

U.S. Fish and Wildlife Service

Captive Rearing of Pacific Lamprey

FY 2012 Annual Report



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Columbia River Fisheries Program Office
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On the cover: Larval Pacific lamprey being sorted in preparation for transfer to captive rearing vessels at Eagle Creek National Fish Hatchery. Photo taken in July 2012 by Jeff Jolley.

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Abstract – Pacific lamprey *Entosphenus tridentatus* are declining in the Columbia River Basin. We used a group of captive larval Pacific lamprey at Eagle Creek National Fish Hatchery to investigate appropriate rearing vessels and feeding regimes for captively reared larvae. Round fiberglass tanks containing sediment were used to rear larvae and were a great improvement over previous designs; limited escapes and mortality have been observed. We evaluated four feed types (algae, leaves, yeast+larval fish food, and salmon analogs) on growth of larvae. Experiments are ongoing and potential growth will be evaluated. We investigated health of lamprey by screening two samples of wild-caught lamprey from the Clackamas River drainage. Larvae were relatively pathogen free although several types of bacteria were detected. Protocols for the establishment of wild-origin lamprey at captive facilities are needed to minimize risk to co-housed species.

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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 lampreys 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. Several critical uncertainties have been formalized regarding the basic life history and ecology of lampreys (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

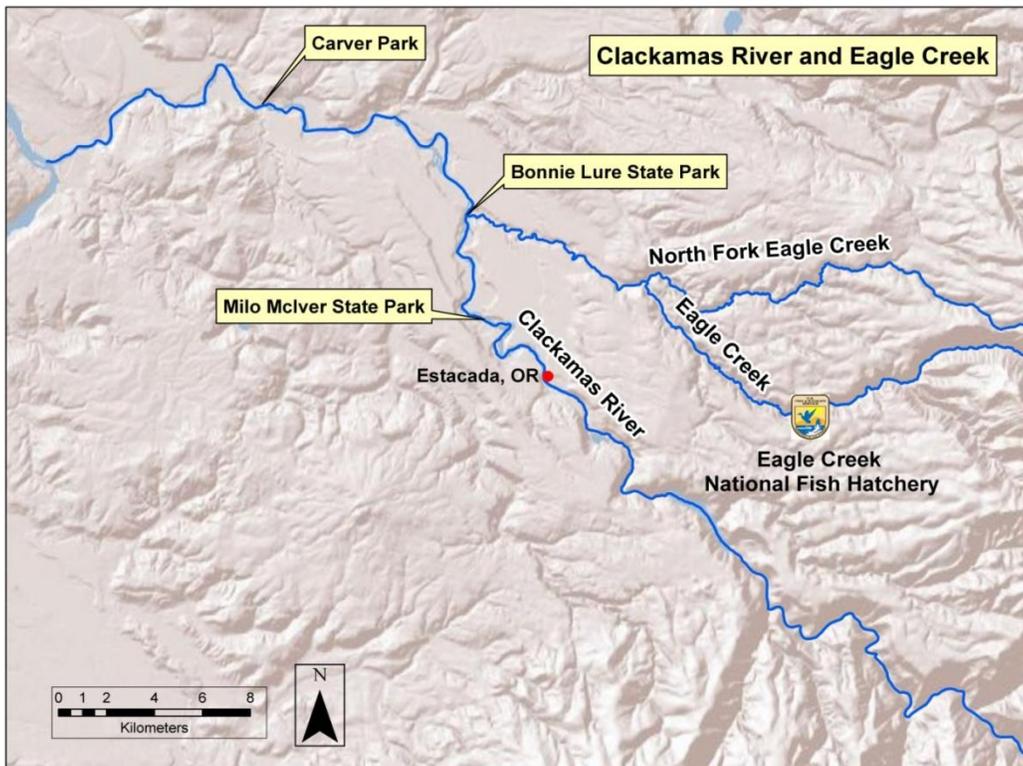


Figure 1. Study area in Eagle Creek and the Clackamas River.

demonstrated. Developing appropriate holding vessels that contain rearing habitat and feed that provides the required nutrition for the growth of robust individuals is necessary. In addition, animals transferred from the wild to captivity may be vectors of pathogens that may potentially infect a research or hatchery 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 the utility of various holding configurations, 2) investigate growth responses of different feeding regimes, and 3) test efficacy of quarantine and antibiotic treatments to treat Pacific lamprey larvae for common bacterial pathogens.

Methods and Results

Rearing configuration

Larval Pacific lampreys (n=160) were collected from the North Fork of Eagle Creek using an AbP-2 backpack electrofisher (ETS Electrofishing, Verona, WI) on 11 July 2011 (Figure 1). Larvae were transported to Eagle Creek National Fish Hatchery and housed in 20 rearing vessels. The vessels were circular fiberglass tanks (Figure 2; 34.3 cm diameter, 40.6 cm deep) with a standpipe (3.8 cm diameter), set at 27.9 cm of depth, which was screened for the



Figure 2. Fiberglass rearing vessels for larval Pacific lamprey at Eagle Creek National Fish Hatchery in 2012.

water outflow (Figures 2, 3). All vessels contained 5-7 cm of sand substrate. The substrate source was a spoil pile excavated from Gibbons Creek on Steigerwald National Wildlife Refuge (Clark County, WA). Substrate was screened to remove large gravel and sun-dried for 1 week. 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 (lower raceways) and supplied with Eagle Creek water in a flow-through system (Figure 4).

The captive holding configuration was chosen based on comparisons of different vessels in 2011, and progressed adaptively as aspects of the configuration were found to be inadequate (Jolley et al. 2012). In addition, because *Vibrio* spp. was isolated in a 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). Shade screens were used in summer to moderate temperatures, and reduce algal growth in the rearing vessels.



Figure 3. Overhead view of a rearing vessel for larval Pacific lamprey showing screened standpipe and substrate at Eagle Creek National Fish Hatchery in 2012.

when tanks overtop due to outflow obstructions. Larval lampreys seem opportunistic and adept at exploiting avenues of escape. 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. The fiberglass circular tank design has been a clear improvement over previous vessels (Jolley et al. 2012). Increasing the number of rearing vessels may be warranted.

Feeding experiments

Prior to initiation of a feeding experiment, the group of lampreys described above was inventoried, and baseline length and weight information was collected on 26 July 2012. Individual lampreys were anesthetized with tricaine methanesulfate (MS-222; 50 mg/L), measured (TL in mm) and weighed (wet weight in g), and given a unique visible

Evaluation of the holding vessels is ongoing. Initial escapes were high in the first week upon transfer from the wild (26/160, 16%). Adjustments in flow rate and water level (i.e., shortened stand pipes) were made that virtually eliminated further escapes. There were four escapes the following week (4/134, 3%), and subsequently no additional escape events detected through the end of the reporting period (30 September). Although larval lampreys periodically escaped from the vessels (3%), the rate of escape was dramatically reduced from the previous year (i.e., >70% loss rate; Jolley et al. 2012). Apparently, lampreys either squeeze through small gaps in outflow openings or escape

Table 1. Number and mean TL (mm) of Pacific lamprey larvae in each feeding trial at Eagle Creek

Tank	Treatment	Mean TL (mm)	Number
1	No food	90.6 (6.7)	7
2		83.4 (3.1)	7
3		91.6 (5)	7
4		86.0 (5)	6
5	Algae	82.6 (5.4)	7
6		84.3 (3.9)	7
7		83.6 (4.7)	7
8		89.3 (6.2)	6
9	Leaves	86.0 (6.0)	7
10		84.4 (7.1)	7
11		101.0 (1.2)	7
12		84.7 (4.2)	6
13	Salmon analog	85.4 (6.9)	7
14		88.4 (4.6)	7
15		87.4 (6.5)	7
16		96.0 (5.4)	6
17	Larval fish food/yeast	88.1 (6.8)	7
18		86.4 (3.6)	7
19		80.5 (4.8)	6
20		80.5 (3.4)	6

implant elastomer (VIE) tag (Silver et al. 2009; Table 1).

Due to presumed escapes and/or mortalities, there were 134 remaining larvae which were randomly assigned to each of five feeding regimes: 1) no food, 2) a combination of baker's yeast and larval fish food, 3) ground leaves, 4) algae wafers, and 5) salmon analogs. Each feeding treatment contained four replicates for a total of 20 experimental vessels (Table 1).

Commercially available algae wafers (Kyorin Food Industries, Ltd, Himeji, Japan) were ground and fed at a rate of 0.8 g per lamprey/week. Leaves were collected from common trees that occur in the riparian zone of Eagle Creek where lampreys occur (e.g., Betulaceae, Sapindaceae, and Salicaceae spp.). Leaves were dried in an oven at 100°C for 4 hours and then ground into a powder. The ground leaves were fed at a rate of 0.8 g per lamprey/week. This type of feeding regime was previously used by Shirakawa et al. (2009). A combination of commercially available baker's yeast and larval fish food (Gemma Wean, Bio-Oregon, Longview, WA) was fed at a 9:1 ratio of yeast: larval fish food at a rate of 0.8 g per lamprey/week (Polkinghorne et al. 2001; McGree et al. 2008). Salmon analog pellets (Bioanalog salmon custom diet, Bio-Oregon, Longview, WA) were ground and fed at a rate of 0.8 g per lamprey/week. Lampreys were fed once per week. Prior to feeding, the measured quantity of food for each rearing vessel was suspended in approximately 500 ml of water and allowed to soak. Suspensions of leaves and water and algae and water were soaked for approximately 24 h, while the more soluble treatments of salmon analog, and yeast and larvae fish food suspensions were soaked for approximately 10 to 15 minutes. Soaking presumably increased the chance that food will sink and therefore be available to filter feeding larval lamprey, rather than floating and flowing out of the container (Limm and Power 2011). Proximate analyses of food types are given in Table 2.

Lampreys were monitored weekly for mortalities on the sediment surface. All mortalities were individually identified and frozen. Detrital buildup and related algal and fungal growth were periodically skimmed from the sediment surface with a fine mesh aquarium net, as judged necessary to maintain reasonable water quality. Lampreys will be examined for potential growth after four months of the feeding trials, as well as at the end of the experiment.

Feed	Percent protein	Percent lipid	Percent ash	Percent moisture
Salmon analog	52.6	10.6	10.5	7.8
Yeast + larval fish food	64.4	15.3	8.4	6.2
Algae	30.7	3.7	4.6	7.5
Leaves	13.9	2.5	4.5	7.8

Table 2. Proximate composition of food types for larval lamprey at Eagle Creek National Fish Hatchery in 2012.

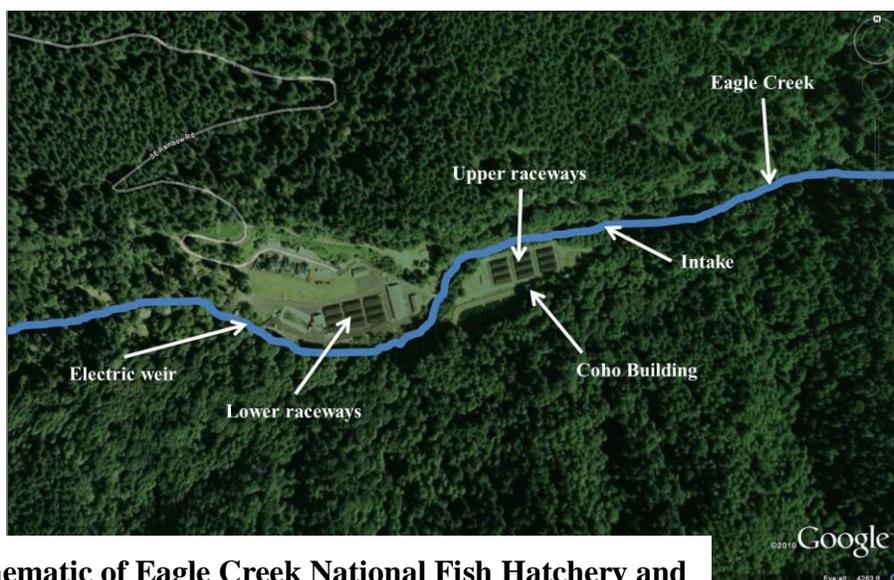
Stable Isotope Analyses

At the end of the feeding experiment we will analyze the carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotopes in larval lamprey muscle tissue to determine if larval lampreys are assimilating the different food types. This analysis may help determine whether any somatic growth in larvae during the experiment was a primary effect of the different types of feed, or was secondarily influenced by variations in growth and productivity of bacterial, algal and fungal communities in the vessels of the various feed groups. Additional nutrients are available sourced from Eagle Creek water and bacterial and microbial communities become established in the sediments of each tank as evidenced by the presence of macroinvertebrates. However, control

tanks may help separate growth effects from allochthonous inputs. The stable isotope ratios of C and N are increasingly being used to provide information about energy flow through aquatic food webs (Vander Zanden and Rasmussen 1999). We will determine baseline $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for each food type as well as a baseline values for muscle tissue from a sample of larval lamprey (N=10) from Eagle Creek. These results will provide baseline values for comparison and inference. At the conclusion of the feeding experiment, the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values from muscle tissue of each larva will be quantified and compared to the baseline larval values. The presence of the unique isotopic signatures from the different food types will provide evidence that the food has been assimilated into the larva. From this we can determine the relationship between food type and patterns of larval growth. The mean particle size of the different food items will be quantified and related to known information on size-selection and digestion efficiencies of larval lampreys (Moore and Mallatt 1980).

Health and disease screening

An additional group of larval Pacific lamprey were collected from the North Fork Eagle



Creek (n=30) and from Clear Creek near Carver Park (Clackamas River tributary, n=30) on 2 July 2012 (Figure 1). Fish were transported alive to the Lower Columbia Fish Health Center (Willard, WA) where they were euthanized using an overdose of MS-222 (750 mg/L), and placed on ice for health and pathogen screening (K. Lujan, LCFHC). Health screening results were

Figure 4. Schematic of Eagle Creek National Fish Hatchery and vicinity.

negative for a variety of common fish

pathogens, including infectious hematopoietic virus (IHNV), viral hemorrhagic septicemia (VHS), and *Aeromonas salmonicida* (furunculosis), all significant salmonid disease concerns. The bacteria *Vibrio vulnificus* was not detected in these lampreys, although samples from 2009 tested positive (Jolley et al. 2011, Table 3, 4). Several other bacteria were detected including *A. hydrophila* and *Salmonella ser pullorum*. A study is currently in development that will test the efficacy and feasibility of common fish antibiotics to treat bacterial infection of larval Pacific lamprey (e.g., *Vibrio* spp., *Aeromonas* spp.). Explicit protocols are needed that guide the establishment of wild-origin lamprey at captive facilities. Appropriate health screenings, quarantine, and antibiotic procedures need to be developed and implemented that minimize pathogen risk to other fish in the facility and the surrounding watershed.

Table 3. Results of fish health examination for Pacific lamprey larvae collected from North Fork Eagle Creek, 2 July 2012 by the Lower Columbia River Fish Health Center (K. Lujan, USFWS).

U.S. FISH & WILDLIFE SERVICE
 LOWER COLUMBIA RIVER FISH HEALTH CENTER
 201 Oklahoma Road
 Willard, WA 98605
 Phone: 509-538-2400
 Fax: 509-538-2404

FISH HEALTH REPORT 2012

FISH SOURCE			FISH EXAMINED
Location: North Fork Eagle Creek County: Clackamas Contact Person: Jeff Jolley Affiliation: USFWS Phone: (360) 604-2500			Species: Pacific lamprey Age: Ammocoetes CHN: W12-116 Number of fish: 30 Date Sampled: 7/02/2012
DISEASE AGENT ¹	SAMPLE SIZE	RESULTS	COMMENTS
IPNV	30	not detected	EPC and CHSE-214 cells
IHNV	30	not detected	EPC and CHSE-214 cells
VHS	30	not detected	EPC and CHSE-214 cells
SVCV	-	not tested	EPC and FHM cells
AS	30	not detected	BHIA medium
YR	30	not detected	BHIA medium
ESC	30	not detected	BHIA medium
BCD	30	not detected	TYES medium
CD	30	not detected	TYES medium
RS	-	not tested	ELISA
WD	-	not tested	Pepsin/Trypsin Digest
Comments	Virus (whole bodies) pooled in 3 fish pools. 8/30 fish (heart tissue) with growth on BHIA medium plates. No Vibrio found. Bacteria keyed out by API: <i>Pseudomonas aeruginosa</i> , <i>Pseudomonas fluorescens</i> , <i>Aeromonas hydrophila</i> , <i>Hafnia alvei</i> , and <i>Enterobacter cloacae</i> .		

¹IPNV Infectious Pancreatic Necrosis Virus, IHNV Infectious Hematopoietic Necrosis Virus, VHS Viral Hemorrhagic Septicemia Virus, SVCV Spring Viremia of Carp Virus, AS Furunculosis (*Aeromonas salmonicida*), YR Enteric Redmouth (*Yersinia ruckeri*), ESC Emphysematous Putrefactive Disease (*Edwardsiella ictaluri*), BCD Coldwater Disease (*Flavobacterium psychrophilum*), CD Columnaris (*Flavobacterium columnare*), RS BKD (*Renibacterium salmoninarum*), WD Whirling Disease (*Myxobolus cerebralis*), CS Salmonid Ceratomyxosis (*Ceratomyxa shasta*).

Table 4. Results of fish health examination for Pacific lamprey larvae collected from Clear Creek, 2 July 2012 by the Lower Columbia River Fish Health Center (K. Lujan, USFWS).

U.S. FISH & WILDLIFE SERVICE
 LOWER COLUMBIA RIVER FISH HEALTH CENTER
 201 Oklahoma Road
 Willard, WA 98605
 Phone: 509-538-2400
 Fax: 509-538-2404

FISH HEALTH REPORT 2012

FISH SOURCE			FISH EXAMINED
Location: Clear Creek (Carver State Park) County: Clackamas Contact Person: Jeff Jolley Affiliation: USFWS Phone: (360) 604-2500			Species: Pacific lamprey Age: Ammocoetes CHN: W12-115 Number of fish: 30 Date Sampled: 7/02/2012
DISEASE AGENT ¹	SAMPLE SIZE	RESULTS	COMMENTS
IPNV	30	not detected	EPC and CHSE-214 cells
IHNV	30	not detected	EPC and CHSE-214 cells
VHS	30	not detected	EPC and CHSE-214 cells
SVCV	-	not tested	EPC and FHM cells
AS	30	not detected	BHIA medium
YR	30	not detected	BHIA medium
ESC	30	not detected	BHIA medium
BCD	30	not detected	TYES medium
CD	30	not detected	TYES medium
RS	-	not tested	ELISA
WD	-	not tested	Pepsin/Trypsin Digest
Comments	Virus (whole bodies) pooled in 3 fish pools. 5/30 fish (heart tissue) with growth on BHIA medium plates. No Vibrio found. Bacteria keyed out by API: <i>Pantoea</i> spp3, <i>Onchrobactrum anthropi</i> , <i>Aeromonas hydrophila</i> , and <i>Salmonella ser pullorum</i> .		

¹ IPNV Infectious Pancreatic Necrosis Virus, IHNV Infectious Hematopoietic Necrosis Virus, VHS Viral Hemorrhagic Septicemia Virus, SVCV Spring Viremia of Carp Virus, AS Furunculosis (*Aeromonas salmonicida*), YR Enteric Redmouth (*Yersinia ruckeri*), ESC Emphysematous Putrefactive Disease (*Edwardsiella ictaluri*), BCD Coldwater Disease (*Flavobacterium psychrophilum*), CD Columnaris (*Flavobacterium columnare*), RS BKD (*Renibacterium salmoninarum*), WD Whirling Disease (*Myxobolus cerebralis*), CS Salmonid Ceratomyxosis (*Ceratomyxa shasta*).

Relationship to the Fisheries Program Strategic Plan

Implementation of this project demonstrates application of the Pacific Region's 2009-2013 Fisheries Program Strategic Plan. The following National goals (NG) and Regional objectives (RO) have been addressed by this project:

- NG1 Open, interactive communication between the Fisheries Program and its partners.
 - RO1.1 Develop and maintain relationships with partners throughout the Pacific Region. *This project has been a collaborative effort between the CRFPO, ECNFH, and LCFHC.*

- NG3 Self-sustaining populations of native fish and other aquatic resources that maintain species diversity, provide recreational opportunities for the American public, and meet the needs of tribal communities.
 - RO3.3 Support the research and fish culture needed to prevent listing or to recover native species listed or proposed for listing under ESA. *Results from this work will help inform conservation of Pacific lamprey.*

- NG8 Assistance is provided to Tribes that results in the management, protection, and conservation of their treaty-reserved or statutorily defined trust natural resources, which help Tribes develop their own capabilities.
 - RO8.1 Recognize and promote the Service's distinct obligations toward Tribes. *This work will aid in the conservation of Pacific lamprey, an important species to Native American tribes.*

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