

**U.S. Fish and Wildlife Service  
Columbia River Fisheries Program Office**

# **Larval Pacific Lamprey Feeding and Growth in a Captive Environment**

*FY 2014 Annual Report*

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***On the cover:*** Larval Pacific lamprey to be assigned an experimental treatment. Photo taken in January 2014 by Christina Uh.

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# LARVAL PACIFIC LAMPREY FEEDING AND GROWTH IN A CAPTIVE ENVIRONMENT 2014 ANNUAL REPORT

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LARVAL PACIFIC LAMPREY FEEDING AND GROWTH IN A CAPTIVE  
ENVIRONMENT  
2014 ANNUAL REPORT

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*Abstract* – Pacific lamprey *Entosphenus tridentatus* are declining in the Columbia River Basin in addition to much of their broader range. To mitigate for reductions in abundance, strategies such as hatchery propagation and captive rearing of larvae are currently being considered. We conducted a series of experiments using captive larval Pacific lamprey at Eagle Creek National Fish Hatchery to investigate the effect of different feeding regimes, including different food types and different food concentrations, on growth of larvae. In our first experiment, we evaluated the growth of larvae given four different food types (algae, leaves, yeast/larval fish food, salmon carcass analogs) and a control group that was not fed. Assimilation of food types was evaluated by tracking unique stable isotope signatures of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  within larval tissues. Results from this experiment indicated that a diet of salmon carcass analog led to positive growth rate. Analyses of stable isotopes showed unique signatures specific to each food type. Isotopic analyses of larval tissues showed signatures that matched those of their specific food treatment. Based on the results of experiment one, we conducted a second experiment to evaluate growth of larvae fed four different quantities of salmon carcass analog. Results from this experiment indicate larval growth rate increased with increasing dosages of food, but positive growth was observed in all feeding treatments. We investigated health of Pacific lamprey by screening a sample of wild-caught larvae from Eagle Creek for common viral and bacterial fish pathogens. Larvae were relatively pathogen free. This work may provide information useful in the development of protocols for establishing wild-origin lamprey at captive facilities that likewise minimizes risk to co-housed species. We successfully reared larval lamprey in captivity with minimal mortality and positive growth.

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# Feeding and Growth of Larval Pacific Lamprey Feeding and Growth in a Captive-Reared Environment

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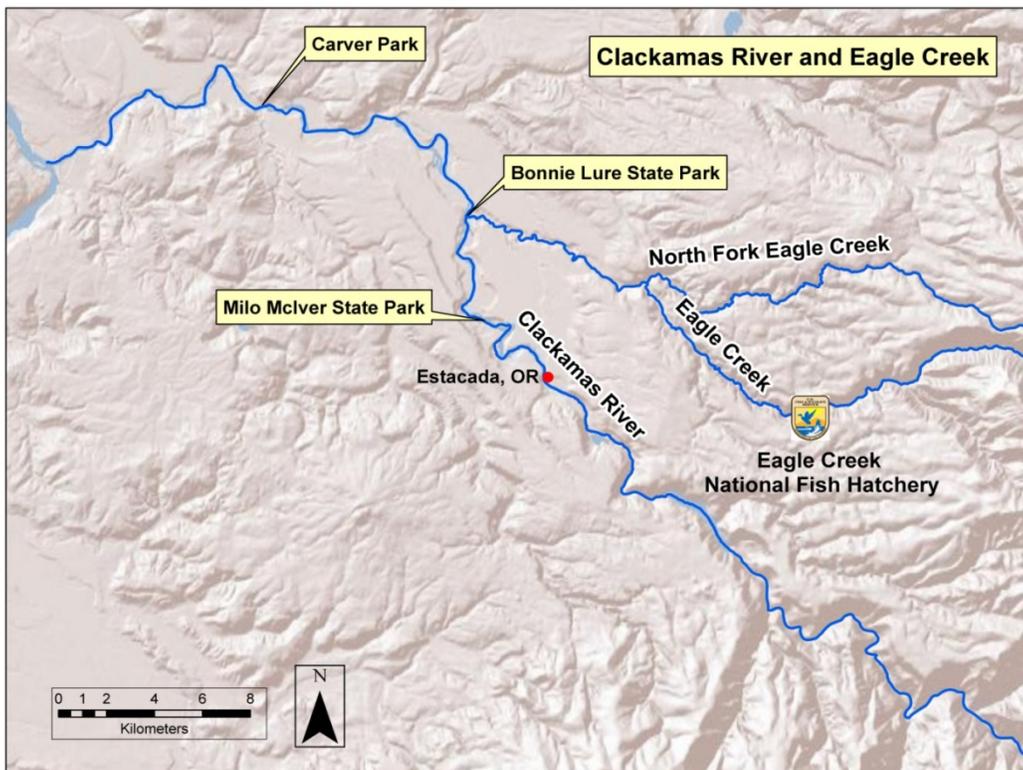
## Introduction

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 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 are cryptic and not readily observed in their natural state, living 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 approximately July to December (McGree et al. 2008) and migrate to the Pacific Ocean where they spend 1 to 3 years before returning to their freshwater spawning grounds to spawn and die.

Lamprey larvae feed by filtering organic material and detritus that is captured from the interstitial water within their burrows (Moore and Mallatt 1980; Sutton and Bowen 1994; Mundahl et al. 2005). The material is entrapped by feeding mucus which fills the pharynx (Moore and Mallatt 1980). Although larval lampreys have been noted to have a high assimilation efficiency of detrital foods (Sutton and Bowen 1994; Mundahl et al. 2005), they are known to successfully remove only a small portion of suspended food particles passing within the current (Mallatt 1983). In captivity, larvae have been fed baker's yeast (Mallatt 1983; Murdoch et al. 1992; Yap and Bowen 2003; Rodriguez-Munoz et al. 2003), larval fish food (Mallatt 1983), ground leaves (Shirakawa et al. 2009), and freshwater algae (Shirakawa et al. 2009). Lamprey larvae possess an unspecialized digestive tract composed of an esophagus, and intestines that are minutely differentiated from the rest of its digestive system (Sutton and Bowen 1994). Their ability to efficiently assimilate detritus is thought to be due to a digestive period that is longer than what is observed in other fish species (Sutton and Bowen 1994).

# Feeding and Growth of Larval Pacific Lamprey Feeding and Growth in a Captive-Reared Environment

Several critical uncertainties have been formalized regarding the basic life history and ecology of lampreys (Luzier et al. 2011). Many of the uncertainties regarding basic biology and ecology of lamprey may be addressed by observation and experimentation using captive animals. Increased knowledge of the biology, population dynamics, ecology, and identification of Pacific lamprey will help managers understand and conserve these important species. The development of Conservation Hatchery programs has been explicitly recommended by several guiding documents from the U.S. Fish and Wildlife Service Pacific Region, including the Pacific Lamprey Assessment and Template for Conservation Measures as a means for providing experimental animals or supplementing depressed natural populations (Luzier et al. 2011). The unique life history of the lamprey poses challenges to captive rearing that are uncommon in



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

traditional finfish culture. Although larval Pacific lamprey have been successfully held for experimentation (Silver et al. 2009; Kurath et al. 2013) and have metamorphosed in captivity (McGree et al. 2008), explicit information on appropriate holding configurations, rearing habitats or substrates, as well as food types and quantities needed for growth of robust individuals in captivity is not known. Thus methods need to be developed for the maintenance and growth of lamprey ammocoetes in captive reared environments.

Traditional feeding and diet studies have employed a number of techniques including gut content analysis (Bowen 1996) and bioenergetics (Harvey et al. 2002). These techniques pose challenges in the ability to identify and quantify homogenous materials in gut contents, thereby

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introducing a high level of uncertainty. The analysis of stable isotopic signatures has progressed as an effective tool for trophic ecologists to examine feeding in organisms and is increasingly being used to provide information about energy flow through aquatic food webs (Vander Zanden and Rasmussen 1999). This technique has many advantages including determination of trophic level, confirmation of food assimilation by tracking unique isotopic signatures (Schlechtriem et al. 2004), and integrating the temporal relationship between food items and consumers (DeNiro and Epstein 1978; Petersen and Fry 1987; Vander Zanden and Rasmussen 2001). Although the technique has only minimally been applied to lampreys (Hollett 1995; Limm and Power 2011; Evans 2012) it has been used successfully in diet validation studies of fish (e.g., rainbow smelt *Osmerus mordax*, Harvey et al. 2002). As such, there is much promise for studies of larval lamprey feeding ecology.

An additional challenge to rearing wild animals in captivity is the possibility of being, vectors of pathogens that may potentially infect a research or hatchery facility. To our knowledge, there are only two published reports of an experimental challenge study with this fish species, showing that lamprey do not appear to be susceptible to the pathogenic agent of bacterial kidney disease (Bell and Traxler 1986) or select rhabdoviruses of the Pacific Northwest (Kurath et al. 2013). With regard to known pathogens of Pacific Northwest fish, the rhabdovirus infectious hematopoietic necrosis virus (IHNV) is the most economically significant viral pathogen causing disease in Pacific salmon hatcheries and trout farms (Wolf 1988; Bootland and Leong 1999). Bacterial infections (e.g. *Aeromonas salmonicida*, the causative agent of furunculosis) may also be of concern (Faisal 2007). Aside from extensive experimentation on lamprey pathology, standard fish pathogen assessments can also reduce risk by screening representative samples of fish before introduction into a captive facility (U.S. Fish and Wildlife Service 2004).

To further the development of explicit protocols for rearing larval lamprey in captivity, we conducted a series of experiments to address the following objectives: 1) evaluate larval growth from four different food types (experiment 1), 2) evaluate larval growth fed different dosages of salmon analog (experiment 2), 3) evaluate assimilation of food types into fish tissue, and 4) conduct screening of larvae for standard fish pathogens.

# Feeding and Growth of Larval Pacific Lamprey Feeding and Growth in a Captive-Reared Environment

## Methods and Results

### *Experimental rearing configuration*

Larval lampreys were reared at Eagle Creek National Fish Hatchery (ECNFH; Clackamas County, Oregon). Eagle Creek is a tributary of the Clackamas River. The experimental rearing configuration consisted of circular fiberglass tanks (34.3 cm diameter, 40.6 cm deep) with screened center standpipes (3.8 cm diameter). Each tank was filled with 5 to 7 cm of sand substrate. Water depth in each tank was about 27.9 cm (Figure 2). 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 at a target flow rate of approximately 2 L/min (Figure 3). Water temperature was not regulated and reflected the natural temperature regime of Eagle Creek. A HOBO tidbit temperature logger recorded water temperature at hourly intervals over the course of the



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

experiments (Figure 4). The experimental configuration was consistent for experiment one and two (described below) and was chosen based on comparisons of different vessels in 2011 (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 Eagle Creek National Fish Hatchery were isolated within the hatchery so that their effluent did not contact other areas of the hatchery as a precautionary approach. Shade screens were used in the summer to moderate temperatures, and reduce algal growth in the rearing vessels. Insulated tarps were utilized during winter months to help prevent freezing and maintain flowing water to all tanks.

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

2009; Table 1). Samples of sediment were collected from experimental rearing vessels at the beginning and end of the feeding trials. Organic content of the sediment was determined using loss-on-ignition methods (Table 2; Heiri et al. 2001).

There were 134 remaining larvae (6% mortality rate) assigned to five feeding regimes, four tanks per regime: 1) no food (control), 2) algae wafers, 3) ground leaves, 4) salmon carcass analogs, and 5) a combination of baker's yeast and larval fish food (Gemma wean), (Table 1). Initial lengths ranged from 59 to 120 mm. Initial wet weight ranged from 0.35g to 2.6 g.

Commercially available algae wafers (Kyorin Food Industries, Ltd, Himeji, Japan) were ground and fed at a rate of 0.8 g/lamprey/week. Leaves were collected from common trees that occur in the riparian zone of Eagle Creek (e.g., *Betulaceae*, *Sapindaceae*, and *Salicaceae* spp.). Leaves were dried in an oven at 100°C for 4 hours and then ground into a

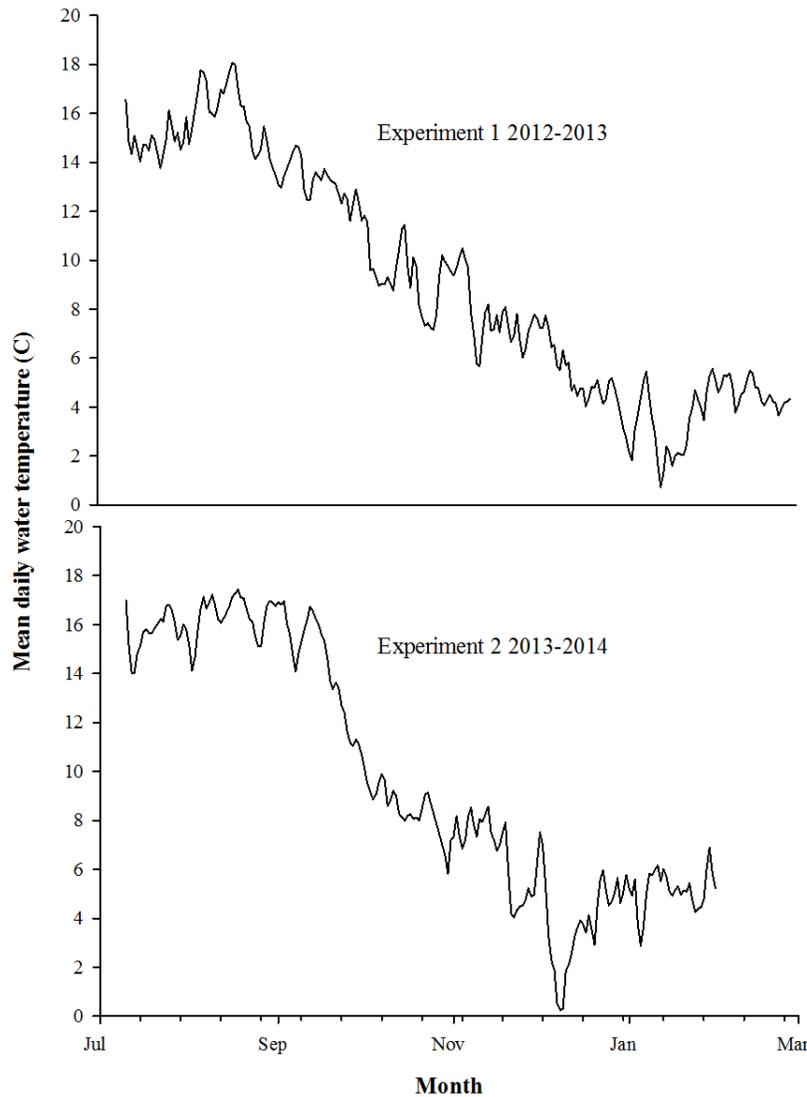
### *Feeding experiment one*

Larval Pacific lampreys (n=160) were collected from North Fork Eagle Creek (near the confluence with Eagle Creek) using an AbP-2 backpack electrofisher (ETS Electrofishing, Verona, WI) on 11 July 2012. Larvae were transported to ECNFH and haphazardly distributed among 20 rearing vessels at a target density of 6.8 fish/m<sup>2</sup>. Prior to the initiation of the experiment, individual lampreys were anesthetized with buffered tricaine methanesulfate (MS-222; 150 mg/L), measured (TL in mm), weighed (wet weight in g), and given a unique visible implant elastomer (VIE) tag (Silver et al.

**Table 1. Number and mean TL (mm) of Pacific lamprey larvae in each feeding trial at Eagle Creek National Fish Hatchery in 2012 (experiment one). Standard errors are in parentheses.**

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

# Feeding and Growth of Larval Pacific Lamprey Feeding and Growth in a Captive-Reared Environment



**Figure 4. Water temperature regime for larval Pacific lamprey feeding experiments in 2012-2013 and 2013-2014.**

Mallatt (1980) report that particles 100-300  $\mu\text{m}$  in size are most readily trapped and absorbed by the mucous cord of the feeding apparatus of larval Eastern brook lamprey *Lampetra planeri*, but there was no size-based selection of particles within the 5-340  $\mu\text{m}$  range. All of the food items in our study fell within this range.

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). Salmon carcass analog pellets (Bioanalog salmon custom diet, Bio-Oregon, Longview, WA) were ground and fed at a rate of 0.8 g per lamprey/week. 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). The mean particle size of the different food items was quantified by measuring three replicates of 100 food particles along the longest axis under a dissecting microscope. Larval fish food particles were the largest (mean = 384  $\mu\text{m}$ ) while yeast particles were the smallest (mean = 58  $\mu\text{m}$ ; Table 3). Moore and

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**Table 2. Sediment organic content of feeding treatments. Standard errors are in parentheses.**

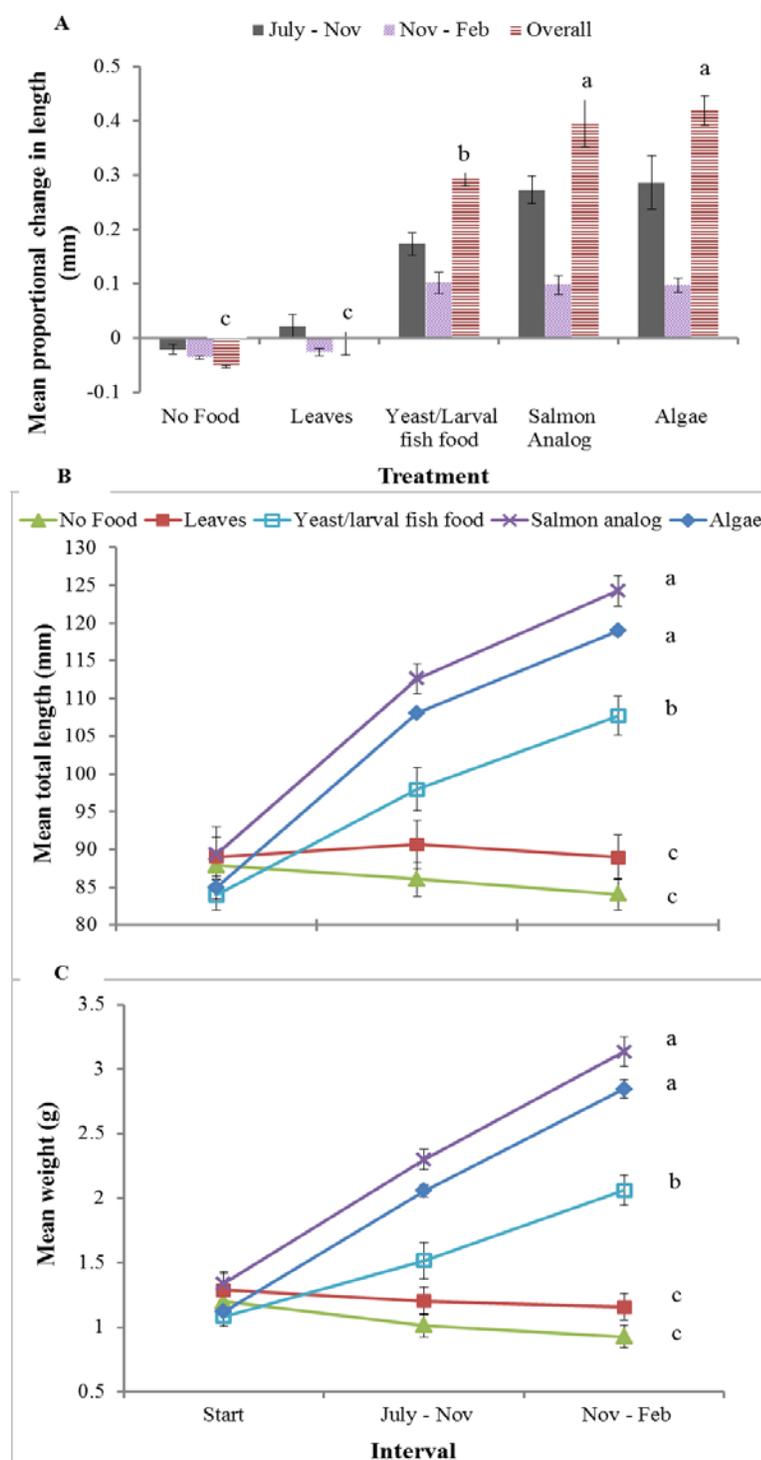
	Treatment	Mean percent organic content	
Experiment 1	No food	1.53 (0.04)	
	Algae	1.81 (0.10)	
	Leaves	2.29 (0.19)	
	Salmon analog	2.34 (0.40)	
	Yeast/larval food	1.78 (0.05)	
	Baseline	1.76 (0.20)	
Experiment 2	No food	0.56 (0.02)	
	Salmon carcass analog	0.2 g/wk	0.79 (0.04)
		0.4 g/wk	0.64 (0.06)
		0.8 g/wk	1.08 (0.21)
		0.8 g/2 wk	0.82 (0.05)
		Baseline	0.48 (0.06)

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 algae were soaked for approximately 24 h, while more soluble salmon analog and yeast and larvae fish food treatments were soaked for approximately 10 minutes. Soaking

presumably increased the chance that food will sink and therefore be available to larvae, rather than floating and flowing out of the container (Limm and Power 2011). Water flow was halted prior to feeding and remained off for approximately 4-6 h after feeding to further reduce loss of food. Larvae were monitored weekly for mortalities on the sediment surface, and 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 were examined for growth at three month intervals during the experiment, and again at the end. At the conclusion of experiment one, larvae were sacrificed and frozen for later isotopic analyses (Objective 3).

Survival was high in experiment one. Of the original 134 larvae, 128 (94%) survived the duration of the study. We compared total length, proportional change in length, weight, and growth rates using ANOVA and Tukey's mean separation ( $\alpha = 0.05$ ) to examine potential differences among food types. There were no differences in mean total length among the treatment groups at the initiation of experiment one (ANOVA,  $P=0.46$ ,  $F=0.97$ ,  $df=4$ ; Figures 5B). Mean proportional change in length varied from  $-0.05$ - $0.00$  (no food and leaves) to  $0.42$  (algae). Algae and salmon carcass analog diets produced the highest proportional change in length, yeast/larval fish food diet was intermediate, and leaves and no food diet was the lowest and negative ( $P<0.01$ ,  $F=283.37$ ,  $df=4$ ; Figure 5A). Mean TL at the conclusion of the experiment ranged from 84 mm (no food) to 124 mm (salmon carcass analogs) and differed among treatments ( $P<0.01$ ,  $F=65.73$ ,  $df=4$ ; Figure 5B). Salmon carcass analog and algae diets produced the longest larvae, the yeast/larval fish food diet produced intermediate size, and leaves and no food diets produced the shortest larvae. Differences in mean weight mirrored differences in mean TL for their corresponding treatments ( $P<0.01$ ,  $F=95.70$ ,  $df=4$ ; Figure 5C).

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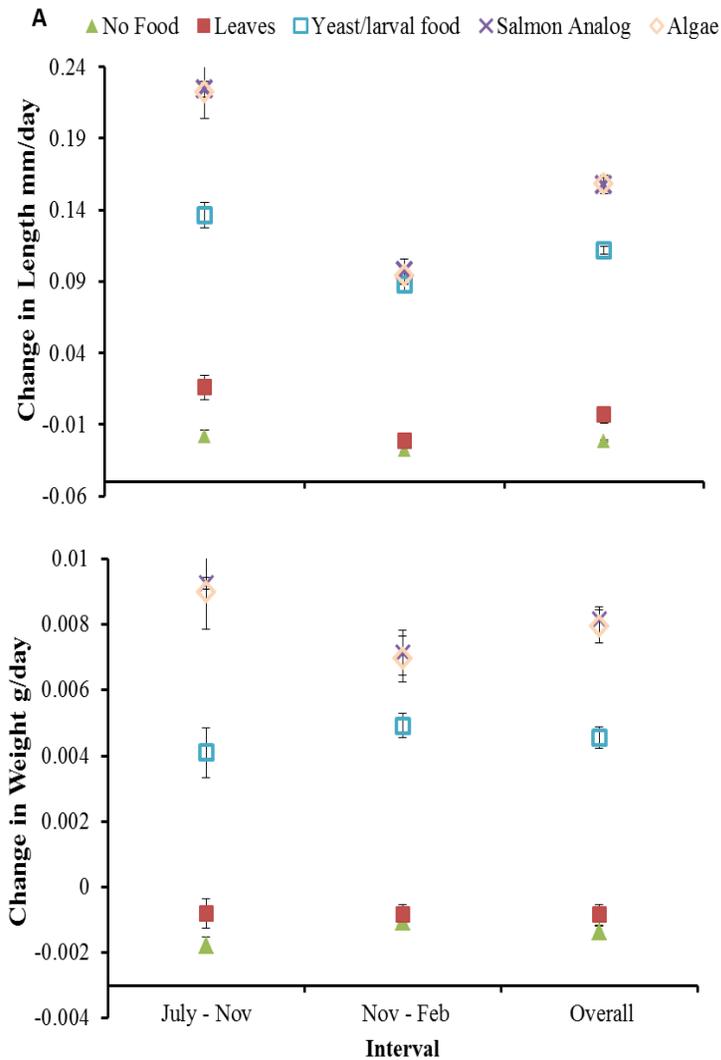


**Figure 5. Proportional change in length (panel A), total length (panel B), and weight (panel C), and standard errors, by time interval and treatment of larval Pacific lamprey at ECNFH, 2012-2013. Lowercase letters represent statistical differences for the overall time period.**

Overall growth rates in length (mean change in length/day) ranged from  $\sim 0.02$  mm/d (no food) to 0.16 mm/d (algae and salmon carcass analog; Figure 6). Overall growth rates in weight (mean change in weight/day) ranged from  $\sim 0.001$  g/d (no food) to 0.01 g/d (algae and salmon carcass analog). Differences in growth rates mirrored those mentioned above for both increases in length (ANOVA;  $P < 0.01$ ,  $F = 381.38$ ,  $df = 4$ ) and increases in weight ( $P < 0.01$ ,  $F = 156.26$ ,  $df = 4$ ). Growth rates were mostly greater during the first interval than in the second (Figure 6). Growth rate in weight decreased in the second interval for larvae fed algae and salmon carcass analogs.

Proximate analysis of one 15 mL sample of each food type was determined using standard methods. Protein content of all food types were determined by the Dumas Method (Method 968.06; AOAC, 2000). Lipid content was determined for salmon analogs and larval fish food using an acid hydrolysis method (Method 954.02; AOAC, 2000), and for leaves and algae using the Randall Method (Method 2003.05; AOAC, 2000). Moisture content was determined through a loss on drying method (Method 930.15; AOAC, 2000). Ash content was determined by measuring the mass of a sample before and after it was heated in a muffle furnace (Method 942.05; AOAC, 2000). Proximate analyses of food types are given in Table 3. Caloric content of food types was quantified using a Model 1341 plain

# Feeding and Growth of Larval Pacific Lamprey Feeding and Growth in a Captive-Reared Environment



**Figure 6. Mean growth rates in length and weight for larval Pacific lamprey in different feed treatments (Experiment 1).**

jacket oxygen bomb calorimeter (Parr Instrument Company, Moline, Illinois). A minimum of four replicates of each food type were analyzed for caloric content in the calorimeter according to manufacturer instructions (Parr 2008). Powdered food types were compressed into pellets (0.8 g) prior to analysis. Caloric values were then incorporated into feed rates to determine the relative energy density for each feeding trial.

Food types were different in proximate composition as well as caloric content. Salmon carcass analogs were generally most energetically dense, having the highest protein content, and the second highest lipid content of all food types. Leaves were most energetically poor, with the lowest protein and lipid content of all food types. We compared mean caloric content of food items using ANOVA and Tukey's mean separation ( $\alpha = 0.05$ ). Mean caloric content of food items ranged from 4.7 kcal/g (salmon carcass analog) to 2.9 kcal/g (leaves; Table 3). Salmon carcass

analog had the highest caloric value followed by larval fish food, yeast and algae, and leaves (ANOVA,  $P < 0.01$ ,  $df = 4$ ,  $F = 95$ ). When adjusted for feeding rates, lamprey fed salmon analog pellets had the most calories available per feeding, (3.7 kcal/lamprey) while those fed leaves had the least amount of calories available (2.3 kcal/lamprey; Table 3). To standardize caloric content to that of the salmon carcass analogs, feeding rates of other food types would need to be increased (Table 3). Organic content at the end of experiment two within the sediment of each treatment was variable and ranged from 1.5% (no food) to 2.4% (salmon carcass analog). Interestingly, organic content was lower in the non-fed treatment (1.5%) than in clean sediment used prior to the study (1.8%; Table 2). Sample sizes were inadequate to statistically compare

# Feeding and Growth of Larval Pacific Lamprey Feeding and Growth in a Captive-Reared Environment

**Table 4. Proximate composition, particle size, caloric content, and feeding rates for larval Pacific lamprey (Experiment 1). Sample size and standard errors are in parentheses.**

Treatment	% protein	% lipid	% ash	% moisture	Mean food particle size (µm)	Mean caloric content (kcal/g)	Feeding rate (g/fish)	Calories (kcal/fish)	Standardized feed	
									rate (g/fish) to carcass analog	Feed rate (g/8 fish)
Algae	30.7	3.7	4.6	7.5	147 (3, 11)	3.68 (5, 0.05)	0.80	2.9	1.0	8.3
Leaves	13.9	2.5	4.5	7.8	283 (3, 29)	2.90 (4, 0.14)	0.80	2.3	1.3	10.5
Carcass analog	52.6	11	11	7.8	254 (3, 27)	4.68 (4, 0.01)	0.80	3.7	0.8	6.5
Yeast/larval food	64.4	15	8.4	6.2	58 (3, 7) <sup>1</sup>	3.85 (5, 0.02)	0.72	2.8	1.0	7.9
					384 (3, 35) <sup>2</sup>	4.16 (4, 0.02)	0.08	0.3	0.9	7.3

<sup>1</sup>Yeast

<sup>2</sup>Larval food

organic content among treatments in experiment one. In experiment two, mean organic content at the end of the experiment ranged from 0.6% (no food treatment) to 1.1% (high food treatment) and was initially 0.5% at the onset of the experiment. Organic content was significantly higher in the high food treatment than it was in the unfed treatment (ANOVA;  $P=0.03$ ,  $df=4$ ,  $F=3.75$ ) at the end of the experiment.

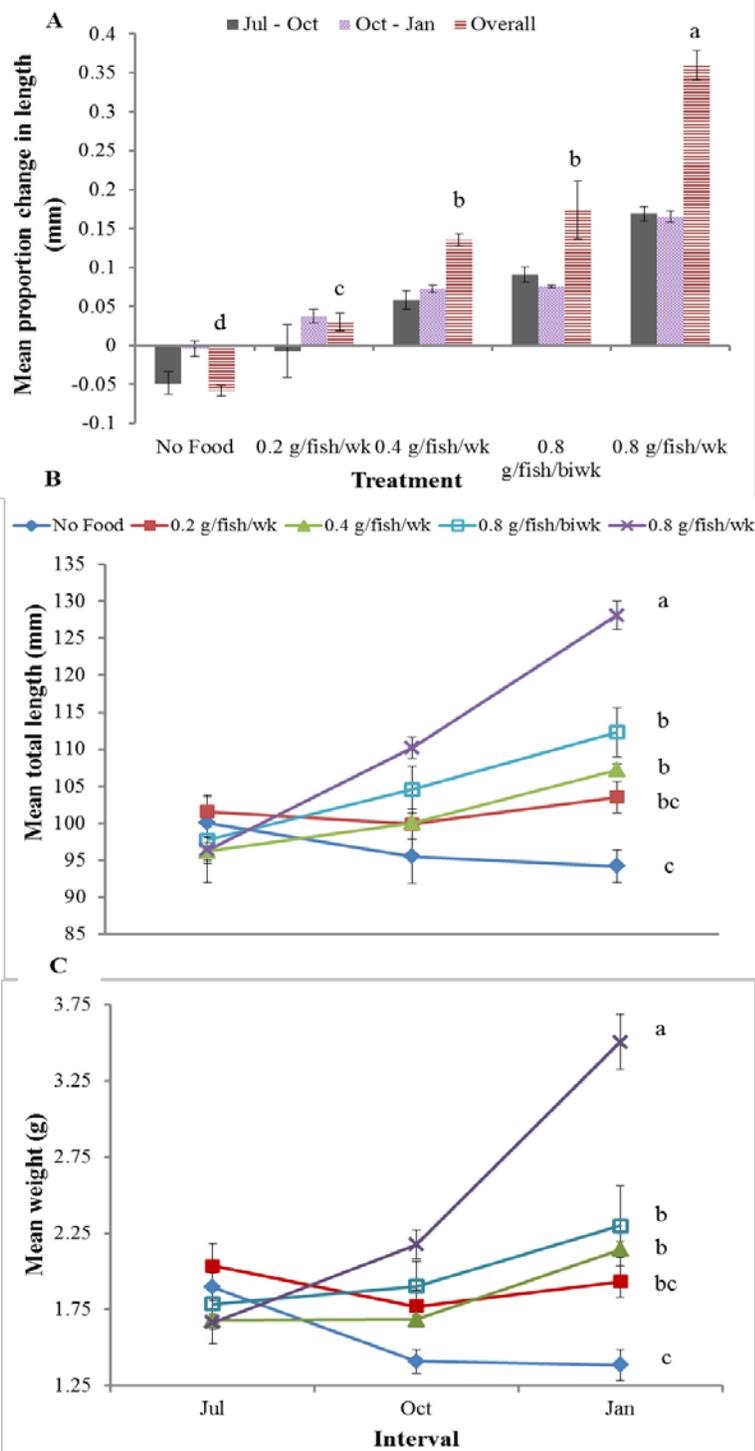
### *Feeding experiment two*

Larval Pacific lampreys (n=160) were collected from the North Fork of Eagle Creek (near the confluence with Eagle Creek) using an AbP-2 backpack electrofisher (ETS Electrofishing, Verona, WI) on 7 July 2013, and transported to Eagle Creek National Fish Hatchery, where they were housed in 20 different rearing vessels at a target density of 6.8 fish/m<sup>2</sup>. Results from experiment one indicated lamprey growth was maximized when fed a weekly dose of salmon analog. Based on this observation, experiment two focused on evaluating the dose response of larval growth in relation to salmon carcass analog food dosages, 1) no food (control), 2) 0.2g/individual, 3) 0.4g/individual, 4) 0.8g/individual, weekly, and 5) 0.8g/individual/biweekly; (Table 4). A biweekly feeding regime was chosen to investigate the practicality of reduced personnel time and effort for feeding. Each treatment had 4 replicates, with each tank initially containing 8 larvae, for a total of 160 individuals. Initial lengths ranged from 59 to 145 mm. Initial wet weight ranged

**Table 3. Number and mean TL (mm) of Pacific lamprey larvae in each feeding trial at Eagle Creek National Fish Hatchery in 2013. Standard errors are in parentheses.**

Tank	Treatment	Mean TL (mm)	Number
1	No food	99.3 (4.6)	8
2		104.8 (6.8)	8
3		101.5 (5.6)	8
4		94.9 (6.7)	8
5	0.2 g/fish/wk	105.8 (6.0)	8
6		100.4 (8.6)	8
7		104.3 (8.1)	8
8		96.1 (8.5)	8
9	0.4g/fish/wk	96.9 (7.2)	8
10		98.6 (8.3)	8
11		93.1 (6.4)	8
12		96.3 (8.3)	8
13	0.8g/fish/wk	93.9 (6.3)	8
14		101 (5.4)	8
15		97.5 (8.1)	8
16		93.3 (9.5)	8
17	0.8g/fish/biwk	94 (5.3)	8
18		114.1 (7.7)	8
19		96.4 (9.4)	8
20		86.6 (6.8)	8

# Feeding and Growth of Larval Pacific Lamprey Feeding and Growth in a Captive-Reared Environment

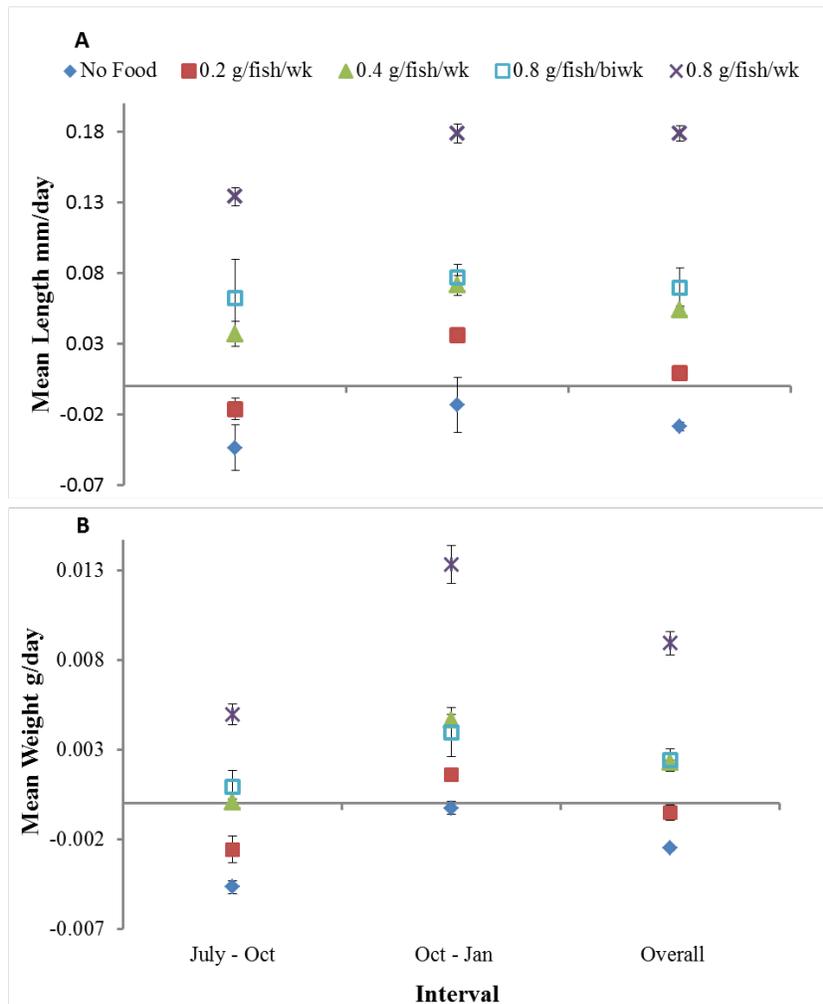


**Figure 7. Proportional change in length (panel A), total length (panel B), and weight (panel C), and standard errors, by time interval and treatment of larval Pacific lamprey at ECNFH, 2013-2014. Lowercase letters represent statistical differences for the overall time period.**

from 0.4g to 4.6 g. As in experiment 1, salmon analog pellets were ground, allowed to soak for approximately 10 minutes, and delivered as a food suspension to each rearing vessel. Water flow was halted prior to feeding, and remained off for approximately 4-6 h afterwards. Feeding, rearing, and maintenance of larvae were generally consistent with the methods described for experiment 1 above. Lampreys were examined for growth at three month intervals during the feeding trials, and again at the end of the experiment.

Survival of larvae was high throughout the study. Of the original 160 larvae 157 were remaining on 31 January, 2013 (98% survival). Statistical comparisons were conducted as outlined in experiment 1. There were no differences in mean TL among the treatment groups at the initiation of experiment one (ANOVA,  $P=0.68$ ,  $F=0.59$ ,  $df=4$ ). Mean proportional change in length varied from 0.06 (no food) to 0.36 (high food treatment). In general, higher amounts of food per individual larvae produced higher growth in both length and weight (Figure 7B, 7C). The high food treatment (0.8 g/week) produced the largest proportional change in length followed by the moderate (0.4 g/week) and bi-weekly food treatment, the low food treatment (0.2g/week), and lastly, the no food treatment which was

# Feeding and Growth of Larval Pacific Lamprey Feeding and Growth in a Captive-Reared Environment



**Figure 8. Mean growth rates in length and weight for larval Pacific lamprey in different feed treatments (Experiment 2).**

Overall growth rates in weight (g/day) ranged from  $-0.005$  g/d (no food) to  $0.013$  g/d (0.8g/fish/wk; Figure 8). The most change in growth in both length and weight occurred during the months of October – January.

Growth of larvae fed 0.8 g bi-weekly was similar to those fed 0.4 g weekly, suggesting a lower feeding frequency still results in positive growth. In general, growth rates were higher in the second interval (October to January) compared to the first (July to October; Figure 8).

### Stable Isotope Analyses

To assess whether the given food items were being assimilated by larvae, we examined stable carbon ( $\delta^{13}\text{C}$ ) and stable nitrogen ( $\delta^{15}\text{N}$ ) isotope ratios of a baseline sample of larval lamprey collected from the North Fork Eagle Creek, each food type, and individual larvae muscle tissue samples at the end of feeding experiment one. Samples were freeze-dried and ground. Carbon and nitrogen content and stable isotopes were measured with an elemental

negative ( $P < 0.01$ ,  $F = 64.32$ ,  $df = 4$ ; Figure 7A). Mean TL of larvae increased in all food dosages and ranged from 94mm (no food) to 128 mm TL (high food) and differed among treatments. The high food treatment produced the longest fish followed by the moderate food and bi-weekly food treatment, and the low and no food treatment producing the shortest fish ( $P < 0.01$ ,  $F = 31.59$ ,  $df = 4$ ; Figure 7B). Mean TL in larval lamprey increased in all food dosages where food was given, and the more food administered per individual lamprey, the more growth was observed; both in terms of length and weight (Figure 7B, 7C). Changes in mean total weight in lamprey closely resembled those in mean TL ( $P < 0.01$ ,  $F = 24.26$ ,  $df = 4$ ; Figure 7C). Overall growth rates in length (mm/day) ranged from  $-0.05$  mm/d (no food) to  $0.18$  mm/d (0.8g/fish/wk; Figure 8).

# Feeding and Growth of Larval Pacific Lamprey Feeding and Growth in a Captive-Reared Environment

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analyzer coupled with a continuous flow isotope ratio mass spectrometer (Delta PlusXP, ThermoFinnigan, Bremen; Brenna et al. 1997; Qi et al. 2003). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values reported are in reference to Pee Dee Belemite (PDB) and Vienna-PDB and atmospheric nitrogen standards, respectively. We compared mean %C, %N,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  using ANOVA and Tukey's mean separation ( $\alpha = 0.05$ ) to examine potential differences among food types, between food types and baseline larvae, and among larvae from different experimental feeding treatments. Using a set of baseline lamprey provides the opportunity to track the assimilation, if any, of a given food treatment through specific isotopic signatures and to establish that baseline signatures provide initial distinction from the food items given.

Examination of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios of food types revealed unique signatures (Table 5). Food type  $\delta^{13}\text{C}$  and %N were unique among all types (ANOVA,  $P < 0.01$ ,  $df = 3$ ,  $F = 2,823$  [ $\delta^{13}\text{C}$ ], 1,138 [%N]). Percent carbon was higher for leaves than the other food types ( $P < 0.01$ ,  $df = 3$ ,  $F = 46.45$ ) and  $\delta^{15}\text{N}$  was highest for salmon carcass analogs, intermediate for leaves and algae, and lowest for yeast/larval fish food ( $P < 0.01$ ,  $df = 3$ ,  $F = 43.64$ ). Tissue from baseline larvae differed in  $\delta^{13}\text{C}$  among all food types except algae ( $P < 0.01$ ,  $df = 4$ ,  $F = 53.87$ ) and %C did not vary ( $P = 0.47$ ,  $df = 4$ ,  $F = 0.92$ , Table 5). Stable isotope values of baseline lamprey ( $\delta^{13}\text{C} = -23.7$ ,  $\delta^{15}\text{N} = -7.4$ ) reflect a largely aquatic source of nutrition (Finlay et al. 1999). Baseline larval tissue was generally higher in overall C but more depleted (i.e., more negative) in the  $\delta^{13}\text{C}$  isotope indicating possible feeding on primary produced items (Limm and Power 2011). Baseline lamprey tissue  $\delta^{15}\text{N}$  was lower than the food types except for the yeast/larval fish food combination ( $P < 0.01$ ,  $df = 4$ ,  $F = 334$ ) and overall %N was highest for baseline lamprey but only differed significantly from leaves ( $P < 0.01$ ,  $df = 4$ ,  $F = 6.1$ , Table 5). Baseline lamprey tissue had the highest overall N content but was the least enriched in the  $\delta^{15}\text{N}$  isotope indicating a nitrogen poor diet. The contrasts in the C and N isotopic signatures likely provide unique signatures that are detectable experimentally. Experimental lamprey tissue  $\delta^{13}\text{C}$  and %C was different among treatments ( $P < 0.01$ ,  $df = 4$ ,  $F = 193$  [ $\delta^{13}\text{C}$ ], 19 [%C]). Lamprey fed yeast had the highest  $\delta^{13}\text{C}$  signature, followed by salmon carcass analogs, algae, unfed, and leaves (the latter two were not different). Percent carbon was highest in those fed salmon carcass analogs and algae, followed by yeast/larval fish food, leaves, and algae (Figure 9; Table 5). Tissue  $\delta^{15}\text{N}$  and %N differed among treatments ( $P < 0.01$ ,  $df = 4$ ,  $F = 257$  [ $\delta^{15}\text{N}$ ], 40 [%N]). Lamprey fed salmon carcass analogs had the highest  $\delta^{15}\text{N}$  followed by yeast/larval fish food and algae, and leaves and unfed. Percent nitrogen was highest in unfed and leaves treatment, followed by algae, yeast/larval food, and salmon carcass analogs (Figure 9; Table 5). The  $\delta^{13}\text{C} : \delta^{15}\text{N}$  ratio for experimental larval tissue versus food type was very similar indicating that isotopes in lamprey tissue tracked the food isotopes and experimental diets were being assimilated into larval lamprey tissues. Tank effects were not detected for feeding treatments ( $P > 0.05$ ,  $df = 4$ ) except for effects on  $\delta^{15}\text{N}$  in the algae and leaves treatment ( $P < 0.05$ ). The lamprey fed salmon carcass analogs had the highest  $\delta^{15}\text{N}$  and second highest  $\delta^{13}\text{C}$  values of any of the treatments.

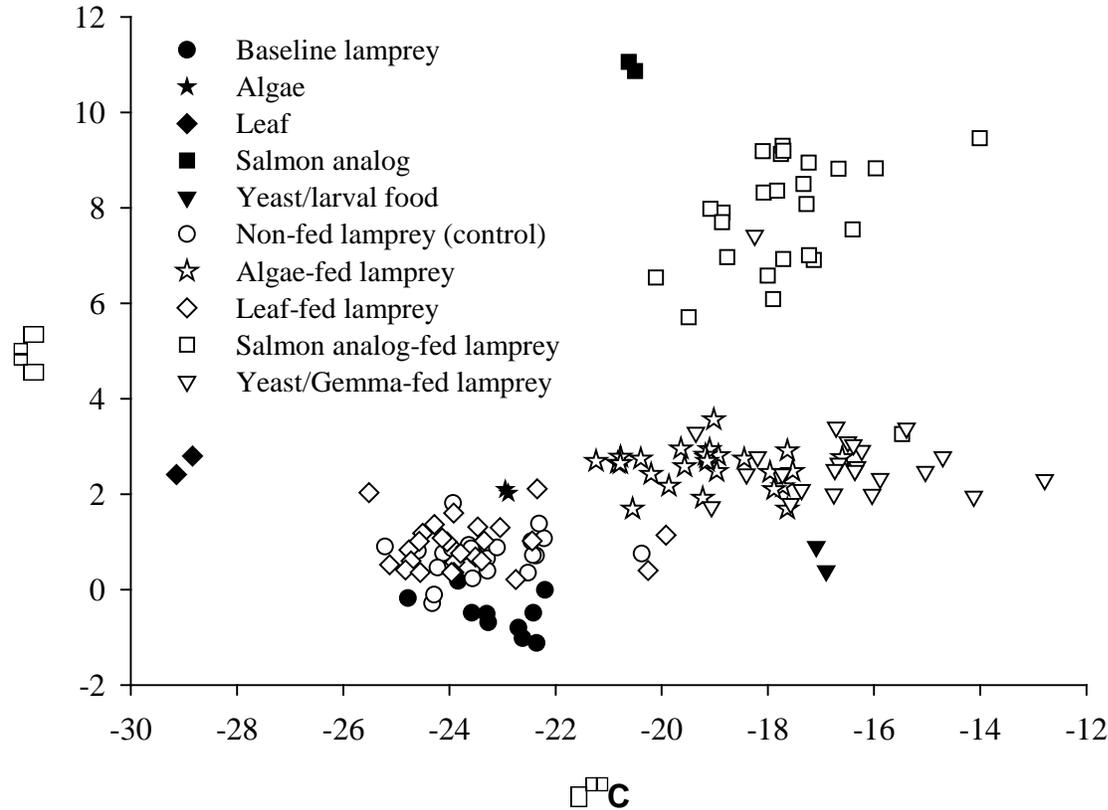
## Feeding and Growth of Larval Pacific Lamprey Feeding and Growth in a Captive-Reared Environment

**Table 5. C and N concentrations in food items, baseline larval Pacific lamprey tissue, and experimentally-fed captive larvae.**

	Number	% C	± SE	% N	± SE	δ <sup>13</sup> C	± SE	δ <sup>15</sup> N	± SE
<b>Food type</b>									
Algae	2	45.02	0.02	4.90	0.00	-22.92	0.03	2.06	0.04
Leaves	2	49.18	0.65	2.02	0.11	-28.99	0.15	2.61	0.20
Salmon carcass analog	2	43.78	0.08	8.37	0.11	-20.56	0.06	10.97	0.10
Yeast/larval fish food	2	45.39	0.20	7.45	0.07	-17.00	0.10	0.65	0.26
<b>Baseline lamprey</b>	20	50.08	1.49	10.24	0.64	-23.68	0.21	-0.40	0.10
<b>Experimental lamprey</b>									
Algae	4	53.16	0.83	9.17	0.22	-22.51	0.11	2.50	0.15
Leaves	4	46.78	1.12	12.46	0.21	-24.10	0.27	1.02	0.20
Salmon carcass analog	4	53.59	1.10	9.13	0.25	-20.67	0.20	7.51	0.26
Yeast/larval fish food	4	49.65	0.95	9.17	0.37	-18.88	0.15	2.73	0.23
No food	4	46.02	0.16	13.02	0.13	-23.60	0.20	0.69	0.11

# Feeding and Growth of Larval Pacific Lamprey Feeding and Growth in a Captive-Reared Environment

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**Figure 9.**  $\delta^{13}\text{C}$ :  $\delta^{15}\text{N}$  ratios from food items, baseline lamprey tissue, and experimentally-fed captive larval Pacific lamprey.

### *Health and disease screening*

A group of larval Pacific lamprey was collected from Eagle Creek (n=30) on 10 July 2013 (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, personal communication). Health screening results were negative for a variety of common fish pathogens, including infectious hematopoietic virus (IHNV), viral hemorrhagic septicemia (VHS), and *A. salmonicida* (furunculosis), all significant salmonid disease concerns. The bacteria *V. vulnificus* was not detected in these lampreys, although samples from 2009 tested positive (Jolley et al. 2011, Table 6). The only bacterium detected was *Hafnia alvei*.

# Feeding and Growth of Larval Pacific Lamprey Feeding and Growth in a Captive-Reared Environment

**Table 6. Results of fish health pathogen examination for larval Pacific lamprey from Eagle Creek in 2013.**

FISH SOURCE			FISH EXAMINED
<b>Location:</b> Eagle Creek <b>County:</b> Clackamas <b>Contact Person:</b> Jeff Jolley <b>Affiliation:</b> USFWS <b>Phone:</b> (360) 604-2500			<b>Species:</b> Pacific lamprey <b>Age:</b> Ammocoetes <b>CHN:</b> W13-110 <b>Number of fish:</b> 30 <b>Date Sampled:</b> 7/10/2013
DISEASE AGENT <sup>1</sup>	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. 4/30 fish (heart tissue) with growth on BHIA medium plates. No Vibrio found. Bacteria keyed out by API: <i>Hafnia alvei</i> .		

<sup>1</sup> **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*).

# Feeding and Growth of Larval Pacific Lamprey Feeding and Growth in a Captive-Reared Environment

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## Discussion, Conclusions and Management Implications

Pacific lamprey larvae can be contained and raised effectively in captivity, at least in small scale situations. The experimental configuration used here may need to be modified for larger scale experimentation. Larval Pacific lamprey showed positive growth in both of our experiments on certain food items and dosages. Survival was high (>95%) after a few initial mortalities in both experiments which suggests that tank configuration is appropriate for this scale of investigation. In addition, nutrients were assimilated into lamprey tissues as evidenced by tracking stable isotope values. The ability to house captive populations of lamprey opens up new avenues for experimentation that may be added to the suite of approaches for Pacific lamprey conservation. Most lampreys that were artificially-fed exhibited some level of growth. The greatest growth occurred on diets of salmon carcass analog or algae, these being the two most caloric dense food items used. Growth of larvae that were fed leaves resembled that of the control group (non-fed) and was minimal and often negative likely due to leaves being a low quality food item. Previous studies have indicated detrital material composed the bulk of assimilated content in larval lamprey (Sutton and Bowen 1994; Yap and Bowen 2003; Mundahl et al. 2005), although algae (primarily diatoms) have also been identified (Sutton and Bowen 1994; Quintella 2000). Shirakawa et al. (2009) experimentally found positive growth of sub-yearling Arctic lamprey *Lethenteron camtschaticum* larvae given a diet of ground leaves and negative growth from those given algae, although comparison to wild larvae suggested a varied diet. Limm and Power (2011) also found increased growth of larvae given leaves compared to control fish in an *in-situ* experiment. Our findings are in contrast where leaves were a suboptimal item and algae were beneficial.

Growth rates corresponded to energy densities of the food items; salmon carcass analogs provided the highest energy density while leaves provided the least. Limm and Power (2011) also found increased growth of larvae given fish flakes which represent a higher quality food item in terms of carbon and nitrogen content. Using the results of calorimetry work, determination of specific amounts of food to be given in order to provide similar energy content to that of the salmon analog can be found. Yeast has been the standard for feeding larval lamprey in previous studies (for examples, see Mallatt 1983, Murdoch et al. 1991; 1992) but our results suggest that this is a sub-optimal item in comparison to salmon carcass analog. Future work focused on manufacturing a lamprey-specific, cost-effective food item that contains the appropriate nutrition would be beneficial.

Growth rates appeared to be inconsistently varied by season. Rates were higher during the second interval of the experiment two but higher during the first interval in experiment one. Exact effects of electrofishing on larval lamprey are not well known. It is possible slower growth rates during the first interval of the experiment two could be explained as a result of electrofishing. Although this effect has been shown for other fishes (Dwyer and White 1997;

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Dwyer et al. 2001; Muth and Ruppert 1997), this was not consistent with the findings of the first experiment. In addition, it is possible these results reflect the temperature regime occurring during the intervals of highest growth; they both coincide with periods of higher temperatures. The findings of experiment one directed our follow-up experiment of varying feeding dosages of one food item, salmon carcass analogs, on growth of lamprey. Different feeding rates produced different growth rates but growth was mostly positive and salmon carcass analogs appeared to be a quality food item based on growth. Lamprey growth occurred in all treatments with the exception of the control, suggesting lamprey will grow even when only minimal amounts of a high quality food, item such as salmon carcass analog, are given. Those treatments with higher feeding rates resulted in higher growth rates, reinforcing the thought that lamprey do not regulate intake of food according to its availability (Moore and Mallat 1980). Higher energy density food items available to lamprey corresponded to higher growth rates. Changes in larval weight were found to mimic changes in length. Lamprey fed at a rate of 0.4g per week and 0.8g every other week showed similar changes in growth, suggesting the possibility of extending frequency of feeding to once every two weeks. The results of this investigation suggest that additional growth could be achieved by increasing the dose of salmon analog; however, the exact dosage in which growth is no longer maximized or when larvae reach satiation remains unknown. In addition, elevated feeding levels may lead to other issues such as tank maintenance and fouling. Further adjustments and evaluation of feeding dosages may be warranted depending on project goals. For example, developing feeding regimes to sustain larvae for use as surrogates in studies relating to wild fish as opposed to artificial propagation of fast-growing progeny may warrant different feeding approaches.

The stable isotopic evaluation of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) provided a useful tool to verify nutrient assimilation and has rarely been applied to feeding studies of larval lamprey. Our results are the first of their kind for captive-reared Pacific lamprey. Feeding habits of larval lamprey can be equivocal and ambiguous and much previous work has been done on gut contents where breakdown of food items can make identification challenging (Sutton and Bowen 1994). Information on other captive populations does not exist but isotopic values were similar to those found by other researchers in wild populations. Limm and Power (2011) also confirmed assimilation of nitrogen from larval Pacific lampreys that were fed fish flakes through elevated  $\delta^{15}\text{N}$  values. The baseline sample of lamprey in our study indicated a low trophic position as indicated by the less-enriched  $\delta^{15}\text{N}$  values and displaying  $\delta^{13}\text{C}$  values that may correspond to both terrestrial based plants ( $\sim 28\text{‰}$ ) or algae, which may vary widely ( $\sim 45\text{‰}$  -  $\sim 20\text{‰}$ ) depending on the source of the dissolved  $\text{CO}_2$  (Rosenfeld and Roff 1993; Fry 1996). A similar low trophic position was reported for larval least brook lamprey *Lampetra aepyptera*, American brook lamprey *Lethenteron appendix*, and sea lamprey *Petromyzon marinus* in Great Lakes tributary streams (Evans 2012). These larvae had  $\delta^{15}\text{N}$  values indicative of consumption of primary-produced plants and detrital materials. Evans (2012) also noted a size-dependent shift in isotopic

## Feeding and Growth of Larval Pacific Lamprey Feeding and Growth in a Captive-Reared Environment

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values suggesting an ontogenetic diet shift in larval lamprey. Experimental feeding in our study of captive fish produced enriched  $\delta^{15}\text{N}$  values and in some cases less-depleted  $\delta^{13}\text{C}$  values that are not indicative in naturally feeding wild fish. The elevated  $\delta^{15}\text{N}$  values and increased growth rates of larvae fed salmon carcass analogs poses unique ecological questions such as the relative influence of the availability of salmon carcasses in natural streams on larval nutrition. Bilby et al. (1996) found enrichment in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of many aquatic macroinvertebrates in streams where coho salmon *Oncorhynchus kisutch* spawned (and thus decomposing carcasses were available) compared to streams without coho salmon. In addition, they reported elevated  $\delta^{15}\text{N}$  values ( $6.4 \pm 1.4$ ) for larval Pacific lamprey in streams where coho salmon were present but didn't capture larval lamprey in streams where coho salmon were absent which precluded comparisons between the streams. Kucheryavyi et al. (2007) hypothesized that alternative life histories of Arctic lamprey exist. Those ammocoetes that have access to the rich nutrient sources provided by salmon carcasses may bypass the parasitic stage, transforming from larvae to adult because they were able to acquire the necessary nutrients in the larval stage. Finally, determination of whole body isotopic values required sacrifice of our fish at the conclusion of the feeding study. Non-lethal techniques exist for other fish (Fincel et al. 2012) and work to perfect these techniques in larval lamprey would be ideal, especially for fish with a conservation concern.

Temperature can affect growth and metabolism of fish (Mommsen 1998) but the exact influence on larval lamprey is generally unknown. Water temperature was not controlled in our experiments and was variable although all treatments experienced the same thermal regime. Larval sea lampreys have a thermal niche of  $17.8\text{-}21.8^\circ\text{C}$  (Holmes and Lin 1994) and Meeuwig et al. (2005) report optimal survival of sub-yearling larval Pacific lamprey at  $18^\circ\text{C}$ . Previous studies have indicated that cooler temperatures may slow down and reduce assimilation efficiencies in the guts of larvae (Moore and Mallat 1980). A logical progression of the work presented here is to conduct feeding trials with manipulated temperature regimes. However, flexibility to house lampreys in this way does not currently exist at ECNFH.

Larvae sampled from Eagle Creek were healthy and disease free. Explicit protocols are needed that guide the establishment of wild-origin lamprey at captive facilities. Some work has been completed assessing larval lamprey susceptibility to viruses (Kurath et al. 2013) but more research needs to be completed to evaluate the ability of antibiotics to treat bacterial infections of concern (e.g., *Aeromonas salmonicida*, *Vibrio* spp.; Jolley et al. 2012, 2013). 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. Current U.S. Fish and Wildlife Service guidelines need to be examined for adapting these protocols to non-traditional culture fish like lamprey (U.S. Fish and Wildlife Service 2004).

# Feeding and Growth of Larval Pacific Lamprey Feeding and Growth in a Captive-Reared Environment

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## *Management implications*

We demonstrated a successful establishment of a group of wild-sourced, captive Pacific lamprey larvae. Wild fish were assessed for pathogens and no evidence of disease concerns were observed in captivity. Survival was high and feeding regimes were identified that led to good growth (and poor growth). Specific food types and dosages were identified that may induce growth characteristics that are desirable depending on project goals (e.g., experimentation relating to wild ecology and conservation, artificial propagation). Nutrient assimilation of food items was confirmed. Variations on feeding frequency were investigated and provide practical results relating to maintenance costs (e.g., staff time). The consideration of the use of conservation hatcheries in managing and conserving Pacific lamprey is warranted and our results provide valuable information to incorporate in the conservation decision making process.

## ***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.*

# Feeding and Growth of Larval Pacific Lamprey Feeding and Growth in a Captive-Reared Environment

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## **Acknowledgements**

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