dams and reservoirs.

Provision for appropriate adult passage rates will be a long, slow and expensive endeavor. During this time, local populations will likely continue to decline. Not only might this affect genetic diversity of the lamprey populations, but it will also hamper ecological integrity and further reduce tribal harvest opportunities. Actions are needed to address this unfortunate situation. Beginning in 2012, two tribes, the Confederated Tribes and Bands of the Yakama Nation and the Confederated Tribes of the Umatilla Indian Reservation, in coordination with the U.S. Fish and Wildlife Service, have undertaken research in the artificial propagation of Pacific lamprey. The objective is three-fold: (1) to develop sufficient numbers of juveniles at appropriate sizes that can be used for research, including determination of downstream passage and survival, (2) to provide juveniles for supplementation as part of a larger recovery strategy, as determined appropriate, and (3) to compare and contrast efficacy and effectiveness of juvenile and adult supplementation restoration approaches.

To date, these efforts have been preliminary but researchers demonstrated great promise in advancing lamprey supplementation methods and outcomes. The translocation of adults into tributary streams appears to be a cost-effective means for supporting and rebuilding local populations (Close et al. 2009; Ward et al. 2012), as is demonstrated in the Umatilla River (Oregon, USA) and Snake River tributaries (Idaho, USA). Artificial propagation is a new and exciting front, which in recent years has begun to reveal many interesting secrets of the cryptic Pacific lamprey. Although artificially propagated metamorphosed juvenile has not been produced to date, we anticipate generating them in the near future (1-3 years).

Adult maturation

Pacific lamprey enter the Columbia River Basin in the year prior to spawning, hence broodstock collection often occurs in the summer one year prior to artificial propagation. For artificial propagation programs thus far, the adults have been collected in trapping efforts at mainstem dams in the Columbia River (Ward et al. 2012) and overwintering of broodstock is accomplished at existing hatcheries (Close et al. 2009; Lampman et al. 2013). Preferably the water supply will reflect the water sources that maturing lamprey would encounter naturally. For example, when broodstock are held on well water or in cold spring water sources (<10°C) throughout this holding period, asynchronous or delayed reproductive maturation can occur (A. Jackson, Confederated Tribes of Umatilla Indian Reservation, and R. Lampman, Yakama Nation, unpubl. data). Genetic evidence using parentage analyses indicates that in at least two instances, an overwintered adult that was released in one year, have spawned with an individual that was released in the same stream but in a subsequent year, indicating that some of the translocated adults can overwinter twice prior to spawning (J. Hess, Columbia Inter-Tribal Fisheries Commission, pers. comm.). Whether this is a natural phenomenon or an artifact of holding the adults for an extended period is unknown.

Secondary sexual characteristics start to develop during April as sex steroid and thyroid hormone levels increase (Mesa et al. 2010). At this time, identification of sex becomes possible based on secondary sexual characteristics, namely the appearance of pseudoanal fin (a prominent swelling behind the vent) and the development of gonads with a soft distended abdomen (Fig. 22-1). Holding adult fish in shallow tanks during this time period allows frequent and non-invasive monitoring of their sexual maturation progress and is convenient for sorting fish. As lamprey get close to spawning, the distance between the two dorsal fins (interval) shortens and eventually closes completely as shown in Fig. 22-2 (Hardisty 2006; Clemens et al. 2009). Mean dorsal fin intervals of successfully spawned Pacific lamprey in 2014 were -2.9 mm (i.e. the two dorsal fins are inverted into each other) for males (range of -7 to 2 mm) and 0.6 mm for females (range of -3 to 5 mm). Although dorsal fin interval was one of the best indicators for assessing spawning readiness, the interval was not conclusive by itself; some individuals with closed or inverted dorsal gaps were not ready for propagation whereas some with positive intervals (up to 5 mm) were successfully spawned.

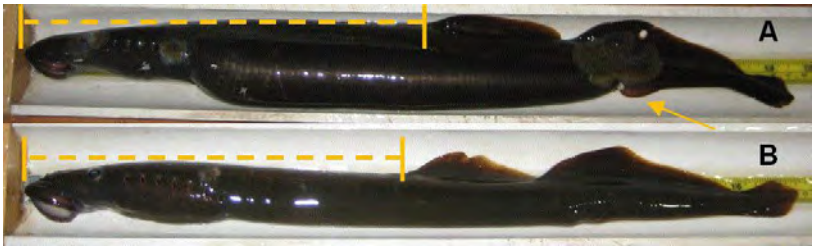


Fig. 22-1. Morphological comparison of the spawn ready Pacific lamprey female (A) and male (B). Spawn ready female has the psuedoanal fin (arrow) and distended abdomen from mature eggs. The interval between snout and the first dorsal fin anterior insertion point is also shown with a dotted line (which is measured for mid-point ratio).

For adults monitored by Yakama Nation biologists in 2013-14, over the course of approximately one year, total length decreased by 23.3% and 27.1% and weight decreased by 42.9% and 40.2%, for males and females, respectfully (Fig. 22-2). While male girth decreased, female girth stayed the same or increased slightly as the gonads matured. Accordingly, females showed an increase in condition factor [fish weight (g) x 10^5 / fish length $(mm)^{2.6}$] of roughly 35% (Fig. 22-2). The distance between the snout and first dorsal insertion generally increases relative to overall length in females, but this ratio ("mid-point ratio") stays virtually the same in males (Fig. 22-1, Fig. 22-2, and Fig. 22-3).

Both observations from the wild and in the laboratory indicate that adult lamprey can spawn on multiple occasions. Genetic evidence indicates that Pacific lamprey are both polygynous and polyandrous (Hess et al. 2014). There is also evidence for polygyny and polyandry in sea lamprey (*Petromyzon marinus*) for all streams (N=4) that were examined in a recent study (Scribner and Jones 2002). Moreover, based on the dispersion of larval full and half-siblings, it appears that sea lamprey females are able to spawn in multiple nests in different areas of the stream. Visual observations and radio telemetry indicated that both male and female Pacific lamprey can spawn several times over the course of several days (R. Lampman, Yakama Nation, unpubl. data). This is reflected in Yakama Nation artificial propagation activities in that milt can be obtained repeatedly from the majority (87% in 2014) of spawning males over the course of roughly two weeks (up to 16 d later). In about 16% of the females spawned in 2014, eggs were not all released on the first spawning event and the remaining eggs could be stripped up to 7 d later.

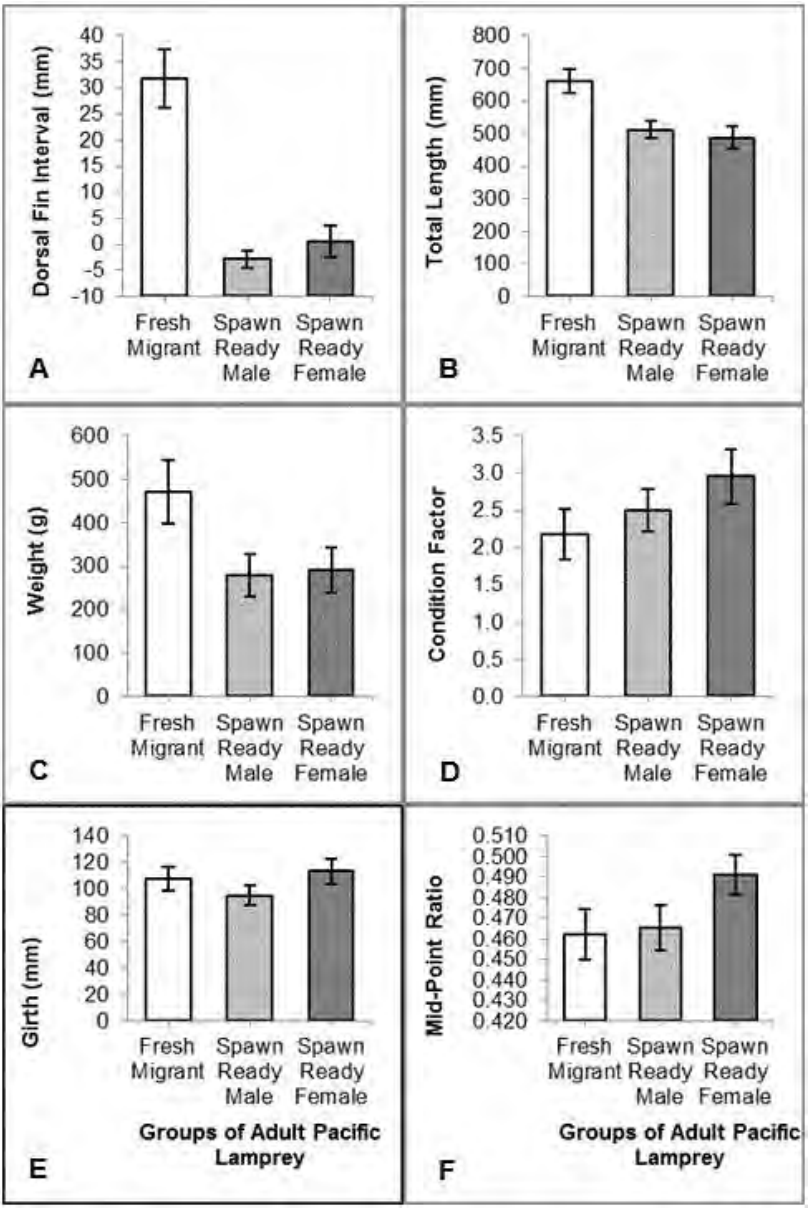

Dorsal Fin Interval (mm)
40
35
30
25
20
15
10
5
0
-5
-10
Fresh Migrant
Spawn Ready Male
Spawn Ready Female
A
Total Length (mm)
800
700
600
500
400
300
200
100
0
Fresh Migrant
Spawn Ready Male
Spawn Ready Female
B
Weight (g)
600
500
400
300
200
100
0
Fresh Migrant
Spawn Ready Male
Spawn Ready Female
C
Condition Factor
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Fresh Migrant
Spawn Ready Male
Spawn Ready Female
D
Girth (mm)
140
120
100
80
60
40
20
0
Fresh Migrant
Spawn Ready Male
Spawn Ready Female
Groups of Adult Pacific Lamprey
E
Mid-Point Ratio
0.510
0.500
0.490
0.480
0.470
0.460
0.450
0.440
0.430
0.420
Fresh Migrant
Spawn Ready Male
Spawn Ready Female
Groups of Adult Pacific Lamprey
F

Fig. 22-2 (previous page). Measurements of adult Pacific lamprey when first captured in Lower Columbia River as fresh migrants in 2013 (n=540) and as spawn ready males (n=24) and females (n=25) the following year for dorsal fin interval (A), total length (B), weight (C), condition factor [weight (g) x 10^5 / length $(mm)^{2.6}$] (D), girth [at first dorsal insertion point] (E), and mid-point ratio [ratio of the interval between snout and first dorsal fin anterior insertion point to total length] (F). Error bars represent 1 SD.

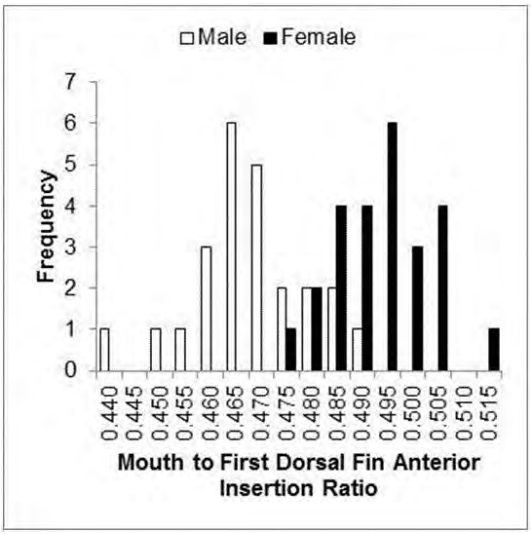


Fig. 22-3. Ratio of the interval between snout and first dorsal fin anterior insertion point to total length for spawn ready Pacific lamprey males (n=24) and females (n=25) from 2014.

Fertilization

The timing of Pacific lamprey gamete harvest is critical. We have observed that forcefully stripping the gametes can result in adult mortality, damaged gametes, and unsuccessful egg development. For this reason it is important that the adults are at a high plane of anesthesia and that the gametes are allowed to release with minimal pressure. If females are not spawned in time, they will die without releasing the eggs. However, eggs from freshly dead females may still be viable (M. Mesa, U.S. Geological Survey, unpubl. data). For example, on three occasions in 2014, eggs from freshly-dead females were mixed with fresh milt and exhibited 59 - 97% development to the morula stage (M. Moser, National Marine Fisheries

Service, unpubl. data). However, only 16 – 24% of these eggs survived to hatching. The ability to obtain eggs in this way and to spawn fish multiple times is important for maximization of genetic diversity and gamete production when broodstock are limited. As a result of spawning adult fish multiple times (as opposed to euthanizing fish prior to spawning), the number of overall spawning events increased by 84%, the spawning matrix was amplified to a much higher ratio of female:male crossings, and the range of spawning dates was substantially increased (Fig. 22-4).

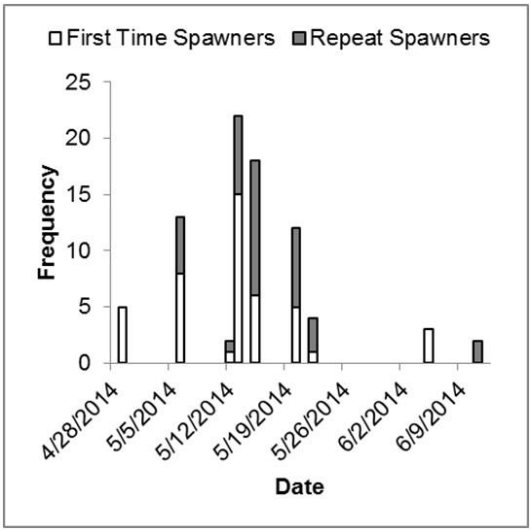


Fig. 22-4. Number of Pacific lamprey that were artificially propagated in 2014 by date, separated by groups of first time spawners (n=44) and repeat spawners (n=37).

We have been developing fertilization protocols over the past three years. Prior to this, work on lamprey fertilization was conducted on Arctic lamprey (*Lethenteron camtschaticum*) by Japanese researchers (Kobayashi 1993; Fukutomi et al. 2002; Yamazaki et al. 2003; Hara 2008), on sea lamprey by Great Lakes researchers (Langille and Hall 1988; Fredricks and Seelye 1995; Ciereszko et al. 2000; Ciereszko et al. 2002) and Spanish researchers (Rodriguez-Munoz and Ojanguren 2002; Rodriguez-Munoz et al. 2001), and on European river lamprey (*Lampetra fluviatilis*) by Finnish researchers (Vikström 2002). Based on their findings, we conducted trials to test the efficacy of mixing different dilutions of gametes for different lengths of time and in different containers. The timing of water introduction and the

effects of rinsing fertilized eggs were also tested. The adhesive nature of the fertilized eggs was addressed in trials that involved exposing newly fertilized eggs to anti-adhesion solutions (pineapple juice and tannic acid).

The culmination of these investigations in 2012-14 was a protocol that yields maximal fertilization success while minimizing egg loss from damage or adhesion (Jackson and Moser 2014; Jackson et al. 2015; Lampman et al., in press). This recommended protocol is the following: 1) dry the anesthetized female body with a towel and hand strip female eggs using gentle but firm pressure from anterior to posterior end (Fig. 22-5) – if flow of eggs halts midway and blood is discharged, stop immediately and return the female to recovery (to spawn the rest of eggs at a later date), 2) dry the anesthetized male body with a towel thoroughly and hand strip milt from males in a similar fashion – again if blood is discharged, stop immediately and return the male to recovery, 3) combine the milt (from one or multiple males) directly with eggs, and immediately add culture water 1-1.5 times in weight to egg weight (a 2-5% solution of milt is recommended to maximize fertilization), 4) gently mix the gametes for 0.5-1 min, 5) add a 1% solution of fresh pineapple juice and gently mix the gametes in the culture water for 1-2 min, 6) gently rinse eggs three times with culture water, and 7) install fertilized eggs in incubation container. For the mixing, we had equal success with combining all ingredients in a Ziploc bag or mixing them together in a disinfected round plastic bowl using a feather.

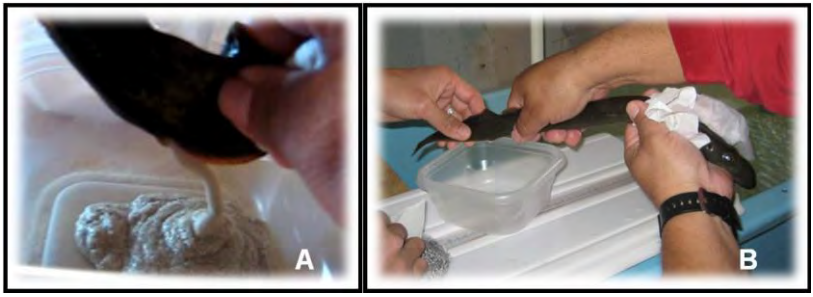


Fig. 22-5. Dry stripping of Pacific lamprey female eggs (A) and male milt (B). One person holding and bending the tail upwards (as seen in A) can help the gametes discharge smoothly and make the task much easier.

Incubation

Development of incubation methodologies for Pacific lamprey was informed by existing salmonid techniques (Piper 1982) and emerging lamprey-specific methods developed by Japanese (Hara 2008), Great Lakes (Piavis and Howell 1969; Langille and Hall 1987; Fredricks and Seelye 1995; Richardson and Wright 2003), and Finnish researchers (Vikström 2002). Initially we tried to incubate eggs in upwelling jars [McDonald type and Eagar type (North Salt Lake, Utah, USA)], static trays, incubation troughs, downwelling buckets, and modified upwelling Heath trays.

McDonald jars were able to hold many eggs, but flows were turbulent near the bottom pipe and resulted in egg damage and loss. Modified Eager upwelling jars (fitted with a 540 micron mesh screen on the bottom instead of filter cloth and <350 micron mesh screen on the top) allowed for gentle, distributed flow (1-2 L/min) that did not damage the eggs. However, silty water could plug the screens depending on water source, and it was difficult to safely remove prolarvae for monitoring. In addition, newly-fertilized eggs that were not adhered to the bottom could become buoyant during egg hardening and get trapped on the upper screen.

Static trays were easy to monitor but daily maintenance was labor-intensive and regular water exchanges could result in damaged eggs from excessive shocking. Because water is not flowing through such systems, water temperature could fluctuate. Buffering temperature by holding the trays in a flowing water bath was necessary. Modification of the static trays to allow some flow through still did not alleviate the need to do regular, labor-intensive, and potentially damaging water exchanges. In general incubation containers that reduced physical damage to eggs and prolarvae and provided gentle even flow worked the best.

Incubation troughs allowed for lateral water flow, were relatively maintenance-free and could accommodate large numbers of eggs. If the screen mesh (<350 micron) for each compartment was completely sealed to the trough, prolarvae losses were minimal and it was easy to monitor development. Adding filter media (such as coconut fiber spawning mat) provided more surface area for egg adherence and prevented egg clumping. Prolarvae could also shelter under the mats and reduce energy expenditure associated with constant swimming. Downwelling buckets allowed for gentle distributed flow (0.3 L/min), but fungus could still develop if the small buckets were not maintained every few days to remove fungus. This system allowed for many eggs to develop and the

hatched prolarvae were able to volitionally migrate into a larger incubation trough.

Heath trays are commonly used in salmonid production (Piper 1982). Standard Heath trays were modified by lining both the tray top and bottom with polyethylene or nylon mesh (<350 micron and 750-800 micron, respectfully). This ensures that neither eggs nor prolarvae are lost through the top screen while allowing prolarvae to move downward through the bottom screen. The holding reservoir at the bottom of Heath Trays was consequently used to isolate and hold hatched prolarvae. The trays worked well with large numbers of eggs, were generally maintenance free, featured gentle flows (6-11 L/min), and allowed for easy prolarvae collection. The heavy, cumbersome trays were difficult to handle for monitoring purposes, with risks of spilling eggs and prolarvae during the tray removal and replacement. Prolarvae could also escape through incomplete mesh seals or the gap between the tray and rack. In summary, modified Heath trays are recommended for high production and low maintenance, while incubation troughs or downwelling buckets provide ease of access for monitoring and research or in cases where limited numbers of eggs are being incubated.

Pacific lamprey incubation timing is typically around 2 weeks, depending on temperature (Yamazaki et al. 2003) and most eggs hatch within just a few days from each other. In 14.2°C water, Pacific lamprey eggs hatched primarily in 14-15 d at Prosser Fish Hatchery, which equates to 199 cumulative degree days (CDD) and is consistent with past studies on Pacific lamprey ranging 184-262 CDD (Close et al. 2001; Yamazaki et al. 2003; Meeuwig et al. 2005) and on sea lamprey ranging 184-217 CDD (Wigley 1959; Piavis 1971). For all incubation methods, mesh used to prevent impingement or entrainment of various developmental stages (Fig. 22-6) needs to be appropriately sized. Mesh size smaller than 750-800 micron will contain all eggs, but > 900 micron mesh will result in loss of the smallest eggs (Fig. 22-7). As a result, mesh sizes of 750-800 micron can be used to effectively separate prolarvae from eggs. Even within a few hours of hatching, prolarvae can be very active and make attempts to pass through small cracks (Jackson and Moser 2014). For prolarvae (6 mm), 540 micron mesh will not prevent escapement for all larvae: on average 38.9% passed through 540 micron mesh in three replicate trials using 30 prolarvae per trial (M. Moser, National Marine Fisheries Service, unpubl. data). For this reason, mesh sizes 250-350 micron are recommended to contain all prolarvae (Fig. 22-7). Use of clean water with minimal turbidity helps reduce clogging of the fine mesh screens.

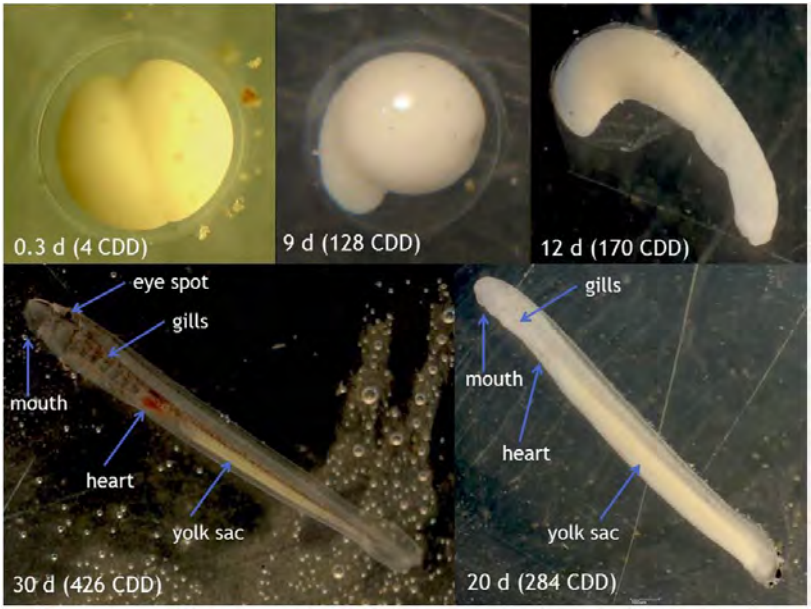
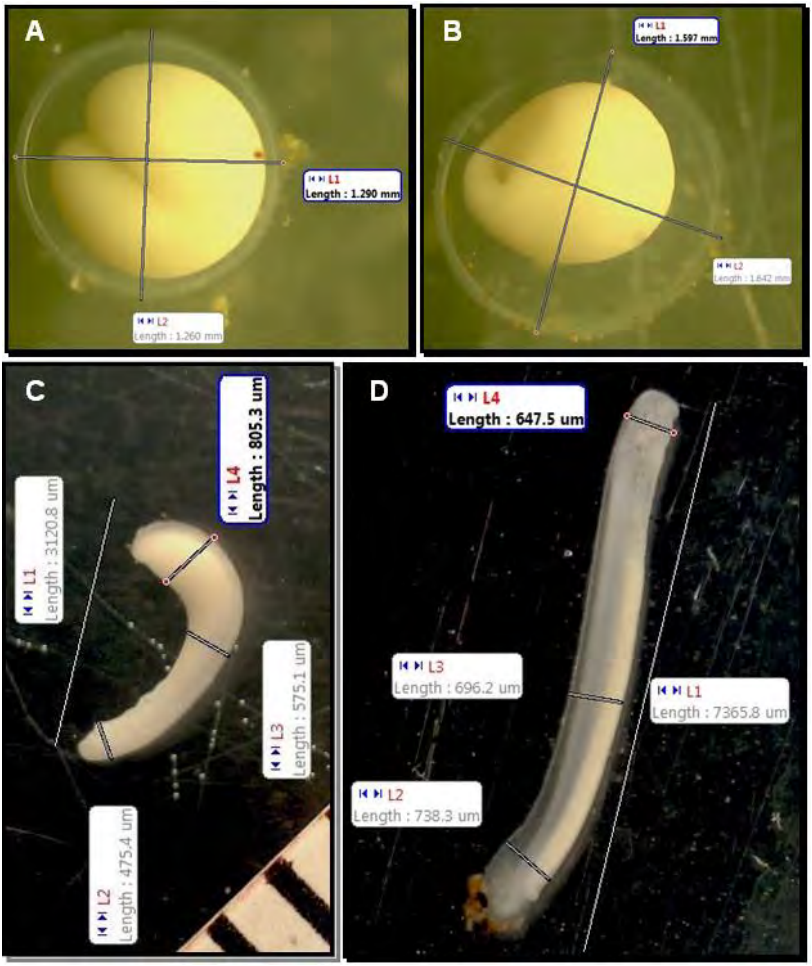


Fig. 22-6. Overview of Pacific lamprey development from egg embryo to burrowing larva [approximate number of days post fertilization and cumulative degree days (CDD) shown in the lower left corner of each photo].

Fig. 22-7 (next page). Size dimensions of Pacific lamprey fertilized egg/embryo and prolarvae in development. (A) Stage 2 (based on Piavis 1971): egg/embryo with two-cell development 0.3 d post fertilization (long diameter 1290 µm; short diameter 1260 µm). (B) Stage 12: egg/embryo with head development 7 d post fertilization (long diameter 1642 µm; short diameter 1597 µm). (C) Stage 14: hatched prolarva 13 d post fertilization (long diameter 3121 µm; short diameter 805 µm). (D) Stage 16: gill-cleft prolarva 20 d post fertilization (long diameter 7366 µm; short diameter 738 µm).



Prolarvae to Larvae Transition

The transition to first feeding is a critical period in lamprey development, as in many fish species (Hjort 1914). The development of methods to count prolarvae and larvae is therefore critical to understanding survival during this key life stage. Manually counting thousands and millions of prolarvae is enormously time consuming and, more importantly, stressful to the fish; hence, an automatic counting device for

aquatic organism called XperCount (XpertSea, Quebec, Canada) was tested by Yakama Nation personnel with technical assistance from the company in 2013 and 2014. In 2013, calibrations were made to the counting program using a combination of manual counting (for numbers smaller than 1000) and extrapolation through subsampling (for numbers larger than 1000). The best method we discovered for subsampling was volumetric subsampling. We swirled the prolarvae for a few seconds to homogenize their distribution within a 10-liter water, subsampled up to 50 ml (0.5%) of the water, counted the number of prolarvae in the sample, and used the ratio of water sampled to estimate the total number of prolarvae. Unfortunately, the precision range was typically as high as ±20%, so five or more measurements were taken to calculate the mean value.

Fig. 22-8. Prolarvae (25 d post fertilization) with well-defined yellow yolk sac (photo taken on 9 June 2014). Mean total length was 6.0 mm for this offspring group.

XpertSea developed a new program in 2014, and a "biomass factor" (an index value to estimate the biomass of prolarvae) was calculated for multiple development stages between 14 d and 32 d post fertilization

(corresponding to beginning of hatching and predominantly burrowing life stage, respectfully) using 500 prolarvae for each measurement (Fig. 22-8 and Fig. 22-9). Five measurements were taken to attain the mean value. Based on this value (Fig. 22-10), we were able to significantly improve accuracy and precision in our total estimates for prolarvae numbers. The maximum number of prolarvae the device could potentially count at a time with 95% accuracy was 160,000 for 14 d prolarvae and 26,000 for 32 d larvae based on the biomass factor calculated (C. Andrews, XpertSea, pers. comm.).

Fig. 22-9. Larvae (31 d post fertilization) with fading yolk sac (photo taken on 6 June 2014). Mean total length was 8.5 mm for this offspring group.

Part of the reason that this period is so critical to lamprey is that they not only acquire feeding ability, but they also must switch habitats and assume a burrowing lifestyle. At about 30 d post fertilization, Pacific lamprey in the wild move from a coarse substrate with swift velocities to silt-laden low velocity areas where they burrow (Brumo 2006). In a hatchery setting (Prosser Fish Hatchery), burrowing behavior first began at 26 d post fertilization (~11 d post hatching) and the majority of larvae (>95%) finished burrowing by 33 d post fertilization (~19 days post hatching), corresponding to CDD of 369 and 469, respectfully (Fig. 22-11). This is consistent with other studies on initial burrowing of Pacific lamprey (Close et al. 2001; Meeuwig et al. 2005). This is also in line with sea lamprey studies of gut formation, which occurred sometime between 23 d and 36 d post fertilization (Richardson and Wright 2003), and tests on burrowing timing (Rodriguez-Munoz et al. 2001).

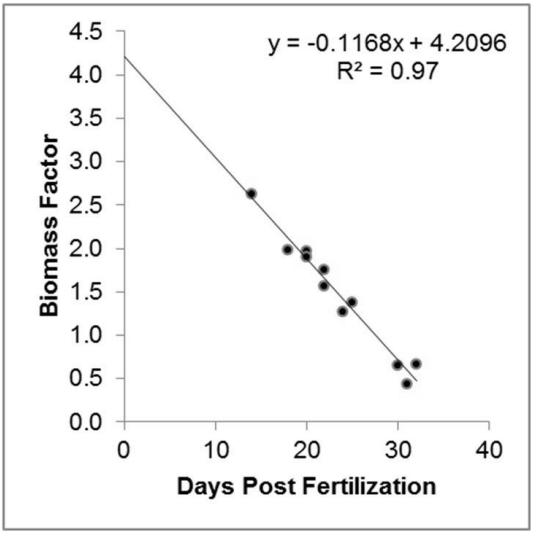


Fig. 22-10. Biomass factor value calculated for groups (N=11) of newly hatched Pacific lamprey prolarvae in days post fertilization using XperCount device (XpertSea, Quebec, Canada).

The exact timing of first feeding, food type and particle size requirements, and optimal substrate and flow for this transitional life stage are unknown. However, observations from artificial propagation provide some insights. Coconut fiber spawning mats can provide dark cover, flow refuge, and small interstitial spaces on the tank bottom important for the

earliest stages, while fine sediment underneath provides habitat for burrowing stages (Fig. 22-12). While larger grain size fine sediment (>500 micron) should be reserved for older larvae (Quintella et al. 2007), provision of small grain size fine sediment (<500 micron) early in the process allows burrowing of early larvae to take place when they are first ready. If no substrate is provided during this critical period, growth and survival are extremely low (Lampman et al. 2014b; Jackson and Moser 2014).

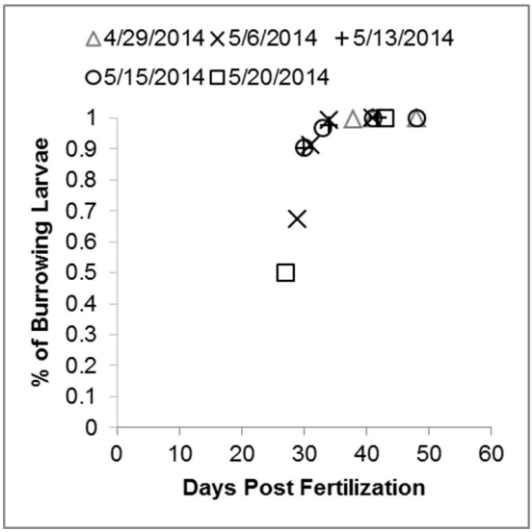


Fig. 22-11. Percent of burrowing larvae in relation to days post fertilization for offspring from multiple spawning events (N=16; date of spawning event shown in the legend).

For all fish culture operations, minimizing density can reduce disease and increase growth and survival. Therefore, identifying the optimal densities to maximize production while minimizing losses is important for all life stages. We have found that observed mortality rates remained very low (<1%) for prolarvae at a density of 50,000 individuals /m^2 (roughly 125 g/m^2) while densities of 100,000 individuals /m^2 (roughly 250 g/m^2) or higher produced significantly more observed mortality ranging from 2.5-10%. However, there may be elements of culture that help early larvae to cope with high density. In experiments where algal mats were placed in tanks in addition to spawning mats, mortality rates remained low even under density levels higher than 100,000 individuals /m^2. When larvae

start feeding (which may coincide with the start of the burrowing behavior), the most optimal food types, particle sizes, and ration at first feeding need to be investigated.

Fig. 22-12. Prolarvae placed in flow through incubation troughs on top of fine sediment (<500 micron) and underneath the coconut fiber spawning mat (which is temporarily lifted out of water for the photo). Density level in this compartment was 165,000 individuals /m^2 (=29,630 individuals).

The burrowing nature of the larvae also makes the screening of the outflow critical, as they will try to work their way through the screens. Using a high surface area of fine mesh outlet screens (250-300 micron) positioned well above the substrate and maintaining low flow rates (<10 liter / min in 300 liter tanks) at this stage will help minimize escapement and impingement. Once larvae grow for 3-4 months, the screen mesh size can be increased to 400-500 micron. However, young of the year larvae display extremely variable growth rates, so caution is needed in increasing the mesh size too soon.

Larval Rearing

The larval (ammocoete) stage of Pacific lamprey begins upon completion of yolk absorption and this stage resides in fresh water for up to seven years before the onset of metamorphosis. The metamorphosis generally occurs over several months and then the juveniles migrate to the ocean. Given the duration of the larval life stage in lamprey, mastering the care of larvae is essential for an effective propagation program. To successfully raise larvae in a controlled setting, their basic requirements of food, water and habitat must be provided. If these needs are not met, growth can be stunted, development can be disrupted, and high mortality can occur.

Various tank styles have been experimentally used for rearing the larvae including plastic storage containers, aquaria, incubation and long troughs, circular tanks and even modified Heath trays. Larval lamprey burrow into substrate, therefore the benthic surface area of the tank is likely more critical than the volume. Shallow tanks with a large footprint will provide more useable rearing area per volume. Deeper tanks with higher water volume may require additional rations to prevent diluted food densities. At the Abernathy Fish Technology Center larvae have been reared effectively with as little as 7 cm of water depth above the substrate, and even lower levels may be possible. Flow rate is typically set to minimal amount for larval lamprey (0.6-1.3 L/min for 25 L tanks) as they naturally prefer slow water habitat. A water turnover time (defined as volume divided by flow rate) of 35-40 min is recommended to preserve the slow water conditions.

The bottom of the tank should be covered with some form of fine substrate (Hanson et al. 1974; Ostrand et al. 2011). A wide variety of substrates have been used successfully for larvae including river sand/silt, beach sand, commercial play sand, and spoils from settling ponds. Rearing larvae without any substrate is known to induce high mortality (especially for small larvae) and at the least will induce severely stunted growth and compromised health due to physiological stress (Lampman et al. 2014c; Jackson and Moser 2014). A variety of alternative substrate media, including loose coconut fiber, spawning mats, nylon filter media, and Bio-Balls, have been tried for young of the year larvae, but none of them resulted in first feeding larval survival for more than several months. Although clayey soil can be rich in organic content (i.e. food source for larval lamprey), its adhesive character severely limits our ability to sift the sediment to effectively extract the fish. As a general rule of thumb, fine sand ($\leq$500 micron particle size) of a depth at least two-thirds of the total

length of the fish have demonstrated to be effective (Rodriguez-Munoz et al. 2003; McGree et al. 2008; A. Gannam, U.S. Fish and Wildlife Service, and R. Lampman, Yakama Nation, unpubl. data). For outside tanks, partial shading (such as mesh cover) is recommended; no shade will result in excessive algae growth in the summer months and complete shade appears to reduce productivity.

Some of the main challenges that substrate places on the culturist are that it obscures the fish from observation which makes it more difficult to detect problems ahead of time. Substrate can make monitoring of the fish laborious and time consuming as it requires sifting and more invasive handling and you cannot simply scoop a net through the water to capture the fish. Finally, the use of substrate also present some challenges for maintaining tank cleanliness, as the inflow of clean water to the culture tank may not improve the conditions of the sediment interstitial water where the lamprey live.

For monitoring larvae, siphon hoses can be used to safely and efficiently extract the fine sediment and larvae out from the tanks, which can be sifted using appropriately sized mesh screens. Wooden framed mesh screens can be floated on water surface at the downstream end of the siphon, enabling larvae to stay under water during the majority of this process. Hand netting of the fine sediment can occasionally damage the larvae, but using hands to gently transfer the fine sediment to nets or mesh screens is another safer alternative, especially for larger larvae. Accuracy of fish weight could be enhanced by placing the larvae (anesthetized or unanesthetized) on a sponge prior to weighing to remove any excess water. With the use of a photarium (Wild Fish Conservancy, Duvall, WA), larval length can be accurately measured to 1 mm accuracy and precision without the use of any anesthetics, greatly reducing overall monitoring time required with minimum stress to the fish (Fig. 22-13). To eliminate the length based bias in condition factor (i.e. mean condition factor decreases with increases in length), the use of a lamprey-specific condition factor equation is recommended: weight (g) x 10^5 / length $(mm)^{2.6}$. Using this modified equation, the length based bias can be eliminated in both wild and hatchery larvae, with a mean value typically around 0.9-1.0 for larvae (R. Lampman, Yakama Nation, unpubl. data).

Fig. 22-13. Use of "photarium" (Wild Fish Conservancy, Duvall, WA) can significantly reduce the amount of time required for larval monitoring as total length of larvae can be accurately measured down to 1mm without any use of anesthetics. Tail region can be seen clearly as well, allowing species identification.

As with culturing any fish, water quality is a critical element of lamprey culture. Pacific lamprey larvae have been reared at a variety of locations with different sources of water. Well water, creek water, and re-circulated dechlorinated municipal water have been used to rear larvae (Mallat 1983; Morman 1987; Murdock et al. 1991). Creek/river water usually holds a lot more natural-origin dissolved nutrients, which may help reduce the amount of artificial feed we need to add to our tanks. Literature suggests that larval lamprey can function well at water temperature ranging between 10-19°C (Reynolds and Casterlin 1978; Meeuwig et al. 2005) with a behavioral preference somewhere between 13 and 20°C (Potter and Beamish 1974; Holmes and Lin 1994). Larvae reared in 9°C water displayed approximately 40% of the growth observed in larvae reared in 15°C water according to a study conducted in 2012 by U.S. Geological Survey (L. Weiland, pers. comm.). As a result, water temperature likely has a strong influence on growth in larvae; therefore, further refinement of optimal rearing temperatures is needed. Although some literature exists on water quality impacts on lamprey (Mallatt 1983;

Mattsoff and Nikinmaa 1988), specific thresholds and acceptable levels for dissolved oxygen, pH, ammonia, nitrite and nitrate in the water when culturing lamprey are largely unknown. Moreover, future investigation should give consideration to the conditions within the substrate rather than just in the water column.

Stopping of water during feeding, typically several hours but sometimes up to 24 h, is a common practice for rearing larval lamprey (Hanson et al. 1974; Mallatt 1983; Swink 1995). This allows more feed (in particular yeast) to settle to the bottom before being flushed out of the tank, maximizing the amount of food that reaches the larval lamprey habitat. Flushing of the sand was recommended by Hanson et al. (1974) when culturing sea lamprey larvae to prevent oxygen depletion and fouling in the substrate in association with water stoppage during feeding. Larval lamprey culture at Abernathy Fish Technology Center employs flushing of the sand substrate with water using a modified spray bar twice a week to improve tank substrate and water quality conditions.

The drawback to this commonly used practice of stopping water is two-fold: 1) fouling can occur requiring the aforementioned tank cleaning and maintenance to reduce excessive build-up (Mallatt 1983; Murdock et al. 1991); 2) risk of forgetting to turn the water back on can cause high mortality due to anoxic conditions. Larvae receiving high ration feed are especially susceptible to high mortality during water stoppage and even 24 h water stoppage can cause mortality in the range of 83-100% (McGree et al. 2008; R. Lampman, Yakama Nation, unpubl. data). However, small larvae (40-60 mm) accidentally left in 50 ml of standing water inside a plastic bowl for over three months during the winter were discovered to be all alive (N=4), so the lack of flowing water for extended periods does not appear to be an issue for larvae when feed is not added to this mix (T. Beals, Yakama Nation, pers. comm.). Lastly, if the tanks are not in temperature controlled rooms, water temperature can rise or drop dramatically during the summer and winter seasons as a result of stopping water. Recent study results (R. Lampman, Yakama Nation, unpubl. data) suggest that stopping water improved growth rates for small larvae (<50 mm), but enhanced growth was not detected for large larvae (>50 mm). Maintaining a reduced minimal flow rate at all times may be an effective way to imitate the effects of stopping water while minimizing the various risks involved with stopping water.

Another critical element of lamprey culture, which influences water quality, is nutrition. While the diet of wild lamprey consists mainly of organic detritus (Moore and Mallat 1980; Yap and Bowen 2003; Mundahl et al. 2005), there is no practical way to provide fish with this type of

forage that is consistent in nutrient content and disease free in an intensive aquaculture setting. Numerous diets have been tested for rearing larvae, including yeast (active dry yeast, brewer's yeast, nutritional yeast), dry leaves, twigs, and compost. A feeding trial conducted at Abernathy Fish Technology Center found a diet of active dry yeast supplemented with a larval fish diet at a ratio of 4:1 provided the fastest growth in larval lamprey (Barron et al., in press). An algae mix was also tried but produced poor growth. In addition, this trial tested the use of a fish oil emulsion as a supplement to the diet, and found it to be an effective method to deliver fatty acids to the larvae. Other ingredients that demonstrated enhanced growth and/or survival includes salmon carcass / analog (Thompson 2007; Uh et al. 2014; Lampman et al. 2014b), wheat flour, alfalfa pellets, and wheat straw (Lampman et al., in press). For some of these alternative feeds, larval lamprey were likely feeding on the seston (plankton, nekton, and detritus) produced as a result of the addition of these feeds instead of ingesting the feed directly.

One important consideration to make is the price of feed. Organic whole wheat flour typically costs less than $2 per kg, whereas active dry yeast costs approximately $10 per kg, and starter larval feed can cost up to $200 per kg. Straw and alfalfa pellets are even cheaper and cost less than 40 cents per kg. If difference in survival and growth rates is minimal (<10%) for different types of feed, cost of the feed can be an important consideration for large scales of lamprey production (especially given the limited budget available for Pacific lamprey). More importantly, incorporation of more natural feeds (such as dry leaves, twigs, organic rich fine sediment, and salmon carcasses, etc.) may help preserve wild qualities of the larval lamprey within the hatchery environment and will be key to successful transitioning of larvae to a field setting.

The amount of the ration appears to be critical to the growth and survival of larvae. Although there may be a minimum threshold of feed (i.e. base feed) needed even in low fish density, in general the ration should be adjusted based on fish weight (Mallatt 1983). A Yakama Nation study in 2014 demonstrated strong logarithmic correlation ($r = 0.881$) between ration and growth rates, and a mixed feed ration of 10-20g / week / fish weight (g) resulted in growth rates of 7-12 mm per month between late July and late September (Fig. 22-14). Active dry yeast was a major ingredient, and constituted roughly 50% of the overall feed. In this same study, a positive linear relationship ($r = 0.706$) was also observed between feed ration per surface area and growth rates; a mixed feed of 400 – 700 g / m^2 resulted in growth rates ranging between 7.5-12 mm / month (Fig. 22-15). As a result, surface area may also be a factor to consider for the amount

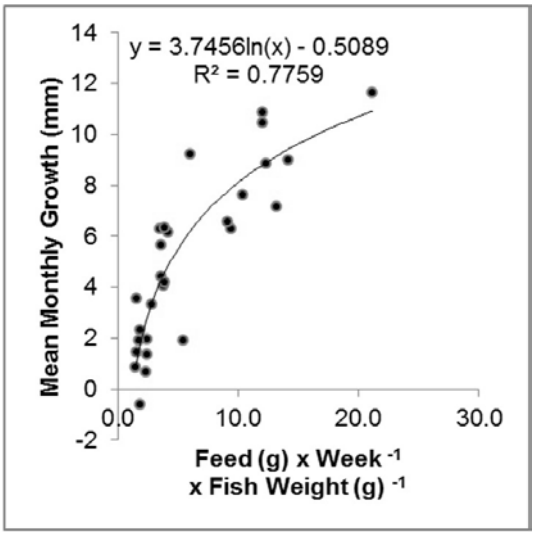


Fig. 22-14. Mean monthly growth (mm) observed for Pacific lamprey larvae reared in various tanks (N=28) at Prosser Fish Hatchery in 2014 in association with the feed (g) per week per overall fish weight (g).

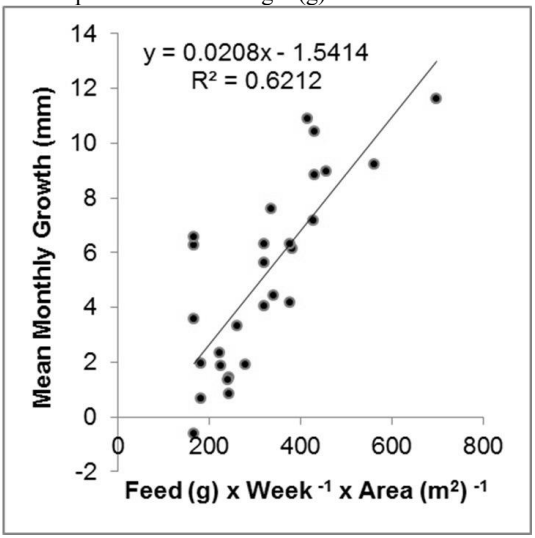


Fig. 22-15. Mean monthly growth (mm) observed for Pacific lamprey larvae reared in various tanks (N=28) at Prosser Fish Hatchery in 2014 in association with the feed (g) per week per area (m^2).

of ration. On the other hand, these high levels of mixed feed can increase tank fouling, especially when water is shut off during feeding, and a balance is needed between ration level, larval biomass and water quality within the substrate where the larvae reside. In addition, enhanced growth well beyond the natural rate of growth observed in wild larvae (which is mostly likely in the range of 2-4 mm / month based on transformation within 3-7 years) may have unknown physiological implications.

Larvae appear to be reasonably tolerant of low levels of feed for extended periods. Although growth rates were negative, McGree et al. (2008) reported a high survival rate (roughly 96%) in large larval Pacific lamprey (~2g individually) that were not fed anything but unfiltered creek water over the course of 3.5 months. Using dechlorinated tap water, death occurred after 7-8 months without food in Pacific lamprey larvae of similar size (Mallatt 1983). In very young sea lamprey larvae, however, Hanson et al (1974) found that feeding too little or not at all appeared to kill most of the larvae within 2 months. With Pacific lamprey, 5-months-old larvae that were reared using only well water without additional feed began dying after 60 days and overall survival rates after 73 days was 51.4% and 73.0% from two tanks with higher survival shown in the tank with organic rich fine substrate (R. Lampman, Yakama Nation, unpubl. data). Despite the lack of supplemental feed, total length and weight still increased slightly by 2.2-3.7 mm and 0-6 mg, respectfully, among the surviving larvae, most likely due to organic content available within the fine substrate provided.

Tank densities are best defined using weight per benthic surface area (g/m^2) due to the immense size difference in larvae among the multiple age classes; newly hatched larvae start off at only 2 mg whereas older larvae can weigh 7 g (roughly 3500 times larger). Studies conducted by US Geological Survey demonstrated that growth rates generally decreased rapidly as density increased over 100 g/m^2 (Mesa 2011). Recent work conducted by the Yakama Nation in 2014 showed a strong logarithmic relationship ($r = 0.651$) between start density (g/m^2) and growth rates (Fig. 22-16), suggesting that growth is dependent to some extent on density. At densities of 100 g/m^2, growth rates were limited to roughly half of the maximum growth rates observed under lower densities. Even under similar fish density and food ration conditions, monthly growth rates were consistently higher (by roughly 2-3 mm) in small aquaria (25 liters) compared to large sized troughs (1160 liters), a trend we have observed consistently over the years (Fig. 22-17). This may potentially be due to the ability of small tanks to retain a higher percentage of the given feed. In addition, although water source is predominantly well water with 13-15

degree water temperature throughout the year, synchronized seasonal fluctuation in growth rates was observed in both sizes of tanks with the highest growth observed during the fall and winter.

Survival rates for 3-month-old and older Pacific lamprey and sea lamprey larvae are generally very high with documented monthly survival rates between 99.3 and 99.8% (Murdoch et al. 1992; Swink 1997; McGree et al. 2008; Uh et al. 2014), which corresponds to annual survival rates of 91.9 and 97.6%. However, survival rates for 0-3 month-old larvae are known to be much lower (Hanson et al. 1974). Studies conducted more recently on 1-3 month-old Pacific lamprey larvae by a consortium of agencies, including U.S. Geological Survey, U.S. Fish and Wildlife Service, Yakama Nation, and Confederated Tribes of Umatilla Indian, demonstrated that overall survival averaged anywhere between 0-50% during this life stage. This high mortality appears to be concentrated between the first-feeding life stage (roughly 30 days post fertilization) and 60-75 days post fertilization, during which high numbers of mortality were often observed on fine sediment surface (R. Lampman, Yakama Nation, and M. Moser, National Marine Fisheries Service, unpubl. data).

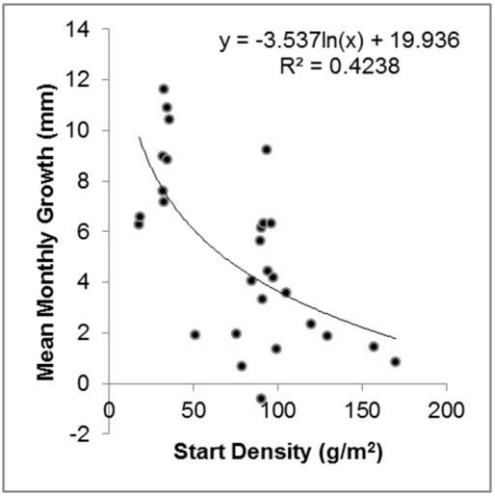


Fig. 22-16. Mean monthly growth (mm) observed for Pacific lamprey larvae reared in various tanks (N=28) at Prosser Fish Hatchery in 2014 in association with the start density (g/m^2).

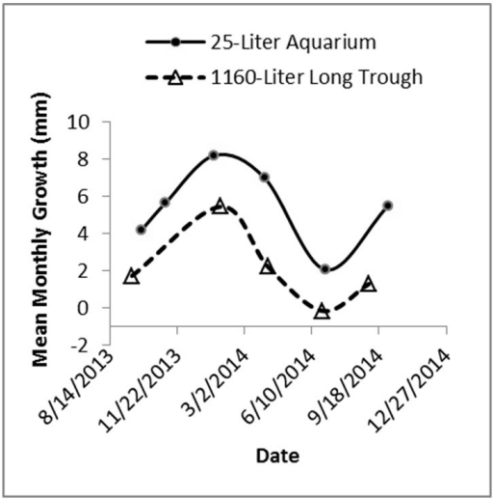


Fig. 22-17. Mean monthly growth (mm) observed for Pacific lamprey larvae hatched and reared in Prosser Fish Hatchery between 2013 and 2014 in two different tank settings.

Abernathy Fish Technology Center documented relatively high survival rates in 2012 (mean survival rate of 88% with a range of 45%-100%) for a 16-week feeding study focusing on this first feeding period. Although it is unclear why survival rates dropped considerably the following year under similar tank conditions, this nevertheless gives us hope that higher survival rates are potentially attainable. In addition, Yakama Nation in 2014 observed improvement in large tank survival rates (up to 68%) for first feeding larvae. This tank with a high survival rate received advanced burial of additional mixed feed (such as salmon carcasses and wheat straw) within the fine substrate prior to larvae transfer and spawning mats over the fine sediment to increase surface area where food particles can accumulate (Fig. 22-18). This period clearly appears to be the bottleneck life stage for Pacific lamprey, at least in the hatchery environment, and more targeted and coordinated research will be needed to investigate the relationship of density, food source, and habitat elements on larval survival at this critical stage.

Fig. 22-18. Incubation troughs (Pentair Aquatic Eco-systems, Apopka, Florida, USA; 2.2 x 0.6 x 0.3 m in length, width, and height, respectfully) installed in 2014, for which buried mixed feed (salmon carcass and wheat straw) was added under the fine substrate and spawning mats were left on top of the fine sediment. Algal mats were also added along tank edges soon after larvae transfer. A secondary outlet screen running along almost the entire length of the tank was built to prevent overflowing from the clogging of the primary screens. Highest survival rate (68%) for 1-3 month larvae was observed with this tank setting.

Holding and Transport of Gametes, Prolarvae, and Larvae

The ability to hold and/or transport early life stages of propagated lamprey is critical to successful hatchery and outplanting programs. In cases where broodstock are limiting, the ability to safely hold and transport gametes will provide flexibility in sharing gametes among hatchery programs and potentially increase genetic diversity of lamprey produced. This is particularly important in situations where there is asynchronous development in males and females. For research purposes and in outplanting of propagated larvae, it is critical that effects of transportation are minimized. Hence, experiments were conducted on both storage and transportation of gametes and on transport of developing eggs and larvae.

Pilot studies of gamete storage (including cryopreservation) were conducted in 2012-14 by biologists from Confederated Tribes of Umatilla Indian Reservation and Yakama Nation. Eggs and milt were held separately for specified periods at room temperature (15°C) or on ice (5°C) without aeration. Testing of milt cryopreservation was conducted using methods developed for salmonids. For each evaluation, eggs were gently combined with an approximately 10% solution of milt in culture water for 0.5-2.0 min and then allowed to rest for 2-3 min. Eggs were then rinsed three times with culture water and incubated in recirculating, UV-irradiated water at 14.5°C (±0.5°C). For each test, gamete viability was scored by 1-4 observers. For milt, 190 uL of hatchery water was pipetted onto a slide and 10 uL of sperm was mixed in and quickly viewed under a compound microscope at 20X magnification. Percent motility was noted by each observer. For eggs, fertilization success was assessed at 18 h (morula stage) and the number of live eggs was also scored after approximately one week (eggs hatch after 14-15 d). For each assessment, we scored at least three sub-samples of approximately 100 eggs each. Treatments were not disclosed to the observers to reduce observer bias.

Few eggs developed when both eggs and milt were held on ice for > 6 h (Jackson and Moser 2014). Fertilization rates were nearly 80% for eggs held 12 h at 14ºC, but few survived to hatching. In contrast, fertilization rates were > 98% for fresh eggs mixed with fresh milt or milt held at 14ºC for 24 h. Yet survival to day 7 was only 77.7% for eggs fertilized with day-old milt. Milt shipped on ice and received 24 h later at 15ºC had estimated motility of 75-90%. Holding milt on oxygen may help to increase survival beyond 24 h.

Initial attempts at lamprey milt cryopreservation using methods developed for salmonids showed some promise. A 1:1 dilution of cryopreservation media and milt had no motility when thawed using standard methods (D. Chase, U.S. Geological Survey, pers. comm.). However when the milt was thawed and combined with an equal amount of activator solution the sperm exhibited approximately 25% motility. Cryopreserved sperm did not appear to be as vigorous as fresh sperm and cryopreserved sperm did not successfully fertilize eggs. Additional research is needed to develop lamprey-specific cryopreservation methods.

Experiments conducted in 2013 and 2014 indicated that lamprey sensitivity to transport and changes in water quality is extremely stage specific (M. Moser, National Marine Fisheries Service, unpubl. data). Gametes that were fertilized and then transported for 2 h without aeration had higher survival to hatching (94.5%) than the same gametes that were transported for 2 h and then fertilized (41.3%). When developing eggs (day 1 and day 3 post-fertilization) were transported without aeration for 7 h and 24 h (simulated shipping), survival was also very high (>98%). These developmental stages were also resilient to changes in water supply following transport at experimental temperatures of 14ºC ±2ºC.

Prolarvae (2, 5 and 11 d post-hatch) transported without aeration for 7 h and 24 h periods exhibited nearly 100% survival if they were maintained in the water supply used for transport; however, nearly all died when they were transitioned to a new water supply during the 4-5 h after transport. Tests with transitions between three different water sources produced the same results. Subsequent experiments to transition larvae at a more gradual rate of change still resulted in high mortality. Providing cover, reducing temperature, and increasing salinity to 3‰ during transport/transitions did not improve survival. These experiments suggest that transferring developing eggs to a new facility can be done safely, but that more research is needed to develop safe transport methods for the sensitive pro-larvae stages.

Disinfection Methods

Standard fish pathogen assessments have been conducted on Pacific lamprey for bacterial kidney disease (Bell and Traxler 1986) and select rhabdoviruses of the Pacific Northwest, including IHNV and VHSV (Kurath et al. 2013), but they do not appear to be susceptible to these pathogenic agents. Other pathogens of concern in the hatchery environment include bacterial infections of *Aeromonas salmonicida* (an etiological agent for furunculosis). *Aeromonas hydrophila* and a Flavobacteria

species have also been documented infrequently, but other pathogens of concern appear to be rare in Pacific lamprey.

Aermonas salmonicida is known to exist within the adult Pacific lamprey populations that are collected from the Lower Columbia River since 1999 and used for supplementation research (S. Onjukka, Oregon Department of Fish and Wildlife, pers. comm.). Upon collection of adult lamprey from Army Corps of Engineer facilities, the fresh migrant adults are transported to receiving facilities in fish transport totes and tanks (200-1100 liter) supplied with oxygen at a rate of 1.5 L/min. Upon arrival, adults are treated with an intraperitoneal injection of oxytetracycline (100 mg/ml or 200 mg/ml products) at a dosage rate of 10 mg/kg of live fish weight. This serves as a precautionary measure to treat any systemic infections that the collected lamprey may have acquired prior to collection and handling. Adults are then placed into holding tanks until spawning. As an additional precautionary measure, adults are treated three times weekly with formalin at a dilution of 1:6000 to prevent any proliferation of fungus, protozoa, and external parasites within the holding tanks.

Development of fungus and other pathogens can result in high mortality of developing eggs and larvae. This is particularly true for recirculating systems. Salmonid culture operations regularly treat fertilized eggs to inhibit proliferation of pathogens in the facility (Piper 1982). However, tolerance of lamprey eggs to such disinfection protocols may differ from salmon. We tested standard salmonid iodophor and formalin disinfection methods. Lamprey eggs showed high survival when exposed to 100 ppm iodophor solution for 10 min right after fertilization (Jackson and Moser 2014). Egg development continued and no negative effects were observed using a 15 min exposure to formalin (1:600 dilution) for eggs 3-10 days post fertilization.

Larvae disinfection was also tested with various concentrations of formalin. No mortality was observed in first-feeding larvae (34-40 d post fertilization) exposed to 1 h formalin treatment at dilutions of 1:12000 and 1:6000. However, mortality was observed after 15-45 min in higher dilution treatment and 90-100% of larvae died at concentrations of 1:3000 and 100% died at a dilution of 1:1500. These higher concentrations correspond to 2× or 4× the concentration typically used on salmon fry, respectively. Prolarvae were also treated experimentally with formalin using 1:6000 dilution, and no mortality have been observed. Prolarvae were also very tolerant of salinities up to 5‰ (M. Moser, National Marine Fisheries Service, unpubl. data). For recirculating systems, UV-irradiation may also help to reduce pathogens.

Prolarvae and larvae may benefit in some cases from the growth of fungus. In the natural environment lamprey are detritivores and feed on decomposing matter. Fungus and the associated microbes may provide an important food source for feed ready larvae. For example, active dry yeast (single cell fungi) is commonly used as a food source for these fish. More research is needed to balance the benefits and detriments of fungal treatments in lamprey culture.

Summary

The role that artificial propagation of Pacific lamprey plays is two-fold. First, development of artificial propagation provides an additional recovery strategy that could be utilized to supplement extirpated populations. Currently, only two strategies exist in regards to recovery of Pacific lamprey: 1) improve habitat and wait for natural re-colonization, which is unlikely given the poor passage conditions that migrating adults currently experience across much of their historical range, and 2) adult translocation, which has been proven an effective method to recover lampreys in selected basins of the Pacific Northwest (Close et al 2009; Ward et al. 2012), but requires many adult brood to be collected and outplanted in the receiving stream. Utilizing artificial propagation will result in fewer adults collected from the wild population. Secondly, developing artificial propagation methods would help produce “test fish” to answer critical research uncertainties that are priorities for managers. Such research is needed to fully understand the complex lamprey life history (tributary habitat utilization, diversion entrainment, dam and reservoir passage, ocean survival, etc.). Without the development of artificial propagation and access to test fish, there will be little increase in knowledge gained (particularly for early life stages), resulting in slow or stalled recovery. Therefore, it is recommended that all of these supplementation strategies be explored to their fullest extent and used in conjunction when possible to allow lamprey recovery to come full circle.

Best management practices for the artificial propagation and early rearing of Pacific lamprey have advanced significantly in the past few years. Progress has been made in techniques and protocols for all life stages, from fertilization to larval rearing. All of this was made possible as a result of incredible advancement in artificial propagation techniques for closely related anadromous lamprey species by researchers from the Great Lakes (Fredricks and Seelye 1995; Ciereszko et al. 2000; Richardson and Wright 2003), Japan (Yamazaki et al. 2003; Hara 2008), Finland (Vikström 2002), and Spain (Rodriguez-Munoz et al. 2001). There were

also local pioneering studies that specifically targeted Pacific lamprey (Close et al. 2001; Meeuwig et al. 2005; Meeuwig et al. 2006; Wade and Beamish 2012), adding even more critical information to the preexisting knowledge base.

The bottleneck life stage in the hatchery setting has been identified as the 1-3 month life stage during which prolarvae transition to burrowing first feeding larvae based on our research (Fig. 22-19). Survival rates from fertilization to hatched prolarvae as well as for 3-month-old and older larvae are high (85-95% and 90-99%, respectfully). By increasing the survival rates only at the bottleneck life stage from 35% (our current average rate) to 80%, cumulative survival rate to 12 month old larvae will theoretically improve from 22% to 49%. As observed with prolarvae, larvae at this early life stage could also be sensitive to impacts from transport and changes in water sources, and more research is needed to understand how this sensitivity changes over time for future larval outplanting purposes.

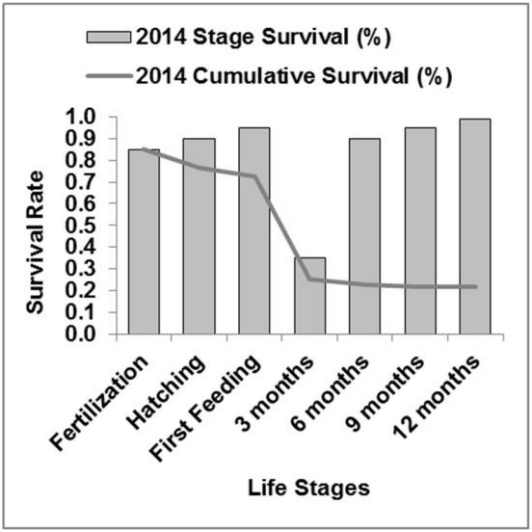


Fig. 22-19. Approximate life stage specific survival rates as well as cumulative survival rates observed for propagated young of the year Pacific lamprey larvae at Prosser Fish Hatchery.

Besides the bottleneck life stage, there is another major obstacle for the intensive culture of larval lamprey. Lamprey spend an extended time in freshwater as larvae, and it is important to understand how much rearing

space is needed to adequately rear a targeted number of larvae. Based on past research, 125 g/m^2 was identified as the density above which survival and growth may be hampered for both prolarvae and larvae life stages (Table 22-1). Adults and eggs can be held at a much higher density (26,000 and 800 g/m^2, respectfully), so space is not an issue for these life stages. Due to their small weight, prolarvae and 3-month-old larvae can also be reared with minimum space (2.0 and 14.0 m^2 for 100,000 individuals, respectfully). Mean percent daily growth for weight stays high (6.6-10.0%) for larvae up to 6 month old, but decreases sharply afterwards (<1.2%). A similar trend is seen in growth rates for length as well. As a result, large scale production of larvae will likely be less efficient for larvae older than 6 months and almost prohibitive for larvae older than one year due to their space requirements (Table 22-1 and Fig. 22-20). Furthermore, this space requirement is only estimated for one year class, and rearing of multiple year classes will obviously require even more space. Smaller target numbers for older larvae/juvenile (macrophthalmia), however, is certainly attainable; for example, 1,000 larvae/juvenile can likely be reared in space smaller than 60 m^2.

Table 22-1. Approximate size, growth, goal density levels, and area needed for 100,000 individuals (approximate equivalent of fecundity for one female) for each life stage of propagated Pacific lamprey. * 3- and 4-year-old larvae (between 140-180 mm length) could likely be a metamorphosed juvemile.

Life Stage	Length (mm)	Mean Monthly Growth (mm)	Weight (g)	Mean % Daily Growth (g)	Goal Density (g/m^2)	Goal Density (#/m^2)	Area (m^2) Needed for 100,000 Individuals
Adults	600	-	400	-	26,000	65	-
Eggs	1	-	0.0010	-	800	800,000	0.13
Prolarvae	6	7.5	0.0025	7.5%	125	50,000	2.0
Larvae (3 month old)	18	6.0	0.017	10.0%	125	7,169	14
Larvae (6 month old)	38	6.7	0.12	6.6%	125	1,027	97
Larvae (9 month old)	50	4.0	0.25	1.2%	125	503	199
Larvae (1 year old)	60	3.3	0.4	0.7%	125	313	319
Larvae (2 year old)	100	3.3	1.5	0.8%	125	83	1205
*Larvae (3 year old)	140	3.3	3.6	0.4%	125	35	2889
*Larvae (4 year old)	180	3.3	6.9	0.3%	125	18	5553

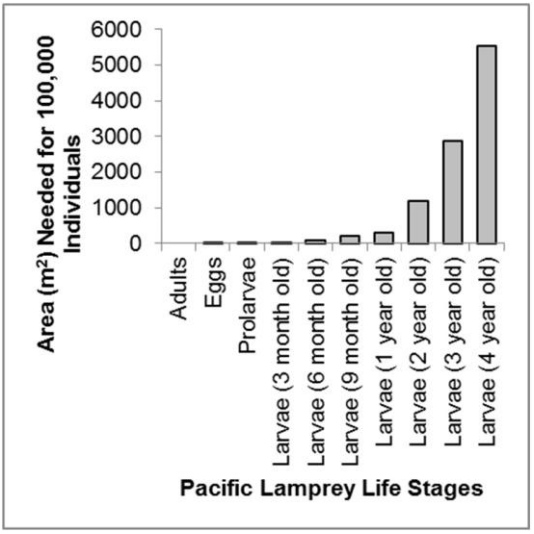


Fig. 22-20. Conceptual model of space (m^2) needed to adequately rear 100,000 individuals (approximate equivalent of fecundity for one female) of Pacific lamprey for various life stages.

Due to the seemingly daunting space requirement for larval lamprey (Table 22-1 and Fig. 22-20), it becomes imperative to take advantage of natural rearing space available. Perennial side channels, acclimation ponds for salmonids (*Oncorhynchus* spp.), as well as irrigation diversions hold abundant rearing space for larval lamprey. For example, over 32,000 m^2 of larval lamprey habitat was identified in diversions within the Yakima Basin (Lampman et al. 2014a), which could theoretically rear up to 500,000 larvae/juvenile. These types of habitat (side channels, acclimation ponds, and diversions) can be monitored intensively to assess survival, growth, and migration over time. The most effective and productive life stages for release will need to be examined. Experimental designs for outplanting strategies have been carefully devised by researchers from the Yakama Nation and Confederated Tribes of Umatilla Indian Reservation and the goal is to initiate experimental outplanting of larvae in 2016.

Aside from the mere production of juvenile and adult hatchery salmon, salmon hatcheries undeniably contributed enormously to our knowledge of salmon biology and its complex life histories. Through hatchery-associated salmon research, a great deal has been learned over the last century to uncover the many mysteries encompassing salmon species and the surrounding ecosystems. New monitoring associated with

supplementation programs, in concert with ongoing hatchery research, will likely reveal other new discoveries about the early life history of lamprey. Hatchery production is not a defendable long-term goal for the recovery of Pacific lamprey. However, too many knowledge gaps and threats (both known and potential) currently exist to adequately manage and protect the species currently facing rapid population decline. Through lamprey aquaculture and supplementation research, we have a momentous opportunity to advance our knowledge of survival and growth, optimal density levels, critical food resources and feeding strategies, migration behavior, environmental sex determination, and demographics. We will also be able to conduct juvenile survival studies at dams and irrigation diversions, for instance, to increase our knowledge of threats to outmigrants. This will ultimately allow identification of factors controlling population growth without extracting limited wild juvenile lamprey. In addition, genetic typing of both hatchery-reared and wild larvae holds great promise for assigning parentage, examining mating systems, and identifying genetic traits that have allowed lamprey to persist for millennia (see Chapter 21 for more detailed discussion on recent advancement in Pacific lamprey genetic analyses and how they can help answer management questions related to supplementation research and species recovery). Finally, these techniques and insights obtained from Pacific Lamprey artificial propagation could also help advance research in other fields or species, such as for biomedical applications and controlling invasive species (Great Lakes sea lamprey).

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