TECHNICAL NOTE

Feeding and Growth of Larval Pacific Lamprey Reared in Captivity

Jeffrey C. Jolley,* Christina T. Uh, Gregory S. Silver, and Timothy A. Whitesel
U.S. Fish and Wildlife Service, Columbia River Fisheries Program Office, 1211 Southeast Cardinal Court, Suite 100, Vancouver, Washington 98683, USA

Abstract
Pacific Lampreys Entosphenus tridentatus are declining in the Columbia River basin as well as in much of their broader range. To mitigate for reductions in abundance, strategies such as hatchery propagation and captive rearing of lamprey larvae are currently being considered. We conducted a series of experiments using captive larval Pacific Lampreys at Eagle Creek National Fish Hatchery to investigate the effect of different food types and different food concentrations on the growth of larvae. In our first experiment, we evaluated the growth of larvae (TL range, 59–145 mm) given four different food types (algae, leaves, yeast–larval fish food, and salmon carcass analogs) and a control group that was not fed. Assimilation of food types was evaluated by tracking unique stable isotope signatures of $\delta^{13}$C and $\delta^{15}$N within larval tissues. Results from experiment 1 indicated that lampreys fed a diet of salmon carcass analog or algae had positive growth (up to 0.16 mm/d and 42% proportional change in length) over approximately 6 months. Isotopic analyses of larval tissues showed unique signatures that matched those of their specific food treatment. Based on the results of experiment 1, we conducted a second experiment to evaluate the growth of larvae fed four different quantities of salmon carcass analog. Results from this experiment indicated larval growth rate increased with increasing rations of food, but positive growth was observed in all feeding treatments. The highest dose of salmon carcass analog resulted in the highest growth (up to 0.15 mm/d and 36% proportional change in length) over 6 months. We successfully reared larval Pacific Lampreys in captivity with minimal mortality and positive growth, which highlights the potential to use captive rearing and propagation as a conservation tool for this ecologically and culturally important species.

Pacific Lampreys Entosphenus tridentatus in the Columbia River basin have declined to a remnant of their historical abundance (Close et al. 2002). The species has been given protected status within Oregon due to declines along the coast and in the Columbia River basin (Close et al. 2002; Kostow 2002). The Pacific Lamprey has a complex life history that includes 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 7 years after hatching where they filter-feed on detritus and organic material (Scott and Crossman 1973; Sutton and Bowen 1994; Dawson et al. 2015). Larvae metamorphose into juveniles from approximately July to December (McGree et al. 2008) and migrate to the Pacific Ocean where they spend 1–3 years before returning to their freshwater spawning grounds to spawn and die (Scott and Crossman 1973).

Our understanding of the basic life history and ecology of Pacific Lamprey has several critical uncertainties (Mesa and Copeland 2009; Luzier et al. 2011). Many of the uncertainties may be addressed by observation and experimentation using captive lampreys. Increased knowledge of the biology, population dynamics, ecology, and identification of Pacific Lamprey will help managers understand and conserve this important species. The development of conservation hatchery programs has been explicitly recommended by several guiding documents from the U.S. Fish and Wildlife Service (USFWS) 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 Pacific Lamprey poses challenges to captive rearing that are uncommon in traditional teleost culture. Although larval Pacific Lampreys 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, and food types and quantities needed for growth of robust individuals in captivity is not available. Thus, methods need to be developed for the maintenance and growth of lamprey larvae reared in captivity.

*Corresponding author: jeffrey_jolley@fws.gov
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Lamprey larvae feed by filtering organic material and detritus 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). 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). Although larval lampreys can 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; Rodríguez-Muñoz et al. 2003; Yap and Bowen 2003), larval fish food (Mallatt 1983), ground leaves (Shirakawa et al. 2009), and freshwater algae (Shirakawa et al. 2009).

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 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 over traditional approaches including the determination of trophic level, the 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; Peterson 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 its application in feeding studies of larval lamprey feeding ecology. Our goal was to evaluate Pacific Lamprey growth of a broad range of sizes over a 6-month time period; specific objective were to (1) evaluate larval growth from four different commonly available and used food types, (2) evaluate larval growth associated with four different rations of a single food type (found to be beneficial to growth from objective 1), and (3) evaluate assimilation of food types into fish tissues using stable isotopic signatures. We hypothesized increased growth rates could be achieved through a quality, high energy-promoting diet compared with a lower energy-producing one.

METHODS

Lamprey source and rearing conditions.—Larval Pacific Lamprey individuals of unknown age were collected from North Fork Eagle Creek near the confluence of the main stem of Eagle Creek (Clackamas River Drainage, Clackamas County, Oregon; experiment 1) on July 11, 2012, and from the main stem of Eagle Creek (experiment 2) on July 7, 2013. Larvae were collected using an AbP-2 backpack electrofisher (ETS Electrofishing, Verona, Wisconsin) and transported to Eagle Creek National Fish Hatchery (ECNFH), Estacada, Oregon, where they were randomly assigned to feeding trials as described below. The rearing configuration for both experiments 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 5–7 cm of sand substrate. Water depth in each tank was about 27.9 cm. The substrate source was a spoil pile excavated from Gibbons Creek on Steigerwald National Wildlife Refuge (Clark County, Washington). Substrate was screened to remove large gravel and allowed to dry in the sun for 1 week. All tanks were placed in rectangular fiberglass troughs (43.2 cm wide × 40.6 cm deep × 4.9 m long) within a raceway and supplied with Eagle Creek water in a flow-through system at a target flow rate of approximately 2 L/min. Water temperature was not regulated and reflected the natural temperature regime of Eagle Creek. A HOBO tidbit temperature logger (Onset Computer Corporation, Bourne, Massachusetts) recorded water temperature at hourly intervals over the course of the experiments. The experimental configuration was consistent for experiments 1 and 2 and was chosen based on comparisons of different rearing vessels in 2011 (Jolley et al. 2012). Shade screens were used in the summer to moderate temperatures and reduce algal growth in the rearing tanks. Insulated tarps were used during winter months (i.e., December and January) to help prevent freezing and maintain flowing water to all tanks.

Experiment 1.—Larval Pacific Lamprey individuals (n = 160) were distributed among 20 rearing tanks at a target density of 6.8 fish/m² (8 fish/tank), a relatively low density that is found in the wild (reviewed in Dawson et al. 2015). Prior to the initiation of the experiment, individuals were anesthetized with buffered tricaine methanesulfonate (MS-222; 150 mg/L), measured (mm TL), weighed (g wet weight), and given a unique visible implant elastomer (VIE) tag (Silver et al. 2009). Prior to initiation of experiment 1 there were 134 remaining larvae (6% loss from mortality or escapement) due to technical failures, which were remedied. The larvae were randomly assigned to five feeding regimes, four tanks per regime, with 6 or 7 fish/tank: (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.

The food items given have been used for feeding larval lampreys in past applications. They were commercially available algae wafers (Kyorin Food Industries, Himeji, Japan; Shirakawa et al. 2009), leaves collected from common birch (Betulaceae), maple (Sapindaceae), and willow (Salicaceae) deciduous trees that occurred in the riparian zone of Eagle Creek (Shirakawa et al. 2009; Limm and Power 2011), salmon carcass analog pellets (Bioanalog salmon custom diet, Bio-Oregon, Longview, Washington), and a combination of commercially available
baker’s yeast and larval fish food (Gemma Wean, Bio-Oregon; 9:1 ratio yeast : larval fish food: Polkinghorne et al. 2001; McGree et al. 2008). Algae wafers, leaves, and salmon carcass analogs were ground into a powder (leaves were dried at 100°C before grinding). The mean particle size of the different food items was quantified by measuring three replicates of 100 food particles each along the longest axis under a dissecting microscope.

Proximate analysis of one 15-mL sample of each food type was determined using standard methods. The protein content was determined by the Dumas method (Method 968.06: AOAC 2000). The lipid content was determined using an acid hydrolysis method for the salmon analogs and larval fish food (Method 954.02: AOAC 2000) or by the Randall method (Method 2003.05: AOAC 2000) for leaves and algae. The 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). Caloric content of food types was quantified using a Model 1341 plain 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’s instructions (Parr Instrument 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. Samples of sediment were collected from experimental rearing tanks at the beginning and end of the feeding trials. Organic content of the sediment was determined using loss-on-ignition methods (Heiri et al. 2001; see Table 1) to describe the characteristics of the rearing environment and potential problems like decomposition, tank cleanliness, and fouling.

Larvae were fed once per week at a rate of 0.8 g of food per lamprey per week. Prior to feeding, the measured quantity of food for each tank 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 of yeast and larvae fish food suspensions were soaked for approximately 10–15 min. Soaking presumably increased the chance that food sank and therefore was available to filter-feeding larval lampreys rather than floating and flowing out of the tank (Limm and Power 2011; Rose and Mesa 2012). Water flow was halted during and after feeding and remained off for approximately 4–6 h to further reduce loss of food. Lamprey individuals were monitored weekly for mortalities on the sediment surface, and all mortalities were individually identified and removed. Detrital buildup and related algal and fungal growth were periodically skimmed from the sediment surface with a fine-mesh aquarium net, when judged necessary to maintain reasonable water quality. Individuals were examined for growth by anesthetizing them and measuring TL and weight at 3 months and at 6 months when the experiment concluded. At the conclusion of experiment 1, larvae were euthanized with an overdose of MS-222 and frozen for later isotopic analyses.

To assess whether the given food items were being assimilated by larvae, we examined stable carbon (δ13C) and stable nitrogen (δ15N) isotope ratios of a baseline sample of larval Pacific Lampreys collected from the North Fork Eagle Creek and of each food type to determine whether a sufficient contrast was apparent, and then compared those ratios with the individual larval muscle tissue samples at the end of feeding in experiment 1. Samples were freeze-dried and ground. Carbon and nitrogen contents and stable isotopes were measured with an elemental analyzer coupled with a continuous-flow isotope ratio mass spectrometer (Delta PlusXP, Thermo Finnigan, Bremen, Germany) (Brenna et al. 1997; Qi et al. 2003). The δ13C and δ15N values reported are in reference to Pee Dee Belemnite (PDB) and Vienna-PDB and atmospheric nitrogen standards, respectively. The baseline sample of lampreys provided the opportunity to track the assimilation, if any, of a given food treatment through specific isotopic signatures and to establish that baseline signatures provided initial distinction from the food items given.

**Table 1.** Percentage of sediment organic content of feeding treatments at Eagle Creek National Fish Hatchery in 2012–2013 and 2013–2014. SE values are in parentheses.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>Mean percent organic content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>No food</td>
<td>1.53 (0.04)</td>
</tr>
<tr>
<td></td>
<td>Algae</td>
<td>1.81 (0.10)</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>2.29 (0.19)</td>
</tr>
<tr>
<td></td>
<td>Salmon analog</td>
<td>2.34 (0.40)</td>
</tr>
<tr>
<td></td>
<td>Yeast–larval food</td>
<td>1.78 (0.05)</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>1.76 (0.20)</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>No food</td>
<td>0.56 (0.02)</td>
</tr>
<tr>
<td></td>
<td>Salmon carcass analog, 0.2 g/week</td>
<td>0.79 (0.04)</td>
</tr>
<tr>
<td></td>
<td>Salmon carcass analog, 0.4 g/week</td>
<td>0.64 (0.06)</td>
</tr>
<tr>
<td></td>
<td>Salmon carcass analog, 0.8 g/week</td>
<td>1.08 (0.21)</td>
</tr>
<tr>
<td></td>
<td>Salmon carcass analog, 0.8 g/2 weeks</td>
<td>0.82 (0.05)</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>0.48 (0.06)</td>
</tr>
</tbody>
</table>

**Experiment 2.**—Growth results from experiment 1 indicated salmon carcass analogs produced positive growth in larvae. Based on this observation, experiment 2 focused on evaluating the dose–response of lamprey larval growth in relation to salmon carcass analog food rations, which were (1) no food (control), (2) 0.2 g/lamprey, (3) 0.4 g/lamprey, (4) 0.8 g/lamprey, all weekly, and (5) a final ration of 0.8 g/lamprey fed every other week. The rationale was to reduce the previous feeding rate by...
50% and 75%, and a biweekly feeding regime was chosen to investigate the practicality of reduced personnel time and effort required for feeding. Each treatment had four replicates, and each tank initially contained eight larvae, for a total of 160 individuals. Individuals were examined for growth by anesthetizing them and measuring TL and weight at 3 months and at 6 months when the experiment concluded.

**Statistical analyses.**—Changes in lamprey larval growth were assessed in a variety of ways. Mean TL at the beginning of each experiment was evaluated for differences using one-way ANOVA. Mean proportion change in length, TL, weight, and daily growth rate was compared among treatments using two-way ANOVA (treatment, tank, and interactive effect). We compared mean %C, %N, δ¹³C, and δ¹⁵N using ANOVA to examine potential differences in signatures among food types, between food types and baseline lampreys, and among experimental lamprey treatments. Tukey’s mean separation procedures were used to identify differences among treatments for all ANOVA. All statistical tests were performed with the Statistical Analysis System (SAS Institute 2010).

**RESULTS**

**Experiment 1**

Survival of lampreys was high in experiment 1. Of the original 134 larvae, 128 (94%) survived the duration of the study. Initial TLs ranged from 59 to 120 mm, and there were no differences in mean TL among the treatment groups at the initiation of experiment 1 (ANOVA: \( P = 0.46, F = 0.97, df = 4 \)). Mean proportional change in length varied from −0.05 to 0.00 (no food and a diet of leaves) to 0.42 (algae). Algae and salmon carcass analog diets produced the highest proportional change in length, the yeast–larval fish food diet was intermediate, and the leaves diet and no food were the lowest and negative (\( P < 0.01, F = 283.37, df = 4 \); Figure 1A). Mean TL at the conclusion of the experiment ranged from 84 mm (no food) to 124 mm (salmon carcass analog diet) and differed among treatments (\( P < 0.01, F = 65.73, df = 4 \); Figure 1B). Differences in mean weight mirrored differences in mean TL for their corresponding treatments (\( P < 0.01, F = 95.70, df = 4 \); Figure 1C).

Overall growth rates in TL (mm/d) ranged from −0.02 mm/d (no food) to 0.16 mm/d (algae and salmon carcass analog). Overall growth rates in weight (g/d) ranged from −0.001 g/d (no food) to 0.01 g/d (algae and salmon carcass analog). Differences in growth rates mirrored those reported above for both increases in length (ANOVA: \( P < 0.01, F = 381.38, df = 4 \)) and increases in weight (\( P < 0.01, F = 156.26, df = 4 \)).

Values for δ¹³C (ANOVA: \( P < 0.01, F = 2.823, df = 3 \)) and %N (\( P < 0.01, F = 1.138, df = 3 \)) were unique among all food types (Table 2). Percent carbon was higher for leaves than for the other food types (\( P < 0.01, F = 46.45, df = 3 \)), and δ¹⁵N was highest for salmon carcass analogs, intermediate for leaves and algae, and lowest for yeast–larval fish food (\( P < 0.01, F = 43.64, df = 3 \)).

Tissue from baseline lamprey larvae differed in δ¹³C among all food types except algae (\( P < 0.01, F = 53.87, df = 4 \)) and %C did not vary (\( P = 0.47, F = 0.92, df = 4 \); Table 2). Baseline
TABLE 2. Number of samples, mean percent concentrations of C and N, and mean isotopic values in food items, baseline larval Pacific Lamprey tissue, and experimentally fed Pacific Lampreys reared in captivity at Eagle Creek National Fish Hatchery, 2012–2013. SE values are in parentheses.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Number</th>
<th>C (%)</th>
<th>N (%)</th>
<th>δ¹³C (%)</th>
<th>δ¹⁵N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Food type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Algae</td>
<td>2</td>
<td>45.02 (0.02)</td>
<td>4.90 (0.00)</td>
<td>-22.92 (0.03)</td>
<td>2.06 (0.04)</td>
</tr>
<tr>
<td>Leaves</td>
<td>2</td>
<td>49.18 (0.65)</td>
<td>2.02 (0.11)</td>
<td>-28.99 (0.15)</td>
<td>2.61 (0.20)</td>
</tr>
<tr>
<td>Salmon carcass analog</td>
<td>2</td>
<td>43.78 (0.08)</td>
<td>8.37 (0.11)</td>
<td>-20.56 (0.06)</td>
<td>10.97 (0.10)</td>
</tr>
<tr>
<td>Yeast–larval fish food</td>
<td>2</td>
<td>45.39 (0.20)</td>
<td>7.45 (0.07)</td>
<td>-17.00 (0.10)</td>
<td>0.65 (0.26)</td>
</tr>
<tr>
<td><strong>Baseline lamprey</strong></td>
<td>20</td>
<td>50.08 (1.49)</td>
<td>10.24 (0.64)</td>
<td>-23.68 (0.21)</td>
<td>-0.40 (0.10)</td>
</tr>
<tr>
<td><strong>Experimental lamprey</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Algae</td>
<td>4</td>
<td>53.16 (0.83)</td>
<td>9.17 (0.22)</td>
<td>-22.51 (0.11)</td>
<td>2.50 (0.15)</td>
</tr>
<tr>
<td>Leaves</td>
<td>4</td>
<td>46.78 (1.12)</td>
<td>12.46 (0.21)</td>
<td>-24.10 (0.27)</td>
<td>1.02 (0.20)</td>
</tr>
<tr>
<td>Salmon carcass analog</td>
<td>4</td>
<td>53.59 (1.10)</td>
<td>9.13 (0.25)</td>
<td>-20.67 (0.20)</td>
<td>7.51 (0.26)</td>
</tr>
<tr>
<td>Yeast–larval fish food</td>
<td>4</td>
<td>49.65 (0.95)</td>
<td>9.17 (0.37)</td>
<td>-18.88 (0.15)</td>
<td>2.73 (0.23)</td>
</tr>
<tr>
<td>No food</td>
<td>4</td>
<td>46.02 (0.16)</td>
<td>13.02 (0.13)</td>
<td>-23.60 (0.20)</td>
<td>0.69 (0.11)</td>
</tr>
</tbody>
</table>

Lamprey tissue δ¹⁵N was lower than in the food types except for the yeast–larval fish food combination (P < 0.01, F = 334, df = 4), and overall %N was highest for baseline lampreys but only differed significantly from leaves (P < 0.01, F = 6.1, df = 4; Table 2). The contrasts in the C and N isotopic signatures likely provided unique signatures that were detectable experimentally.

The δ¹³C : δ¹⁵N ratio for larval tissue associated with various food types were very similar indicating that isotopes in Pacific Lamprey tissue tracked the food isotopes and experimental diets were being assimilated into larval lamprey tissues (Figure 2). Lamprey tissue (from experiment 1) δ¹³C and %C were different among treatments (P < 0.01, F = 193 [δ¹³C] and 19 [%C], df = 4). Lampreys fed yeast–larval fish food had the highest δ¹³C signature, followed by salmon carcass analogs, algae, no food, and leaves (the latter two were not significantly different). Percent carbon was highest in those fed salmon carcass analogs and algae, followed by yeast–larval fish food, leaves, and algae (Table 2). Tissue δ¹⁵N and %N differed among treatments (P < 0.01, F = 257 [δ¹⁵N] and 40 [%N], df = 4). Lampreys fed salmon carcass analogs had the highest δ¹⁵N followed by those fed yeast–larval fish food or algae, and leaves or no food. Percent nitrogen was highest in no food and leaves treatments, followed by algae, yeast–larval food, and salmon carcass analogs (Table 2). The lampreys fed salmon carcass analogs had the highest δ¹⁵N and second highest δ¹³C values of any of the treatments.

Food types were different in size, proximate composition, and caloric content (Table 3). Larval fish food particles were the largest (mean = 384 μm) while yeast particles were the smallest (mean = 58 μm). Salmon carcass analogs were generally the most energetically dense, having the highest protein content and the second highest lipid content of all food types. Leaves were the most energetically poor and had the lowest protein and lipid content of all food types. Statistical analyses on potential differences in proximate composition of food items were not conducted because replicate samples were not analyzed. Mean caloric content of food items ranged from 4.7 kcal/g (salmon carcass analog) to 2.9 kcal/g (leaves). Salmon carcass analogs had the highest caloric value followed by larval fish food, yeast or algae, and leaves (ANOVA: P < 0.01, F = 95, df = 4). When adjusted for feeding rates, lampreys 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, as indicated in Table 3. Sample sizes were inadequate to statistically compare organic content among treatments in experiment 1.

**Experiment 2.**—Survival was high throughout the study, in which 157 of 160 larvae survived (98%). Initial TLs of fish
TABLE 3. Proximate composition, particle size, caloric content, and feeding rates for larval Pacific Lampreys in experiment 1. Sample size (x) and SE values (y) are in parentheses (x, y). Lowercase letters represent statistical differences.

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

*aYeast.
*bLarval food.
FIGURE 3. (A) Proportional change in length, (B) total length, and (C) weight by time interval and treatment of larval Pacific Lampreys at Eagle Creek National Fish Hatchery in experiment 2. Error bars represent ± SE; lowercase letters represent statistical differences for the overall time period.

to increased length and weight (Figures 3B and 4C). The high food treatment produced the longest fish followed by the moderate food and biweekly food treatments, and the low and no food treatments ($P < 0.01$, $F = 31.59$, df = 4; Figure 3B). Changes in mean total weight of lampreys closely resembled those changes seen in mean TL ($P < 0.01$, $F = 24.26$, df = 4; Figure 3C). Overall growth rates in weight ranged from −0.05 g/d (no food) to 0.18 g/d (0.8 g/fish per week). Overall growth rates in weight ranged from −0.005 g/d (no food) to 0.013 g/d (0.8 g/fish per week). The most change in growth in both length and weight occurred during the months of October–January. The overall growth response across increasing feed rations was strongly linear and did not indicate reaching an asymptote across the range of food rations that we examined (Figure 4).

Mean organic content at the end of experiment 2 ranged from 0.6% (no food treatment) to 1.1% (high food treatment) and was initially 0.5% at the onset of the experiment (Table 1). Organic content was significantly higher in the high food treatment than it was in the no food treatment (ANOVA: $P = 0.03$, $F = 3.75$, df = 4; Figure 3A). Mean TL of larval lampreys increased in all food treatments where food was given (range, 98 mm [no food]–128 mm [high food]); higher doses led
to mean TL among the treatment groups at the initiation of experiment 2 (ANOVA: $P = 0.68$, $F = 0.59$, df = 4). Mean proportional change in length (accounting for initial length) varied from −0.06 (no food) to 0.36 (high food). The high food treatment (0.8 g/fish per week) produced the largest change followed by the moderate and biweekly food treatments, the low food treatment, and lastly, the no food treatment, which was negative ($P < 0.01$, $F = 64.32$, df = 4; Figure 3A). Mean TL of larval lampreys increased in all food treatments where food was given (range, 98 mm [no food]–128 mm [high food]); higher doses led

**DISCUSSION**

Pacific Lamprey larvae had positive growth coupled with high survival in both of our experiments using certain food items and ration levels. In addition, nutrients were assimilated into lamprey tissues as evidenced by tracking stable isotope values. These results highlight the ability to contain and raise healthy lamprey larvae in captivity, at least in small scale applications. Aside from periodic tank maintenance the personnel cost was relatively low; weekly visits of 2–3 h were necessary to conduct feeding and cleaning (simple removal of the excessive
FIGURE 5. Water temperature regime for larval Pacific Lamprey feeding experiments at Eagle Creek National Fish Hatchery in 2012–2013 (experiment 1) and 2013–2014 (experiment 2).

detrital layer from the top of sediment). The ability to house captive populations of lampreys opens up new avenues for experimentation that may be added to the suite of approaches for Pacific Lamprey conservation.

Most Pacific Lampreys fed with artificial food exhibited some level of growth, and the most growth occurred on diets of salmon carcass analogs and algae. Food items with higher energy densities available to the lampreys corresponded with higher growth rates. Growth of larvae fed leaves resembled that of the control (no food) group and was minimal and often negative. Previous studies have indicated detrital material comprises the bulk of assimilated content in larval lampreys (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). Positive growth of larvae of subyearling Arctic Lamprey *Lethenteron camtschaticum* given a diet of ground leaves and negative growth from those given algae has been reported, although comparison with wild larvae suggested a varied diet (Shirakawa et al. 2009). Limm and Power (2011) also found increased growth of Pacific Lamprey larvae given leaves compared with control lampreys in an in situ experiment.

Our findings are contrast those results in that leaves were a suboptimal item and algae were beneficial. All of the food items in our study fell within the size range reported as those most readily consumed by European Brook Lampreys *Lampetra planeri* without any size-based selection (5–340 μm; Moore and Mallat 1980) and were therefore palatable to the individuals.

Growth rates of lampreys were varied inconsistently by season. Rates were higher during the second interval of experiment 2 but higher during the first interval in experiment 1. The specific effects of electrofishing on larval lampreys are not well known and it is possible slower growth rates during the first interval of experiment 2 could be explained as a result of being recently captured from the wild by electrofishing. Although this effect has been shown for other fishes (Dwyer and White 1997; Dwyer et al. 2001; Muth and Ruppert 1997), this was not consistent with the findings of experiment 1. Temperature can affect the growth and metabolism of fishes (Mommsen 1998) but the exact influence of temperature on larval lampreys is generally unknown. Quintella et al. (2003) reported slower growth in stream-caught Sea Lamprey *Petromyzon marinus* larvae in winter (when water temperatures were cooler), while Murdoch et al. (1992) observed slower growth of Sea Lampreys during the fall (compared with summer or winter) even when water temperature was held constant in the laboratory.

Water temperature was not controlled in our experiments and was variable, although all treatments experienced the same thermal regime. Larval Sea Lamprey has a thermal niche of 17.8–21.8 °C (Holmes and Lin 1994). Also, 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, the flexibility to house larvae in this way does not currently exist at ECNFH.

The findings of experiment 1 directed our follow-up experiment to determine the effect of varying feeding rations of one food item, salmon carcass analogs, on the growth of Pacific Lampreys. Different feeding rates produced different positive growth rates (except for the control) suggesting lampreys will grow even when only minimal amounts of a high quality food item, such as salmon carcass analog, are given. Those treatments with higher rations resulted in higher growth rates, reinforcing the notion that lampreys do not regulate intake of food according to its availability (Moore and Mallat 1980). Furthermore, lampreys fed at a rate of 0.4 g per week and 0.8 g every other week showed similar changes in growth, suggesting the possibility of extending the frequency of feeding to once every 2 weeks. The results of this investigation showed that additional growth could be achieved by increasing the ration of salmon carcass analog; however, the exact ration at which growth is no longer maximized or when larvae reach satiation remains unknown. The typical growth response curves used in many aquaculture-based studies (Shearer 2000) did not indicate an asymptote that would indicate satiation. Elevated feeding rations may lead to other problems such as tank fouling and the resulting maintenance
required as evidenced by the higher amount of organic material found in the high-dose feeding trial. Further adjustments and evaluation of feeding rations 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 and rearing approaches (Araki et al. 2008).

Maximum growth rates observed in this study were higher than what might be expected to occur naturally. We observed growth rates that, if maintained throughout the year, would have resulted in approximately 58 mm of growth. Morman (1987) reported annual growth rates of 20–28 mm/year in caged Sea Lamprey larvae held at low densities in situ in streams, and Murdoch et al. (1992) reported growth rates of 12 mm/year in captive Sea Lampreys. Our observed growth rates were two to five times higher, especially when lampreys were given a diet of relatively higher quality. These high growth rates were attainable in a likely nonnatural context. In the wild, factors such as seasonal availability of food items with variable quality, thermal regime, and competition all likely factor into natural growth rates of lampreys (reviewed in Dawson et al. 2015).

The stable isotopic evaluation of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) provided a useful tool to verify nutrient assimilation and has rarely been applied to feeding studies of larval lampreys. Our results are the first of their kind for Pacific Lampreys reared in captivity. Feeding habits of larval lampreys can be equivocal and ambiguous, and much previous work has been done on gut contents where breakdown of food items can make their identification challenging (Sutton and Bowen 1994). Information on other captive populations does not exist but isotopic values of baseline lampreys were similar to those found by others in wild populations. Limm and Power (2011) also confirmed assimilation of nitrogen from larval Pacific Lampreys that were fed fish flakes through elevated $\delta^{15}N$ values. The baseline sample of lampreys in our study indicated a low trophic position as indicated by the less-enriched $\delta^{15}N$ values and display of $\delta^{13}C$ values that may correspond to both terrestrial based plants ($\approx 28\%$e) or algae, which may vary widely ($\approx 45\%e$ to $\approx 20\%e$) depending on the source of the dissolved CO₂ (Rosenfeld and Roff 1992; Fry 2006). A similar low trophic position was reported for larval Least Brook Lampreys Lampetra aepyptera, American Brook Lampreys Lethenteron appendix, and Sea Lampreys in the Great Lakes and Ohio River tributary streams (Evans 2012). Stable isotope values of baseline lampreys ($\delta^{13}C = -23.7\%e$, $\delta^{15}N = -0.4\%e$) reflected a largely aquatic source of nutrition (Finlay et al. 1999). Baseline lamprey larval tissue was generally higher in overall C but more depleted (i.e., more negative) in the $\delta^{13}C$ isotope indicating possible feeding on primary produced items (Limm and Power 2011). The analysis of baseline lamprey tissue revealed it had the highest overall N content but was the least enriched in the $\delta^{15}N$ isotope indicating a nitrogen-poor diet (Fry 2006). These baseline (i.e., wild) larvae had $\delta^{15}N$ values indicative of consumption of primary-produced plants and detrital materials. Evans (2012) also noted a size-dependent shift in isotopic values suggesting an ontogenetic diet shift in larval lampreys (over a wider range of sizes that we examined in this study). Experimental feeding in our study of captive fish produced enriched $\delta^{15}N$ values and in some cases less-depleted $\delta^{13}C$ values that are not indicative in naturally feeding wild fish.

The elevated $\delta^{15}N$ values and increased growth rates of lamprey larvae fed salmon carcass analogs pose 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 $\delta^{15}N$ and $\delta^{13}C$ values of many aquatic macroinvertebrates in streams where Coho Salmon Oncorhynchus kisutch spawned (and thus decomposing carcasses were available) compared with to streams without Coho Salmon. In addition, they reported high $\delta^{15}N$ values (6.4 ± 1.4%) for larval Pacific Lampreys in streams where Coho Salmon were present but did not capture larval lampreys in streams where Coho Salmon were absent, which precluded making comparisons between the streams. Kucheryavyi et al. (2007) hypothesized that alternative life histories for the Arctic Lamprey exist. Those larvae that have access to the rich nutrient sources provided by salmon carcasses may bypass the parasitic stage and transform from larvae to adults because they are able to acquire the necessary nutrients during the larval stage. Lipid concentration may ultimately govern these processes and work to understand the relationship between diet, growth, metamorphosis, and energetic content is needed (Jolley et al., in press). Finally, determination of whole-body or muscle-tissue isotopic values required sacrificing our fish at the conclusion of the feeding study. Nonlethal techniques exist for other fishes (Church et al. 2008; Fincel et al. 2012), and work to perfect these techniques in larval lampreys would be ideal, especially for those species for which there is a conservation concern.

Populations of Pacific Lamprey remain depressed and timely conservation of this ecologically and culturally significant species is critical. The use of conservation hatchery programs as a tool has shown promise for other species (Berejikian et al. 2008; Gum et al. 2011; Cochran-Biederman et al. 2015) and has been explicitly recommended for Pacific Lamprey by multiple entities (USFWS, Columbia River Inter-Tribal Fish Commission). Our results highlight the practical possibility of advancing this idea. Further refinement and attention to all areas within this program including, but not limited to, growth, condition, physical requirements, and disease–pathology issues are necessary.

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