

Captive Rearing of Pacific Lamprey

2011 Annual Report

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Introduction

Pacific lamprey *Entosphenus tridentatus* in the Columbia River Basin have declined to a remnant of their historical abundance (Close et al. 2002). Pacific lamprey populations are declining and have been given protected status within Oregon due to declines along the coast and in the Columbia River Basin (Close et al. 2002; Kostow 2002). Pacific lampreys have a complex life history that includes a three to seven year larval (i.e., ammocoete), migratory juvenile (i.e., macrophthalmia) and adult phases (Scott and Crossman 1973). Larvae and juveniles are strongly associated with stream and river sediments. Larvae live burrowed in stream and river sediments for periods up to seven years after hatching, where they filter feed detritus and organic material (Scott and Crossman 1973; Sutton and Bowen 1994). Larvae metamorphose into juveniles from July to December (McGree et al. 2008) and migrate downstream to the Pacific Ocean.

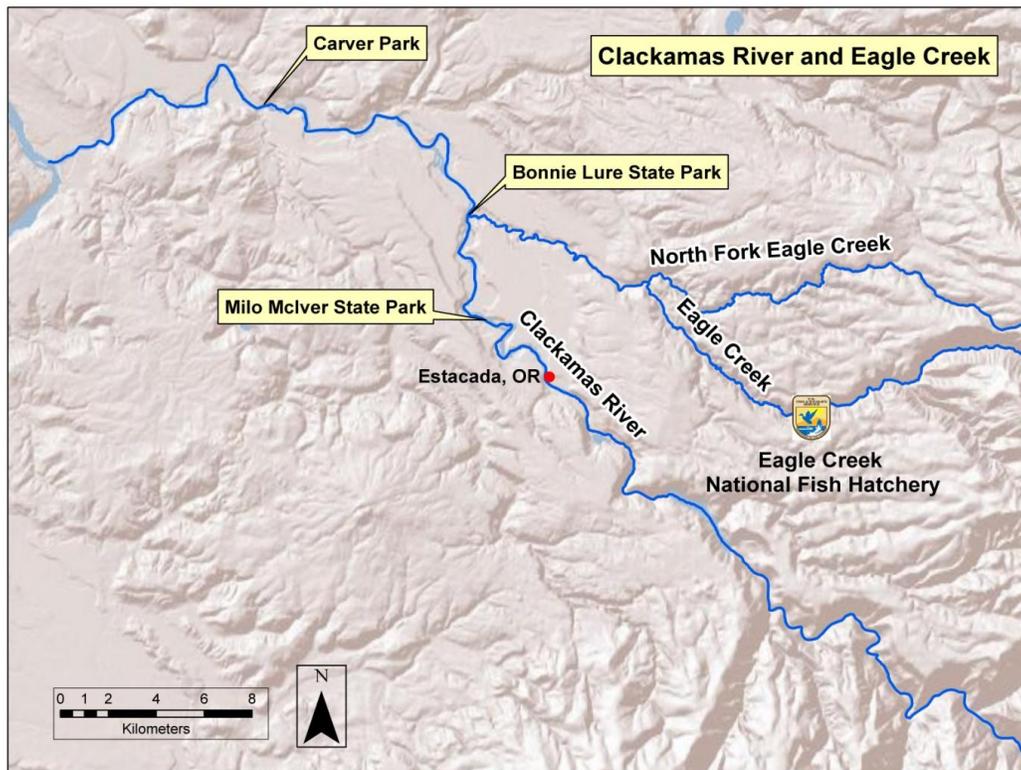


Figure 1. Study area in Eagle Creek and the Clackamas River

Several critical uncertainties have been formalized regarding the basic life history and ecology of lampreys (CRBLTWG 2005; Luzier et al. 2011). The timing, duration, and habitat use at the larval and juvenile life stage are poorly understood. Increased knowledge of the biology, population dynamics, ecology, and identification of Pacific lamprey will help managers understand and conserve these important species. Many of the uncertainties may be addressed by observation and experimentation using captive animals. For example, McGree et al. (2008) improved understanding of Pacific lamprey metamorphosis using captive animals. However, the unique life history of the lamprey poses unique challenges to rearing them in captivity. Although larval lamprey have successfully been held for experimentation and have been shown to metamorphose in captivity, explicit information on rearing and feeding leading to regular growth has not been well demonstrated. Developing appropriate holding vessels that contain rearing habitat and nutrition required for the growth of robust individuals is necessary. In addition, animals transported to captivity from the wild may be vectors of pathogens that can be spread to the rest of the facility. Again, it is unclear if and how lampreys are influenced by many common fish pathogens. Proper care needs to be taken to address these potential issues. Our objectives were to 1) establish a captive group of lamprey larvae at Eagle Creek National Fish Hatchery (ECNFH) and investigate various holding configurations, 2) investigate growth responses of different feeding regimes, and 3) test Pacific lamprey larvae for susceptibility to infection and mortality following exposure to strains of Pacific Northwest fish viruses.

Methods and Results

Captive housing configuration

Larval Pacific lampreys (n=80) were collected from the North Fork of Eagle Creek using an AbP-2 backpack electrofisher (ETS Electrofishing, Verona, WI) on 17 November 2009

(Figure 1). Larvae were transported to Eagle Creek National Fish Hatchery and housed in four different rearing vessels in 2 replicates of each vessel as follows: 1) Plastic tubs (61.5 x 41.2 x 22.4 cm) with screened openings for outflow, 2) Plastic tubs (61.5 x 41.2 x 30.5 cm) with



Figure 2. Larval lamprey rearing tanks at Eagle Creek National Fish Hatchery.

screened openings for outflow, 3) Plastic tubs (61.5 x 41.2 x 30.5 cm) with drilled openings for outflow, and 4) Circular fiberglass tanks (34.3 cm diameter, 40.6 cm deep) with a double standpipe (3.8 cm diameter), set at 27.9 cm of depth, that was screened for the water outflow. All vessels contained 5-7 cm of sand substrate. The substrate source was a spoil pile excavated from the presettling pond below the ECNFH intake. This spoil contained natural Eagle Creek sediments. The average organic content was 5.3% (n = 8

samples, loss-on-ignition methods [Heiri et al. 2001; Jolley et al. 2011]). All vessels were placed in rectangular fiberglass troughs (43.2 cm wide x 40.6 cm deep x 4.9 m long), in a raceway and supplied with Eagle Creek water in a flow-through system (Figure 2).

The captive holding configuration progressed adaptively as aspects of the configuration were found to be inadequate (Jolley et al. 2011). In addition, because *Vibrio* spp. was isolated in



Figure 3. Schematic of Eagle Creek National Fish Hatchery and vicinity

the sample of lamprey screened for pathogens (Jolley et al. 2011), those brought to ECNFH were isolated in the hatchery so that their effluent did not

contact other areas of the hatchery. This was deemed a precautionary and conservative approach (S. Gutenberger, USFWS, personal communication). The lampreys were confined to either the upper raceways or lower raceways sections of the hatchery (Figure 3). Shade screens were used in summer to moderate temperatures. A combination of commercially available baker's yeast and larval fish food (Gemma Wean, Bio-Oregon, Longview, WA) were fed at a weekly rate of 0.27 g yeast + 0.03 g larval fish food per lamprey (Polkinghorne et al. 2001; McGree et al. 2008). Rearing vessels and holding troughs were checked daily for signs of escaped larvae and all observed escapes were noted.

Evaluation of the holding vessels is ongoing. Larval lampreys have escaped from all vessels with the exception of the circular fiberglass tanks. Apparently, lampreys either squeeze through outflow openings or between the screen-vessel connections. In addition, various marine epoxies have failed allowing screens to become detached. Larval lampreys seem opportunistic in exploiting these weaknesses. Escapes likely happen at night as larvae are known to be more active in darkness (Gadomski and Barfoot 1998; White and Harvey 2003) and they were not

observed out of the sediment during daylight hours. Preliminary results indicate that the fiberglass circular tank design is preferable because no larvae have escaped from these vessels. Expansion of the number of rearing vessels may be warranted.

Feeding experiments

Pacific lampreys (n=104) were collected from the North Fork Eagle Creek, of the Willamette River Basin (Clackamas County, Oregon) on 17 November 2009. Lampreys were collected using a backpack electrofisher (described above). This group of lampreys was the first group of lampreys brought into the hatchery for use in developing a captive rearing configuration and protocol (see Jolley et al. 2011).

On 6 August 2010, prior to initiation of a feeding experiment, this group of lampreys was inventoried for baseline length and weight information. Due to escapes and mortalities, there were 86 remaining larvae. Individual lamprey were anesthetized with tricaine methanesulfate (MS-222; 50 mg/L), identified from previous unique visible implant elastomer (VIE) tags (Silver et al. 2009) and randomly assigned to feeding treatment groups (Table 1). Lampreys were measured (TL in mm) and weighed (wet weight in g). In addition, a hydrostatic weight in water (g) was taken for calculation of body density. Body density (BD) was calculated as:

$$BD = \text{wet weight} / ([\text{wet weight} - \text{weight in water}] / \text{density of water})$$

Prior to weighing, gentle pressure and/or the use of a syringe filled with water were used to expel as much air as possible from each lamprey's buccal cavity. Lampreys were weighed using the "weigh below" option on an Ohaus digital scale, where items can be hung from the underside of the scale. Lampreys were attached to a hook and clip apparatus, submerged in a container of water, and the weight in water was recorded (Jolley et al. 2011).

Lampreys were randomly assigned to one of four potential feeding treatments: 1) no food, 2) combination of baker's yeast and larval fish food, 3) ground cottonwood leaves, and 4) algae. Each feeding treatment contained two replicates for a total of eight experimental tubs (Table 1). Any escaped larvae were recovered and placed in an "escape tub" for each feeding treatment. Commercially available algae wafers (Kyorin Food Industries, Ltd, Himeji, Japan) were ground in an electric coffee grinder and fed at a rate of 0.3 g per lamprey. Black cottonwood (*Populus* spp.) leaves collected from the floodplain of the Columbia River were chosen to generically represent the willow family (Salicaceae) that commonly grows in the riparian zone where lampreys occur. This type of feeding regime was previously used by Shirakawa et al. (2009). Leaves were dried in an oven at 100°C for 4 hours and then ground in an electric coffee grinder into a powder. The ground leaves were fed at a rate of 0.3 g per lamprey. A combination of commercially available baker's yeast and larval fish food (Gemma Wean, Bio-Oregon, Longview, WA) were fed at a rate of 0.27 g yeast + 0.03 g larval fish food per lamprey (Polkinghorne et al. 2001; McGree et al. 2008). Prior to feeding, a suspension of water and leaves or water and algae was created by adding the food to 500 mL water and allowing it to soak for approximately 24 h. Soaking presumably increased the chance that the food will sink and therefore be available to filter feeding larval lamprey and decreases the chance that the food floats and flows out of the container (Limm and Power 2011). Lampreys were fed once per week.

Table 1. Number and mean TL (mm) of Pacific lamprey ammocoetes in each feeding trial at Eagle Creek National Fish Hatchery 2010-2011 in three time periods. Standard errors are in parentheses.

Tub	Treatment	August 2010		January 2011		May 2011	
		Mean TL (mm)	Number	Mean TL (mm)	Number	Mean TL (mm)	Number
6	Algae	82.4 (6.6)	10	98.3 (13.1)	4	120.5 (3.5)	2
7		83.3 (4.4)	10	76.6 (3.0)	12	90.0 (-)	1
Escape		.	.	93.5 (5.5)	2	94.5 (2.5)	2
2	Leaf	86.8 (4.6)	12	91.6 (7.4)	5	107.0 (-)	1
8		81.4 (5.2)	10	78.4 (7.4)	7	.	0
Escape		.	.	79.0 (6.6)	4	92.0 (8.0)	2
3	No food	88.3 (5.8)	12	.	0	110.0 (-)	1
4		86.8 (4.9)	10	100.0 (-)	1	.	0
Escape		.	.	94.8 (6.3)	8	.	0
1	Yeast+larval food	83.3 (4.5)	12	.	0	81.9 (4.2)	9
5		85.1 (5.6)	10	83.3 (3.5)	17	77.8 (2.3)	8
Escape		.	.	74.7 (4.2)	7	.	0

Table 2. Mean TL (mm) by treatment group of Pacific lamprey ammocoetes in each feeding trial at Eagle Creek National Fish Hatchery 2010-2011 in three time periods. Standard errors are in parentheses.

Treatment	November 2010		January 2011		May 2011	
	Mean TL (mm)	Number	Mean TL (mm)	Number	Mean TL (mm)	Number
Algae	82.9 (3.9)	20	83.3 (4.0)	18	104.0 (6.9)*	5
Leaf	84.3 (3.4)	22	82.7 (4.3)	16	97.0 (6.8)	3
No food	87.6 (3.8)	22	95.3 (5.6)	9	110.0	1
Yeast + larval food	84.1 (3.4)	22	80.8 (2.8)	24	79.9 (2.4)	17

*Indicates significant difference among time periods.

Lampreys were monitored weekly for mortalities on the sediment surface. All mortalities were identified and frozen. Detrital buildup and related fungal growth were periodically skimmed from the sediment surface with a small mesh net, as judged necessary to maintain reasonable water quality.

Lampreys were examined for potential growth on 3 January and 6 May 2011. Lampreys in each tub were agitated from the sediment, anesthetized, identified, measured (TL) and weighed. Potential differences in growth rate (i.e., initial weight-final weight) were examined by feeding treatment. Substantial mortality and escape occurred throughout the study. Some mortalities (n= 20) and escapes were documented but many were not. Many lampreys were

simply unaccounted for. The experiment started with 86 total lamprey; 67 and 26 lampreys were documented in January and May 2011, respectively. This corresponds to an overall loss rate of 70%. In addition, remaining individually marked larvae did not always correspond to the marks that were assigned to each tub, which suggests potential inter-tub movement. The substantial escape rate along with the possibility of inter-tub movement precluded calculation of individual growth rates. Because individuals could not be absolutely identified, growth rates were pooled by treatment. Exploratory analyses examining growth rate by treatment indicated that algae-fed fish were significantly longer in May than either January or November (assuming complete containment of these fish), although the final length data consisted of 5 individuals (ANOVA, $F=3.33$, $df=2$, $P=0.05$, Table 2). No other differences in mean TL were detected by treatment among time periods. Mean wet weight also differed in the algae treatment, compared to the other treatments in May ($F=5.35$, $P<0.01$), although post-hoc mean separation did not occur. Algae fed fish weighed the most (mean=1.6 g, n=5) while yeast-fed fish weighed the least (mean=0.64 g, n=17). Growth data was equivocal and TL decreased in many instances.

Lamprey disease challenges

Lamprey disease challenges were conducted at the USGS Western Fisheries Research Center in Seattle, WA. A comprehensive report on these results will be forthcoming (G. Kurath, USGS, personal communication) but an abridged version of the methods and preliminary results follow. A pilot study was first conducted to evaluate the holding configuration and handling aspects of larval lamprey. Eight larval lampreys were obtained from a rotary screw trap on the Green River (King County, WA) and held in 4-L challenge buckets without substrate for 14 days. In general, normal behavior was observed and no mortalities occurred. Larvae were

anesthetized with MS-222. Larvae were injected (27 G tuberculin syringe) with 25 μ L of phosphate buffered saline (pH 7.0) into the intraperitoneal cavity and three mortalities were observed 4-7 days after injection. Based on the results of this pilot study, the experiment was modified to minimize the number of injected fish (as the process may lead to increased mortality).



Larval Pacific lamprey (n=357) were collected on 22 June 2011 from the North Fork Eagle Creek using a backpack electrofisher (described above). Fish were placed in aerated coolers

Figure 4. Larval lamprey virus immersion trial. containing ice (2 frozen, 2 L containers each) and transported to the USGS Western Fisheries Research Center. Water temperature in North Fork Eagle Creek was 14.4°C and was 11°C and 13°C in each of the coolers upon arrival at the Western Fisheries Research Center.

Larvae were exposed to three different strains of virus: two strains of infectious hematopoietic necrosis virus (IHNV) representing the U and M genogroups of IHNV that occur in Washington and Columbia River Basin salmonids, and one strain of viral hemorrhagic septicemia virus (VHSV) genotype IVa that occurs in marine fish and salmonids off the Pacific

coast. Fish were exposed to virus by immersion at a moderate (2×10^3 PFU/mL) and high dose (2×10^6 PFU/mL, Figure 4). Each immersion treatment was done in triplicate 4-L challenge buckets with two buckets monitored for mortality. To determine presence of virus, regardless of mortality, a third tank was used for sampling fish at days 6 and 12 post-exposure. Some larvae were also exposed to virus by direct injection. A single tank of 10 individuals for each of the viruses was used. Virus was delivered via intraperitoneal injection (28 G tuberculin syringe) at a concentration of 2×10^4 PFU per fish. All experiments took place at 12° C. Larvae were examined daily for disease symptoms and mortality for 28 days. Examples of clinical disease symptoms may be loss of pigment or possible hemorrhages. To determine and quantify virus presence, dead fish were frozen and stored (-80°C) for later assay and titering. Sub-samples of fish were euthanized at day 6 and 12 post-exposure, shipped fresh on ice to the Lower Columbia Fish Health Center for assay and titering. Mock (i.e., control) treatments were also utilized to examine potential handling effects. Final analyses are ongoing and results will be forthcoming. Preliminary results indicate limited acquisition of any virus by the larvae, and their ability to rapidly clear any injected virus. Pacific lamprey may not be susceptible to or vectors of some common Pacific Northwest salmonid pathogens.

Findings and Recommendations

The initiation of Pacific lamprey captive rearing at ECNFH has the potential to provide many insights into the basic biology and ecology of this important species. The ability exists to successfully hold larval lamprey in captivity and we have identified vessels that show more promise than others. Minimization of screen attachment points, overtopping of vessels, and mesh size opening should minimize escapes. Facilities that prevent lamprey escape, provide protection from extreme climatic events (i.e., excessive summer heating, prolonged sub-zero

temperatures in winter), and allow experimental manipulation of environmental parameters would be beneficial. For example, the ability to vary thermal regimes may provide insight into how this fish may be affected by global climate change, thereby increasing our understanding of ecosystem effects.

Positive growth of larval lamprey in captivity is possible and this topic remains important and should continue to be pursued. We observed positive growth in a small number of surviving lamprey in the algae treatment. The large loss rate resulting from larval mortality and escape greatly hinders our ability to make useful inferences from the growth portion of this experiment. Further experimentation on feeding regimes is necessary. Recent experiments have identified that diet of ground leaves or fish flakes result in positive growth of larval lampreys (Shirakawa et al. 2009; Limm and Power 2011). Moore and Mallatt (1980) investigated size selection of feeding European brook lamprey *Lampetra planeri* and that particles in the 3-150 μm range may be most suitable to ingestion. Follow-up feeding studies should incorporate several aspects: 1) Improved holding vessels that minimize escapes and retain individually marked larvae, 2) A consideration of food particle size as appropriate and ingestible by larval lamprey, and 3) Additional response variables that capture some aspect of the nutrition or physiology of different feeding regimes (e.g., stomach content analysis, lipid analysis, stable isotope analysis of C and N). Professional fabrication of lamprey-specific chambers may be useful or repetition of the experiment using the circular tanks mentioned above may be beneficial.

Preliminary results indicate a low susceptibility of larval Pacific lamprey to three different viruses that occur in the Pacific Northwest. The less-derived, ancestral body form of lampreys show a remarkable lack of susceptibility to certain viruses and ability to clear these viruses rapidly. These results are encouraging and may allow much more flexibility in how

larval lamprey may be reared in a hatchery. For example, if quarantine requirements are lifted from lamprey at ECNFH, exploration of using egg tray stacks as rearing vessels may prove advantageous. Benefits may include tighter control of water flows and more secure vessels (minimizing escapes). The pathology of lamprey will continue to be an important consideration in a captive rearing facility. Information on lamprey susceptibility to pathogens is scarce (Bell and Traxler 1986) and further study is warranted. Understanding the basic biology of lampreys and potential virus resistance may prove valuable in conservation and recolonization efforts as many watersheds in the Pacific Northwest are known hotspots for pathogens (e.g., migratory stocks of salmonids known to carry viruses).

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