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Research Article

Evaluation of Larval Sea Lamprey *Petromyzon marinus* Growth in the Laboratory: Influence of Temperature and Diet

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Conservation aquaculture provides a means for promoting environmental stewardship, useful both in the context of restoring native species and limiting the production of invasive species. Aquaculture of lampreys is a relatively recent endeavor aimed primarily at producing animals to support the restoration of declining native populations. However, in the Laurentian Great Lakes, where sea lamprey *Petromyzon marinus* are invasive, the ability to acquire a reliable source of certain life stages would be a significant benefit to those controlling their populations and studying the species. Here, we apply methodologies developed for Pacific lamprey *Entosphenus tridentatus* restoration to investigate the feasibility of rearing larval sea lamprey under laboratory conditions. In two experiments lasting 3 and 9 months, we tested the effects of different dietary sources and water temperature (ambient and controlled) on the survival and growth of wild-caught larvae. Rearing conditions had no effect on mortality, as larval survival was 100% in both experiments. Growth was significantly affected by water temperature, with the highest average daily growth rates observed at 22 and 15°C (0.14 mm day^{-1}) and lowest at 8°C (0.06 mm day^{-1}). Diets of yeast alone (0.19 and 0.21 g L^{-1}) performed better than those comprising a mixture of yeast and other material when fed 3 times weekly (rice flour, wheat flour, fish meal; 0.19 and 0.32 g L^{-1}). Averaged across the three constant temperatures (8, 15, and 22°C), larvae fed on yeast grew 0.13 mm day^{-1} and 0.01 g day^{-1}, whereas on yeast + fish meal, they grew 0.09 mm day^{-1} and 0.01 g day^{-1}. At ambient temperature (4–20°C), larvae fed on yeast grew 0.15 mm day^{-1} and 0.01 g day^{-1}, whereas those fed on yeast + wheat flour grew 0.13 mm day^{-1} and 0.008 g day^{-1} and those fed on yeast + rice flour grew 0.12 mm day^{-1} and 0.009 g day^{-1}. An experimental duration of 90 days was sufficient to detect significant changes to larval sea lamprey growth stemming from temperature variation. Overall, rearing of sea lamprey in captivity appears feasible at low density (31–32 g m^{-2} and 17–25 larvae m^{-2}), but uncertainties remain regarding the most appropriate means of providing adequate feed for these fish in high-density conditions.

1. Introduction

The global aquaculture sector is valued at ~$290 US billion $year^{-1}$ and projected to grow 5.5% annually through 2030, primarily providing for the production of aquatic-based human food, nonhuman animal feed, and a range of commercial products [1]. In the past two decades, advances in technology and standards of practice have expanded the role of aquaculture. Specifically, aquatic organisms can now be cultured to offset wild captures of declining populations, restore degraded habitats by shifting to terrestrial-based culture, and aid in species recovery [2–4]. The development of "conservation aquaculture"—defined as "*the use of human cultivation of an aquatic organism for the planned management and protection of a*

natural resource"—holds significant promise to support the sustainable use of natural resources and species requiring management intervention [5].

Lampreys are a feature of temperate freshwater and inshore marine ecosystems globally, yet >25% of species are considered "at risk" of extirpation in at least some portion of their range [6]. Major threats to lampreys include habitat loss and fragmentation, pollution, and overexploitation [7]. In some geographic locations, lampreys are highly valued as a seasonal food source, which has resulted in the development of aquaculture programs to support a sustainable harvest and bolster dwindling captures [8–10]. Over the last decade, aquaculture research on Pacific lamprey *Entosphenus tridentatus* has generated significant advances in the development of methods to culture embryos and larvae for both research and stocking efforts in the Columbia River basin of the Pacific Northwest region of North America [11]. Coincident with the decline of many native lamprey species, in the Laurentian Great Lakes, an invasive population of sea lamprey *Petromyzon marinus* established itself as one of the most destructive pests in the world's largest freshwater system [12]. Sea lamprey invasion led to the implementation of a bi-national (US and Canada) control program that seeks to maintain the abundance of sea lamprey at levels low enough to be economically tolerable to the lake's multi-billion-dollar fishery [13]. Ironically, because of the efficacy of this control program, which aims to control populations by killing the filter-feeding sea lamprey larvae before they become large enough to metamorphose into parasitic juveniles and prevents adults from accessing spawning habitat using barriers [14], access to certain life stages of sea lamprey for research purposes has become severely limiting [15, 16]. Limited access to sea lamprey for study was recognized as an issue decades ago [17], but early attempts at rearing them were unsuccessful [18]. These limitations have hindered research efforts toward the development of new control tactics (e.g., gene editing [19], "green" pesticides [20], and supplemental controls [21]). Thus, there is a need to develop methods to culture sea lamprey under controlled conditions, particularly to provide access to and knowledge of metamorphosing and juvenile life stages.

Larval lamprey grow slowly over an extended period of time, typically requiring at least 3–5 years to reach the point where they undergo metamorphosis [22]. Therefore, the establishment of net positive growth of larvae over relatively short timeframes (i.e., 3–6 months) is key to enable rapid testing of new protocols and methods for rearing sea lamprey. Methods for culturing and rearing Pacific lamprey have progressed quickly in recent years; for example, specific guidelines were developed and outlined for culturing all life stages, in terms of flow rates, water volume and height, tank surface area, sediment characteristics, and feed type and rates [23]. Therefore, these existing protocols can be leveraged to facilitate efforts to culture sea lamprey for research.

In this study, the type, quantity, and frequency of larval feed provided to larval sea lamprey were based on experiences rearing Pacific lamprey larvae [11, 24–27]. In those studies, active dry yeast was used as a base feed at a rate of 0.5–1.5 g L week^{-1}, and supplements (e.g., Otohime, Reed Mariculture Inc., alfalfa pellets, brown rice flour, or wheat flour) were added at a ratio of 2 : 1–4 : 1 (yeast to supplement ratio). In Pacific lamprey, larval growth and survival were found to be considerably greater in tanks provided with supplements compared to tanks only receiving yeast. Research on Pacific lamprey also found that distributing a week's worth of feed over three application periods resulted in higher growth rates than the application of feed once or twice per week [27]. Larval Pacific lamprey held at densities greater than 100–200 g m^{-2} of substrate surface area showed considerably lower growth rates [11, 28]. Based on Barron et al. [29, 30], high growth rates for larval Pacific lamprey were detected in a wide range of water temperatures between 14 and 19°C, but as larvae grew and aged, the optimal growth temperature was found at a lower, narrower range (14−16°C). In this study, our aim was to determine the feasibility of rearing larval sea lamprey in captivity using similarly prepared feeds and a range of temperatures. Specifically, we collected larvae from the wild and exposed them to both ambient and controlled water temperatures, as well as a range of dietary treatments, over a 3- to 9-month time frame to measure these effects on growth and survival.

2. Materials and Methods

2.1. Experiment 1—Growth and Survival in Relation to Diet at Ambient Water Temperature

2.1.1. Experimental Design. Larval sea lamprey ($n = 120$; length = 90–110 mm total length (TL)) were collected from Baldwin River, MI, with backpack electrofishers (ABP-2; ETS, Madison, WI; Jubar et al. [31]) between 11 and 15 July 2020 by the US Fish and Wildlife Service, Ludington Biological Station, Ludington, Michigan (MI). Larvae were then transported to the US Geological Survey Great Lakes Science Center, Hammond Bay Biological Station (HBBS), Millersburg, MI on 16 July 2020. The experiment began on 1 August 2020 and was terminated on 12 May 2021. Larvae were weighed (g) and measured (mm TL) at three points: Day 0, Day 90, and Day 270. Larvae were randomly assigned to six outdoor circular tanks (Table 1) with a surface area of 1.17 m^2 and a total volume of 711 L ($n = 20$ tank^{-1}: density = 17.1 m^{-2}, biomass = 32.3 g m^{-2} a medium density as defined by [11]). Tanks were provided with sand collected from Schmidt Creek, Presque Isle County, MI, to a depth of 25 cm, with water height of 15.2 cm. Water volume was 172 L. Tanks were continuously supplied with Lake Huron water at ambient temperatures at a rate of 1.8 L min^{-1}, giving a turnover rate of 90 min. Water temperature ranged from 20°C in August to less than 4°C December through May. Tank lids were opened from 08:00 to 16:00 hr. Water temperature and dissolved oxygen levels (DO) were measured 5 days week^{-1} using a dissolved oxygen meter (YSI Pro 20).

Three feed treatments were tested (Table 1): active dry yeast (Red Star, Milwaukee, WI; 150 g week^{-1}); yeast and brown rice flour (Anthony's, Glendale, CA; 75 g week^{-1} + 150 g yeast); and yeast and organic whole wheat flour (War Eagle Mill, Rogers, AR; 75 g week^{-1} + 150 g yeast). Weekly food rations were distributed over 3 days (Monday, Wednesday,

Table 1: Summary of experimental design for both Experiments 1 and 2.

Collection locality	Experiment 1 Baldwin river	
	Days 0–90	Days 90–270
Density (abundance, individuals m^{-2})	17.1	40
Density (biomass, $g\,m^{-2}$)	32.3	165.4
Sediment depth (cm)	25	2–4
Water depth (cm)	15.2	33
Water temperature (°C)	3.3–14.6	0–4.2
Flow rate ($L\,min^{-1}$)	1.8	4
Water turnover rate (min)	90	39
Feed source	Yeast, yeast + brown rice flour (2 : 1 ratio), yeast + whole wheat flour (2 : 1 ratio)	Yeast, yeast + brown rice flour (2 : 1 ratio), yeast + whole wheat flour (2 : 1 ratio)
Weekly feed amount (g)	Yeast (150 g), yeast (150 g) + brown rice flour (75 g), yeast (150 g) + whole wheat flour (75 g)	Yeast (25 g), yeast (25 g) + brown rice flour (12.5 g), yeast (25) + whole wheat flour (12.5 g)
Weekly feed ration ($g\,L^{-1}$)	0.21, 0.32, 0.32	0.17, 0.25, 0.25
Feed frequency	3× weekly	1–2× weekly
Biofilm disturbance	Every 14 days	None
Collection locality	Experiment 2 Pigeon river	
Density (abundance, individuals m^{-2})	25	
Density (biomass, $g\,m^{-2}$)	31.2	
Sediment depth (cm)	7	
Water depth (cm)	10.2	
Water temperature (°C)	8, 15, 22	
Flow rate ($L\,min^{-1}$)	0.25	
Water turnover rate	56	
Feed source	Yeast, yeast + fish meal (1 : 1 ratio)	
Weekly feed amount (g)	Yeast (2 g), yeast (1 g) + fish meal (1 g)	
Weekly feed ration ($g\,L^{-1}$)	0.19	
Feed frequency	3× weekly	
Biofilm disturbance	None	

and Friday) and dispersed evenly across the water surface without mixing with water. Feed typically floated on the surface for ~13 min before dispersing through the water column or accumulating on the substrate, and some portion of feed was lost through the outflow while floating on the surface. To ensure anoxic conditions did not develop, once every 14 days, biofilm growing on the substrate was disturbed with a wooden rod by inserting it 3–5 cm into the substrate and dragging it through the sand. Suspended biofilm eventually settled back into the substrate or was removed via the outflow. It was not known if DO levels would decrease due to high feeding rates, and low oxygen content was expected to negatively impact larvae; therefore, we progressively increased the feeding rate. For the first two feeds, only 50% of the ration was supplied to each tank, and for the next two feeds, 75% of the ration was supplied. After recording no change in DO levels prior to and after feeding, 100% rations were supplied starting 7 August 2020.

To prevent experimental tanks from freezing over winter, at Day 90, larvae were moved to six smaller, separate rectangular tanks (Table 1) with a surface area of 0.5 m^2 and volume of 150 L ($n = 20\,tank^{-1}$, density = 40 m^{-2}, biomass = 165.4 $g\,m^{-2}$). These tanks were filled with sand collected from Lake Huron to a depth of 2–4 cm and supplied with Lake Huron water at ambient temperatures circulating at a rate of 4 $L\,min^{-1}$. Water height was set at 33 cm, giving a turnover rate of 39 min. Each tank received the same feed type; however, the rations were reduced to 1/6th due to low ambient water temperatures (1−5°C): rations were reduced to 25 g of yeast and 12.5 g of brown rice flour or whole wheat flour per week. During November, weekly food rations were distributed over 2 days, dropping to just 1 day $week^{-1}$ December–April. During feedings, the water supply was shut off for 1–2 hr to allow for the dispersion of the feed. Feed was added to the surface and immediately agitated with a plastic mixing paddle for 1 min. All experimental tanks developed a biofilm 1 cm thick or greater. The biofilm was not disturbed during this period. On 15 February 2021, five larvae were removed from half of the tanks for use in a separate study. Experimental protocols involving the handling of fishes were carried out in accordance with US federal guidelines for the care and use of animals as described in the American Fisheries Society Use of Fishes in Research Committee [32].

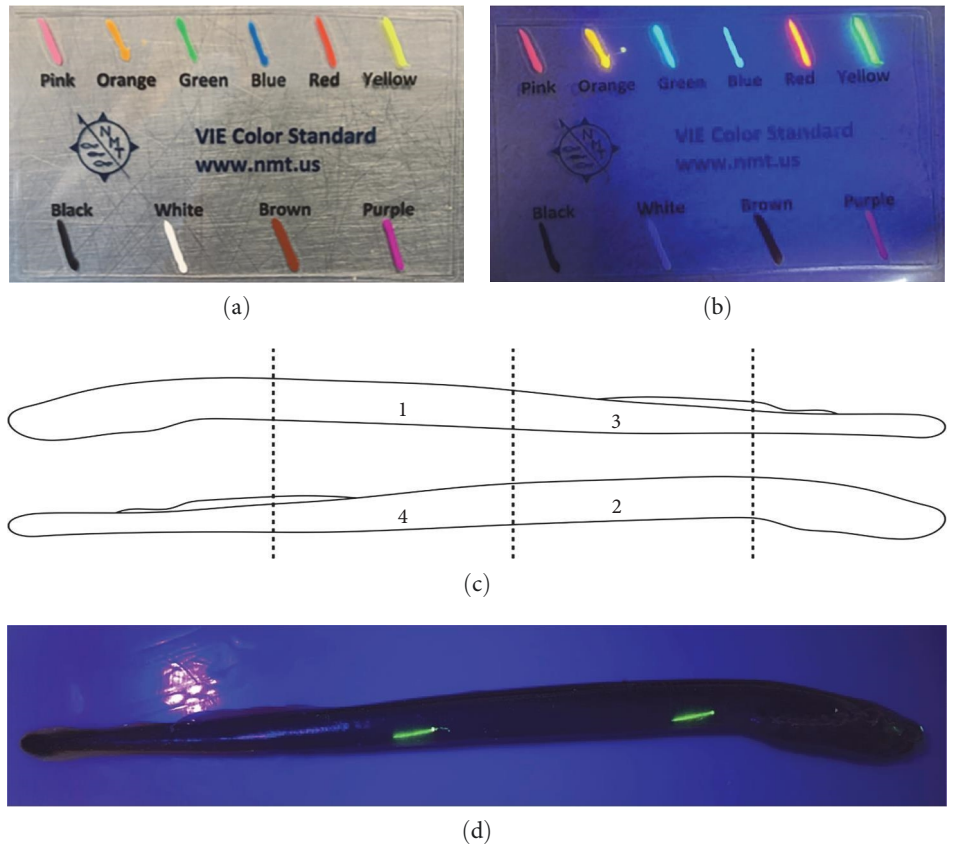

FIGURE 1: Marking scheme for larval sea lamprey *Petromyzon marinus* using visible implant elastomer (VIE) tags. VIE tag colors appear differently under visible light (a) and when fluoresced at 405 nanometers (nm) (b). Using five fluorescent colors and four body locations (c), it is possible to individually mark 150 larvae. For example, injecting yellow VIE beneath translucent skin at two locations on the right side of a larva provides the unique code Y2Y4 (d).

2.1.2. Data Treatment and Analysis. Growing degree days (GDD) were calculated using Equation (1):

$$\text{GDD} = T_{\overline{X}} - T_{\text{base}}, \tag{1}$$

where $T_{\overline{X}}$ is the mean daily temperature, and T_{base} is 5°C. We selected the base temperature from previous studies of sea lamprey growth that concluded little to no growth was achieved below 5°C [33]. Any days with a negative GDD value were recorded as zero. A one-way analysis of variance (ANOVA) was used to test for differences in larval size between feed treatments at the outset of the experiment. A repeated measures ANOVA with Greenhouse–Geisser correction was used to compare larval size (length and weight) between three time periods: 0, 90, and 270 days. Condition factor (K) was also calculated using a lamprey-specific correction factor [11] using Equation (2):

$$K = \frac{W \times 10^5}{L^{2.6}}, \tag{2}$$

and compared between sample periods, where W is the wet weight of a larva (g), and L is the length (mm TL). Post hoc Tukey honestly significantly different (HSD) tests were used to identify any significant effect of feed treatment on growth, using an $\alpha = 0.05$.

2.2. Experiment 2—Growth and Survival in Relation to Diet and Water Temperature Regimen

2.2.1. Experimental Design. Larval sea lamprey ($n = 108$; length = 90–110 mm TL) were collected from the Pigeon River, Cheboygan County, MI, with backpack electrofishers (ABP-2 ETS, Madison, WI) between 30 and 31 August 2022 and brought directly to HBBS. Larvae were anesthetized using a 0.1 g L^{-1} solution of buffered tricaine methanesulfonate MS222 (with sodium bicarbonate) and weighed (g), measured (mm TL) and individually tagged subcutaneously using visible implant elastomer (Northwest Marine Technology Inc., WA). Tags were prepared according to the manufacturer's instructions and combinations were derived using the program SalaMarker (MacNeil et al. [34]; Figure 1). Larvae were then transferred to a recovery tank and monitored for 24 hr. The experiment occurred between 14 September 2022 and 19 December 2022. Larvae were randomly distributed among 36 tanks, each with a surface area of 0.12 m^2 and a volume of 10.8 L ($n = 3$ $tank^{-1}$; density = 25 m^{-2}; biomass = 31.2 g m^{-2}; Table 1). Tanks were provided with commercial play sand (Quikrete premium play sand, Atlanta, GA) sifted to <500 μm and a depth of 7–7.5 cm and continuously

supplied with Lake Huron water at a rate of 250 mL min^{-1}. Water height was set to 10.2 cm, providing a turnover rate of 56 min. Water temperature and DO levels were measured daily using a dissolved oxygen meter (YSI Pro20 YSI Inc., OH).

Two feed treatments (yeast, Red Star, WI; yeast and 50% menhaden fish meal by weight, Omega Protein Inc., VA) and three temperature treatments (8, 15, and 22°C) were distributed across the 36 experimental tanks in blocks to minimize tank effects, resulting in an $N = 6$ for each feed and temperature combination. Feed ration was provided as either 2 g of yeast per week or 1 g yeast + 1 g fish meal, distributed over 3 days (Monday, Wednesday, and Friday) by adding directly to the tank water surface (Table 1). Water inflow to tanks was turned off for 2 hr, and feed was mixed at the surface using a plastic paddle for 1 min. At the conclusion of the experiment, larvae were recovered from the sediment using a net and anesthetized to re-identify individuals using VIE tags. Larvae were weighed, measured, and returned to holding tanks to recover. All procedures were carried out in accordance with Michigan State University's Animal Care and Use Committee requirements (ID: PROTO202200101).

2.2.2. Data Treatment and Analysis. To determine if between-study comparisons were appropriate, a one-way ANOVA was used to test for differences in initial larval size between treatments. The change (Δ) in length, weight, and condition factor (K) from the outset to day 96 was compared between treatments using a two-way ANOVA that included feed treatment and temperature regimen as factors as well as their interaction. Post hoc Tukey HSD tests were used to identify the significant effect of feed and temperature on growth, using an $\alpha = 0.05$. Levene's Test of Homogeneity of Variance was used to compare the Δ in length, weight, and condition factor (K) in Experiment 2.

3. Results

Larval sea lamprey differed significantly in starting size between Experiments 1 and 2 (ANOVA, length: $p < 0.001$; weight: $p < 0.001$; K: $p < 0.001$). In Experiment 1, larvae were larger and had a mean initial length of 104.9 ± 2.7 mm TL, weight of 1.88 ± 0.22 g, and mean K of 0.82 ± 0.14, whereas in Experiment 2, larvae measured 99.9 ± 5.4 mm TL, 1.26 ± 0.22 g, and had a K of 1.05 ± 0.09.

3.1. Experiment 1—Growth and Survival in Relation to Diet at Ambient Water Temperature. Diet influenced growth in larval sea lamprey, with the highest length and mass gain observed in the size of the animals fed yeast. Larvae did not differ in size across treatments at the start of the experiment (length: $p = 0.567$; weight: $p = 0.476$; K: $p = 0.22$).

Diet had a significant influence on the change in larval size, with larvae fed on yeast being significantly longer and heavier than larvae fed on yeast + wheat flour or rice flour supplements (Tukey HSD, $p < 0.001$, both; Figure 2). We found no differences in either the length or weight of larvae that were provided with either wheat flour or rice flour supplements (Tukey HSD, $p = 0.486$ and $p = 0.459$, respectively). The condition factor was not significantly different between pair-wise tests of feed treatments after either 90 or 270 days. No mortality occurred during the experiment.

Larval sea lamprey were significantly larger following 270 days of rearing in the laboratory compared to Day 0 (length: $p < 0.001$; weight: $p < 0.001$; K: $p = 0.005$; Figure 2). However, most of the growth was restricted to the first 90 days, likely because there were 0 GDD after this (Days 0–90 = 572 GDD, 3.3–14.6°C; Days 90–270 = 0 GDD, 0–4.2°C; Figure 3). After 90 days, larvae were significantly longer and heavier than at the outset of the experiment, but there was no appreciable growth between 90 and 270 days (length: $p = 1$; weight: $p = 0.076$). Over the first 90 days, larvae grew an average of 35.3 ± 4.2 mm TL and 2.19 ± 0.87 g, but between 90 and 270 days, they grew 0.5 ± 0.2 mm TL and decreased in weight by 0.11 ± 0.05 g.

3.2. Experiment 2 —Growth and Survival in Relation to Diet and Water Temperature Regimen. Diet and water temperature both had a significant effect on larval growth in terms of length ($p < 0.001$), mass ($p < 0.001$), and K ($p < 0.001$) (Figure 4). After a period of 90 days, larval length increased significantly (diet: $p = 0.014$; temperature: $p < 0.001$; Figure 4(a)); however, larval mass was only significantly affected by water temperature (diet: $p = 0.257$; temperature: $p < 0.001$; Figure 4(b)). The longest larva after 90 days was fed on yeast alone (133 mm TL), and the greatest increase in length was also observed in an individual fed on yeast alone (+34 mm). Larvae reared at 15 and 22°C were significantly longer than those at 8°C (Tukey HSD, $p < 0.001$, both). No differences in length between larvae reared in 15 vs 22°C water ($p = 0.976$; Figure 4(b)) were observed, but larvae reared at 15°C were heavier than those in 22°C ($p = 0.035$; Figure 4(b)). A weak but nonsignificant interaction between diet and temperature was present (length: $p = 0.097$; mass: $p = 0.954$).

Across all treatments, larvae did not differ in size at the beginning of the experiment (length: $p = 0.813$; mass: $p = 0.9119$; K: $p = 0.843$); however, it was noted that larvae reared in warmer water conditions (15 and 22°C) exhibited higher levels of variation in growth after 90 days compared with animals reared at 8°C. Levene's test indicated that the variance in Δ length ($p < 0.001$) and Δ weight ($p = 0.038$) were not equal between temperature treatments, but the variance in Δ K was equal ($p = 0.647$). One larva was not recovered at the end of the experiment and was presumed dead or escaped.

4. Discussion

The rearing of larval lampreys to address management objectives (e.g., stocking to bolster low population size) has been an ongoing research effort for several lamprey species in recent decades [15, 23]). However, laboratory-based efforts involving long-term maintenance of wild-caught Great Lakes larval sea lamprey are typified by low rates of growth. Previous studies obtained growth rates of just 0.03 and 0.07 mm day^{-1} over the course of 12 months [35, 36]. Using a combination of prepared, commercially available food sources, as well as constant or ambient temperatures, we were able to

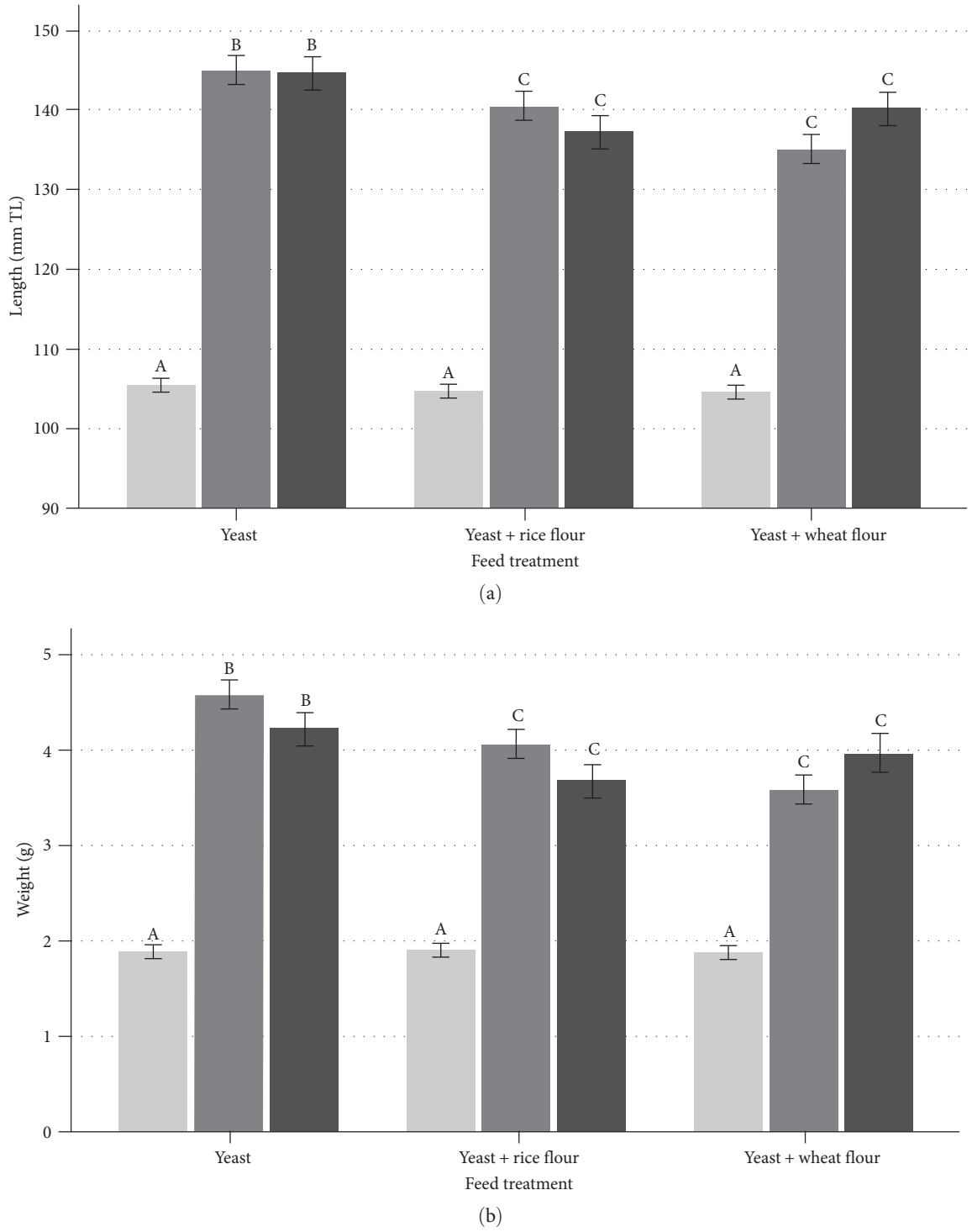

FIGURE 2: Mean and confidence intervals for the length (a) and body mass (b) of larval sea lamprey *Petromyzon marinus* in Experiment 1 (ambient temperature) at the outset (white bars) and after 90 days (gray, 3.3–14.6°C) and 270 days (dark gray, 0–4.2°C) of rearing. Letters denote significant differences between treatments.

demonstrate that net positive growth and 100% survival of larval sea lamprey are possible in a controlled environment. Under ambient temperatures over a 9-month period, we observed average growth rates of 0.15 mm day^{-1} (yeast), 0.13 mm day^{-1} (yeast + wheat flour), and 0.12 mm day^{-1} (yeast + rice flour). Using controlled temperatures over a 3-month period, we obtained similar growth rates for yeast (0.13 mm day^{-1}) and yeast + fish meal (0.09 mm day^{-1}). Barron et al. [28] reported much higher daily growth rates (0.25–0.4 mm day^{-1}) for young-of-the-year (YOY) Pacific lamprey reared in captivity under constant temperature and receiving some portion of fish effluent and similarly high growth rates (0.39–0.54 mm day^{-1}) were observed using

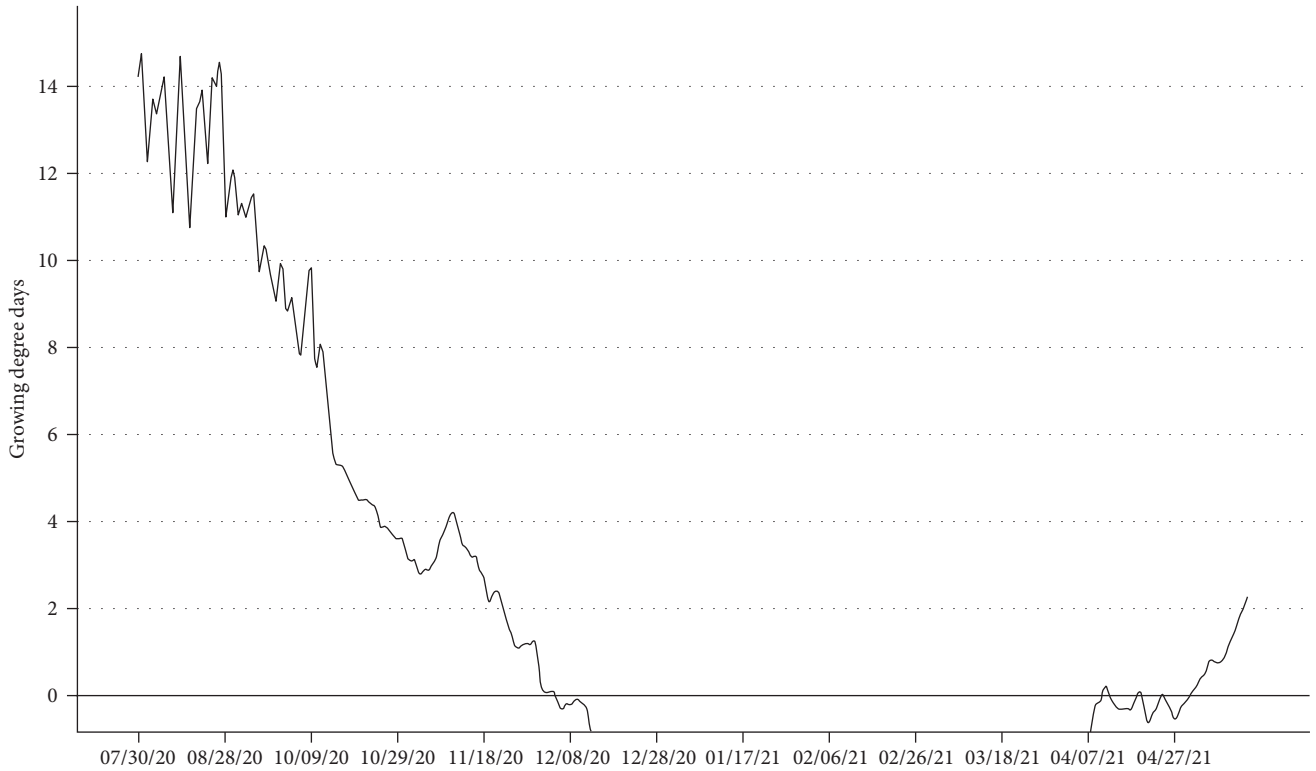

FIGURE 3: Growing degree days (GDD) with a base temperature of 5°C during Experiment 1. The black horizontal line indicates the point at which no larval sea lamprey *Petromyzon marinus* growth was expected to occur.

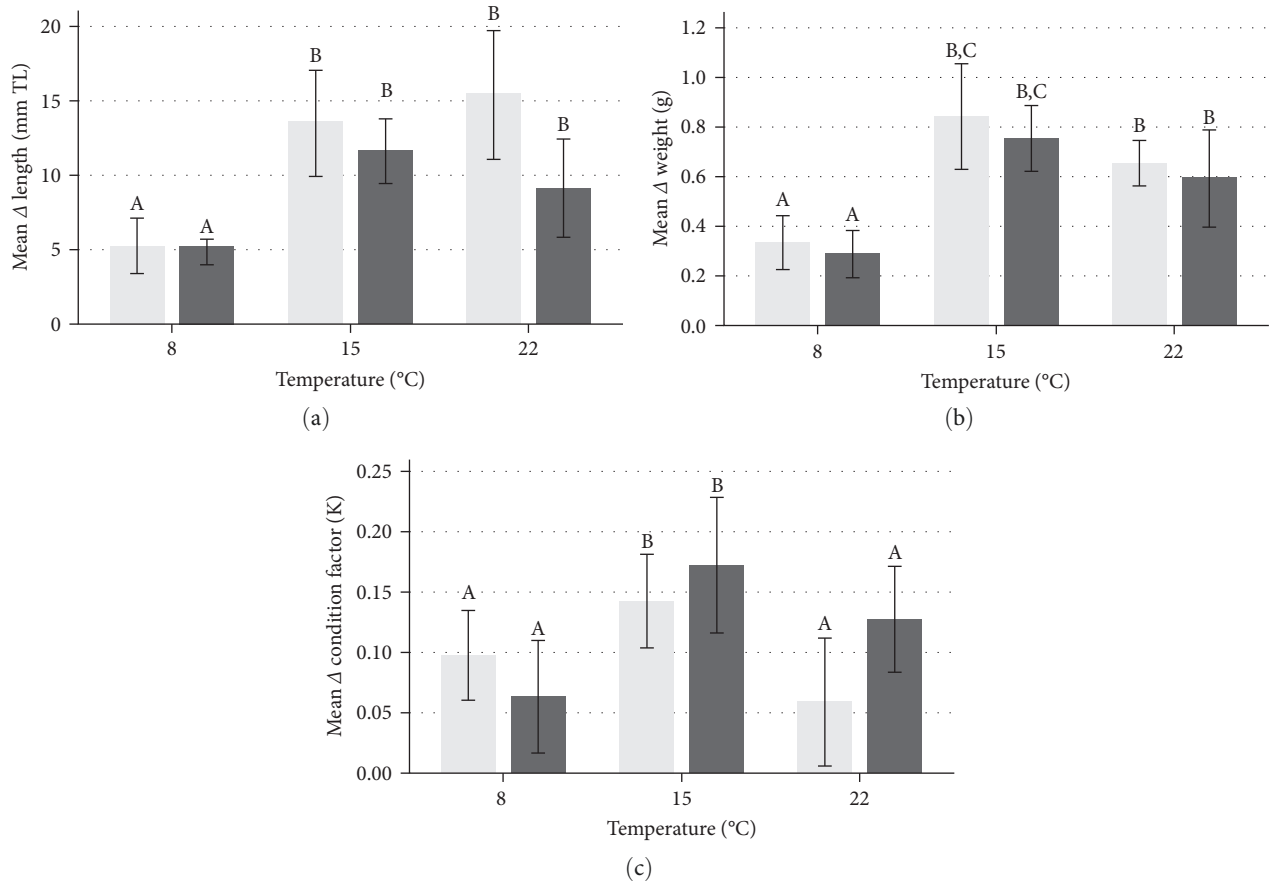

FIGURE 4: Mean difference in length (a), weight (b), and condition factor (c) with confidence intervals for larval sea lamprey *Petromyzon marinus* reared under controlled temperatures (8, 15, 22°C) and provided with yeast (white bars) or yeast + fish meal (gray bars) as a food source in Experiment 2. Letters denote significant differences between treatments.

larger sized 3-year-old larvae which grew from ~66 mm to 99–113 mm after only 84 days of rearing [28]. Lampman [25–27] also observed higher daily growth rates (0.28–0.58 mm day^{-1}) from laboratory studies spanning 2–3 months using artificially propagated Pacific lamprey YOY and age-1 larvae with a variety of supplemental feeds. Using wild-caught Pacific lamprey larvae (64–145 mm TL), Jolley et al. [37] observed mean daily growth rates of up to 0.18 mm day^{-1} using ambient temperatures and a salmon carcass analog over a period of 6 months. In our study, a relatively short period of time (90 days) was sufficient to observe significant changes to the animal's size, both in terms of length and weight. Larval lampreys grow slowly in natural environments, typically only increasing ~100 mm over the course of 3–5 years [22], which would average ~0.05–0.09 mm day^{-1}. Establishing protocols that produce observable and statistically significant changes in larval size over short timeframes can help to enable the rapid testing of a range of environmental conditions necessary to reveal optimal growth conditions for sea lamprey.

Based on measurements of growth (length, weight, condition factor) under our tank conditions, the addition of supplementary feeds may not be necessary in rearing large sea lamprey larvae. We found that the addition of supplementary food sources in combination with yeast had no significant positive impact on growth over yeast alone. Sutton and Bowen [38] found larval sea lamprey assimilate food slowly, suggesting more food supplied in a single period may not necessarily equate with higher rates of growth. We also found that the condition factor was not influenced by feed type. This result contrasts with findings in Pacific lamprey rearing studies that showed higher growth and/or higher proportions of whole-body lipids when animals were given supplementary feed (e.g., Otohime, alfalfa pallets, wheat, and other flour products) in addition to yeast [24–27]. Jolley et al. [37] observed higher growth in larvae provided salmon carcass analog, which had the highest protein and second highest lipid content of the different diets provided. Thus, the benefit of supplemental feed to sea lamprey may be influenced by the nature of the supplement itself. Future studies with larval sea lamprey should address important methodological differences employed in the study of larval Pacific lamprey growth. Specifically: (1) reduce the height of water above the sediment (e.g., feed may have been diluted due to higher water volume), (2) increase feed ration (e.g., 3–6 times higher feed rate for Pacific lamprey, and (3) increase flow rates (e.g., 10 times lower flow rates may have resulted in suboptimal dissolved oxygen levels).

Temperature has a profound effect on the growth of larval lampreys, with variation in growth rates within species evident when their geographic range encompasses multiple climatic regimes or several degrees of latitude [22, 39]. In the Great Lakes region, larval sea lamprey inhabiting warmer tributaries of Lakes Ontario and Erie exhibit higher growth rates compared to larvae inhabiting the colder tributaries of Lakes Huron, Michigan, and Superior [22]. Growth of larval lamprey in temperate regions is also strongly seasonal, with maximum growth occurring in summer-fall, declining and then ceasing in winter before resuming in spring [40]. In our study, we observed larval growth under both constant temperature and ambient temperature conditions. Water temperatures in Experiment 2 were consistent with stream temperatures in May through October in the Great Lakes; we observed the lowest growth rates at 8°C which corresponds to spring temperatures. Larvae in Experiment 2 were longest when held at 22°C but heaviest and with a higher condition factor at 15°C, which is consistent with the estimated fundamental thermal niche of sea lamprey as falling close to or between 17.8 and 21.8°C [41]. Similarly, larval Arctic lamprey *Lethenteron camtschaticum* also exhibited the highest growth rates at 18°C [42], and Pacific lamprey larvae grew quickly at 14−19°C [28, 30], suggesting larval lamprey growth may be optimized under either "coldwater" or "coolwater" conditions.

Recent work demonstrated that an accurate method for estimating the length-at-age of sea lamprey in the Great Lakes includes GDD in the highest-performing model, with 5°C assumed to be the species' base temperature in that study [33]. Our data support the validity of this assumption, as little to no growth was evident in our captive larvae between November and May when ambient water temperature ranged from 0 to 4.2°C. However, it must be noted that during the period when colder temperatures were experienced, food rations were lower, and densities were higher. So, these conditions could conceivably have had an effect. Regardless, European brook lamprey *Lampetra planeri* were found to have extremely low assimilation efficiencies of lipid (8%), protein (3.9%), and carbohydrates (6.2%) at 5°C [43], and it is reasonable to anticipate similar low efficiencies occur in larval sea lamprey. Consistent with this expectation, larval sea lamprey assimilation efficiency was found to be highest in natural streams in May–October (72%) and lowest in November–March (53%) [38]. Despite the apparent simplicity of the external morphology of larval lampreys, these animals are capable of thermoregulatory behavior similar to other vertebrates by moving to detect preferred temperature regimes [44]. Although coldwater temperatures (e.g., 5°C) appear not to be detrimental to the health of larval sea lamprey, in the context of rearing the species quickly, growth is likely to be most rapid when water is provided at temperatures >15°C. Additional studies using a more finely graded range of temperatures would be useful in determining if a narrower optimal temperature for growth exists.

Larval lamprey growth in the wild has been associated with a multitude of biotic and abiotic factors operating at the micro- and macrohabitat scale [22, 45]. Likely, the relative importance of any given factor and their varied interactions will fluctuate spatiotemporally. For example, larval sea lamprey rely on allochthonous material to fuel growth in some rivers where riparian forests are dense [46] but use autochthonous material and aged organic matter to a greater extent in rivers surrounded by more agriculture [47, 48]. In our study, we found no interaction between the source of food and the temperature to which larval sea lamprey were exposed to. These data suggest that the composition of the food was of lesser importance in fueling larval growth than the prevailing temperature, supporting the notion that the

availability of a source of ingestible material may be more critical than the nature of the material itself. If true, this result could indicate that in a captive-rearing environment, larvae derive most of their energy from bacterial and/or fungal populations breaking down and adhering to ingested particles [49] and that warmer environmental temperatures may be more conducive to bacterial and/or fungal growth. In Experiment 1, larvae fed yeast alone received a lower quantity of food compared to the other treatments, yet they were, in fact, larger at the end of the experiment. This finding indicates more food does not equate to increased growth and suggests that perhaps, in this instance, a greater quantity of food was, in fact, negatively impacting larvae (e.g., due to poor substrate conditions or dilution of preferred yeast content). As well as determining the upper threshold for the concentration of feed provided, future studies could examine the exact nature of what larval lampreys are ingesting vs. assimilating to fuel growth relative to what is provided to them.

Larval lamprey growth can be influenced by animal density to a significant degree, with those experiencing high densities subject to slower growth [22]. The mechanism that underpins density-dependent growth remains elusive, and there have been studies that indicate interference competition via physical [50] or chemical [51] cues may play a role. At the conclusion of Experiment 2, our data showed that larval sea lamprey reared in warmer water exhibited a greater degree of body size variation among individuals compared to those held in cooler water, despite the food ration and larval abundance being the same between temperature treatments. These data suggest several possible phenomena were occurring. First, resource competition could be apparent in tanks experiencing warmer conditions (e.g., some individuals were better able to exploit available food sources, reducing access by others), or second, some larvae have higher activity rates and were increasing rates of movement through the substrate disturbing others and reducing the overall time spent feeding and assimilating food [50]. The standard metabolic rate of larval Pacific lamprey increases with temperature [52], and in our study, the two warmer temperature treatments encompass the sea lamprey's fundamental thermal niche (17.8–21.8°C; [41]). Therefore, lastly, the warmer conditions may have resulted in increased metabolism that, via interactions with food availability (negative or positive), differentially influenced the growth of individuals. For example, an animal with high metabolism and access to sufficient food could grow quickly, whereas an animal with high metabolism and insufficient access to food could lose mass. Assimilation efficiency would also be greater in larval sea lamprey at higher temperatures, resulting in more efficient nutrient absorption and growth [38].

The implications of variation in larval sea lamprey growth rate revealed here are also important to sea lamprey control. For example, rapidly growing larvae could metamorphose prior to planned pesticide applications on a 3–4-year cycle, as was suspected in the Chippewa River, east-central MI [53]; therefore, a clearer understanding of growth rate would improve the planning of treatments. The growth rate could also potentially be under selection by the constant and severe mortality caused by pesticide applications, and understanding the genetic components of growth may be important in limiting the evolution of pesticide resistance [54]. There are also possibilities that variation in growth rate is sex-specific [55, 56] or associated with feeding rate as a parasitic juvenile, perhaps resulting in less effective control (in terms of reduced fecundity of the population) or individuals that are disproportionately more damaging to the fishery. Larval rearing of sea lamprey for research and management on a large scale will require identifying and optimizing a range of factors that influence growth, not only those highlighted here. However, prior research on Pacific lamprey has provided a significant jumpstart on this process and highlights that knowledge exchange between those involved in lamprey aquaculture for conservation and control can be extremely beneficial.

Data Availability

Data are available on request.

Disclosure

Any use of trade, product, or firm name is for descriptive purposes only and does not imply endorsement by the US Government.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] FAO, *The State of World Fisheries and Aquaculture 2022*, FAO, Rome, 2022.

[2] J. T. Patterson, "The growing role of aquaculture in ecosystem restoration," *Restoration Ecology*, vol. 27, no. 5, pp. 938–941, 2019.

[3] D. D. Mizuta, H. E. Fröhlich, and J. R. Wilson, "The changing role and definitions of aquaculture for environmental purposes," *Reviews in Aquaculture*, 2022.

[4] K. Overton, T. Dempster, S. E. Swearer, R. L. Morris, and L. T. Barrett, "Achieving conservation and restoration outcomes through ecologically beneficial aquaculture," *Conservation Biology*, Article ID e14065, 2023.

[5] H. E. Froehlich, R. R. Gentry, and B. S. Halpern, "Conservation aquaculture: shifting the narrative and paradigm of aquaculture's role in resource management," *Biological Conservation*, vol. 215, pp. 162–168, 2017.

[6] P. S. Maitland, C. B. Renaud, B. R. Quintella, D. A. Close, and M. F. Docker, "Conservation of native lampreys," in *Lampreys: Biology, Conservation and Control*, M. F. Docker,

Ed., vol. 1 of *Fish and Fisheries Series*, pp. 375–428, Springer, 2015.

[7] M. C. Lucas, J. B. Hume, P. R. Almeida et al., "Emerging conservation initiatives for lampreys: research challenges and opportunities," *Journal of Great Lakes Research*, vol. 47, no. Supplement 1, pp. S690–S703, 2021.

[8] D. A. Close, M. S. Fitzpatrick, and H. W. Li, "The ecological and cultural importance of a species at risk of extinction, Pacific lamprey," *Fisheries*, vol. 27, no. 7, pp. 19–25, 2002.

[9] R. Vikström, "Notes on lamprey propagation," Regional environment publications 252, pp 29, 2002.

[10] Hokkaido Fish Hatchery, "Manual for artificial hatching of Arctic lamprey," Inland Water Resources Department, pp 8, 2008.

[11] R. Lampman, M. Moser, A. Jackson, R. Rose, A. Gannam, and J. Barron, "Developing techniques for artificial propagation and early rearing of Pacific lamprey (*Entosphenus tridentatus*) for species recovery and restoration," in *Jawless Fishes of the World*, A. Orlov and R. Beamish, Eds., pp. 160–195, Cambridge Scholars Publishing, 2016.

[12] M. P. Wilkie, N. S. Johnson, M. F. Docker et al., "Invasive species control and management: the sea lamprey story," in *Conservation Physiology for the Anthropocene—Issues and Applications*, vol. 39B of *Fish Physiology*, pp. 489–579, Elsevier-Academic Press, Cambridge, MA, 2022.

[13] M. Gaden, C. O. Brant, and R. Lambe, "Why a great lakes fishery commission? The seven-decade pursuit of a Canada–U. S. fishery treaty," *Journal of Great Lakes Research*, vol. 47, no. Supplement 1, pp. S11–S23, 2021.

[14] J. E. Marsden and M. J. Siefkes, "Control of invasive sea lamprey in the Great Lakes, Lake Champlain, and Finger Lakes of New York," in *Lampreys: Biology, Conservation and Control*, M. Docker, Ed., vol. 38 of *Fish & Fisheries Series*, pp. 411–479, Springer, Dordrecht, 2019.

[15] M. L. Moser, J. B. Hume, K. K. Aronsuu, R. T. Lampman, and A. D. Jackson, "Lamprey reproduction and early life history: insights from artificial propagation," in *Lampreys: Biology, Conservation and Control*, M. F. Docker, Ed., vol. 2 of *Fish and Fisheries Series*, pp. 187–245, Springer, Dordrecht, 2019.

[16] J. B. Hume, P. R. Almeida, C. M. Buckley et al., "Managing native and non-native sea lamprey (*Petromyzon marinus*) through anthropogenic change: a prospective assessment of key threats and uncertainties," *Journal of Great Lakes Research*, vol. 47, no. Supplement 1, pp. S704–S722, 2021.

[17] L. H. Hanson, E. L. King Jr, J. H. Howell, and A. J. Smith, "A culture method for sea lamprey larvae," *The Progressive Fish-Culturist*, vol. 36, pp. 122–128, 1974.

[18] R. E. Lennon, "Artificial propagation of the sea lamprey, *Petromyzon marinus*," *Copeia*, vol. 3, pp. 235-236, 1955.

[19] D. Ferreira-Martins, J. Champer, D. W. McCauley, Z. Zhang, and M. F. Docker, "Genetic control of invasive sea lamprey in the Great Lakes," *Journal of Great Lakes Research*, vol. 47, no. Supplement 1, pp. S764–S775, 2021.

[20] S. R. Lantz, R. A. Adair, R. Amberg et al., "Next-generation lampricides: a three-stage process to develop improved control tools for invasive sea lamprey," *Canadian Journal of Fisheries and Aquatic Science*, vol. 79, pp. 692–702, 2022.

[21] M. J. Siefkes, N. S. Johnson, and A. M. Muir, "A renewed philosophy about supplemental sea lamprey controls," *Journal of Great Lakes Research*, vol. 47, no. Supplement 1, pp. S742–752, 2021.

[22] H. A. Dawson, B. R. Quintella, P. R. Almeida, A. J. treble, and J. C. Jolley, "The ecology of larval and metamorphosing lampreys," in *Lampreys: Biology, Conservation and Control*, M. F. Docker, Ed., vol. 1 of *Fish and Fisheries Series*, pp. 75–137, Springer, Dordrecht, 2015.

[23] R. T. Lampman, A. N. Maine, M. L. Moser, H. Arakawa, and F. B. Neave, "Lamprey aquaculture successes and failures: a path to production for control and conservation," *Journal of Great Lakes Research*, vol. 47, no. Supplement 1, pp. S201–215, 2021.

[24] J. M. Barron, R. G. Twibell, H. A. Hill, K. C. Hanson, and A. L. Gannam, "Development of diets for the intensive culture of Pacific lamprey," *Aquaculture Research*, vol. 47, pp. 3899–3906, 2016.

[25] R. Lampman, "Development of artificial propagation methods for production of juvenile Pacific lamprey (Entosphenus tridentatus) for the use in research associated with Section 4.2.3 of the Rocky Reach Pacific Lamprey Management Plan," In Yakama Nation Pacific Lamprey Project 2017 annual report. Prepared for the Public Utility District No. 1 of Chelan County, Wenatchee, WA, 35 pp, 2017.

[26] R. Lampman, "Development of artificial propagation methods for production of juvenile Pacific lamprey (Entosphenus tridentatus) for the use in research associated with Section 4.2.3 of the Rocky Reach Pacific Lamprey Management Plan," Yakama Nation Pacific Lamprey Project 2017 annual report. Prepared for the Public Utility District No. 1 of Chelan County, Wenatchee, WA, 22 pp, 2018.

[27] R. T. Lampman, "Development of artificial propagation methods for production of juvenile Pacific lamprey (Entosphenus tridentatus) for the use in research associated with Section 4.2.3 of the Rocky Reach Pacific Lamprey Management Plan," Yakama Nation Pacific Lamprey Project 2018 annual report. Prepared for the Public Utility District No. 1 of Chelan County, Wenatchee, WA, 36 pp, 2019.

[28] J. Barron, R. Headley, and A. Gannam, "Development of artificial propagation methods for production of juvenile Pacific lamprey (*Entosphenus tridentatus*) for the use in research associated with Section 4.2.3 of the rocky reach pacific lamprey management plan," Annual report (Contract No. 15-105). Prepared for the Chelan County Public Utility District, Wenatchee, WA. 2018, 2019.

[29] J. Barron, R. Headley, K. Hawke et al., "Development of artificial propagation methods for production of juvenile Pacific lamprey (*Entosphenus tridentatus*) for the use in research associated with Section 4.2.3 of the Rocky Reach Pacific Lamprey Management Plan," Annual report (Contract No. 15-105). Prepared for the Chelan County Public Utility District, Wenatchee, WA. 2017, 2018.

[30] J. Barron, R. Headley, and A. Gannam, "Development of artificial propagation methods for production of juvenile Pacific lamprey (*Entosphenus tridentatus*) for the use in research associated with Section," 4.2.3 of the Rocky Reach Pacific Lamprey Management Plan annual report (Contract No. 15-105). Prepared for the Chelan County Public Utility District, Wenatchee, WA. 2019, 2020.

[31] A. K. Jubar, R. J. Frank, D. A. Keffer, F. B. Neave, M. J. Symbal, and T. B. Steeves, "Prioritizing lampricide treatments in Great Lakes tributaries and lentic areas during 2000–2017," *Journal of Great Lakes Research*, vol. 47, Supplement 1, pp. S238–S246, 2021.

[32] American Fisheries Society, *Guidelines for the Use of Fishes in Research*, American Fisheries Society, Bethesda, Maryland, 2014.

[33] H. A. Dawson, C. Higgins-Weier, T. B. Steeves, and N. S. Johnson, "Estimating age and growth of invasive sea

lamprey: a review of approaches and investigation of a new method," *Journal of Great Lakes Research*, vol. 47, no. Supplement 1, pp. S570–S579, 2021.

[34] J. E. MacNeil, G. Dharmarajan, and R. N. Williams, "Sala-Marker: a code generator and standardized marking system for use with visible implant elastomers," *Herpetological Conservation and Biology*, vol. 6, no. 2, pp. 260–265, 2011.

[35] S. P. Murdoch, F. W. H. Beamish, and M. F. Docker, "Laboratory study of growth and interspecific competition in larval lampreys," *Transactions of the American Fisheries Society*, vol. 120, pp. 653–656, 1991.

[36] S. P. Murdoch, M. F. Docker, and F. W. H. Beamish, "Effect of density and individual variation on growth of sea lamprey (*Petromyzon marinus*) larvae in the laboratory," *Canadian Journal of Zoology*, vol. 70, pp. 184–188, 1992.

[37] J. C. Jolley, C. T. Uh, G. S. Silver, and T. A. Whitesel, "Feeding and growth of larval Pacific lamprey reared in captivity," *North American Journal of Aquaculture*, vol. 77, no. 4, pp. 449–459, 2015.

[38] T. M. Sutton and S. H. Bowen, "Significance of organic detritus in the diet of larval sea lamprey in the Great Lakes Basin," *Canadian Journal of Fisheries and Aquatic Science*, vol. 51, pp. 2380–2387, 1994.

[39] I. C. Potter, "Ecology of larval and metamorphosing lamprey," *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 37, pp. 1641–1657, 1980.

[40] I. C. Potter, "The life cycles and ecology of Australian lampreys of the genus *Mordacia*," *Journal of the Zoological Society of London*, vol. 161, pp. 487–511, 1970.

[41] J. A. Holmes and P. Lin, "Thermal niche of larval sea lamprey, *Petromyzon marinus*," *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 51, pp. 253–232, 1994.

[42] H. Arakawa and S. Yanai, "Upper thermal tolerance of larval Arctic lamprey (*Lethenteron camtschaticum*)," *Ichthyological Research*, vol. 68, pp. 158–163, 2021.

[43] J. W. Moore and I. C. Potter, "A laboratory study on the feeding of larvae of the brook lamprey *Lampetra planeri* (Bloch)," *Journal of Animal Ecology*, vol. 45, no. 1, pp. 81–90, 1976.

[44] W. W. Reynolds and M. E. Casterlin, "Behavioral thermoregulation by ammocoete larvae of the sea lamprey (*Petromyzon marinus*) in an electronic shuttlebox," *Hydrobiologia*, vol. 61, no. 2, pp. 145–147, 1978.

[45] C. E. Torgersen and D. A. Close, "Influence of habitat heterogeneity on the distribution of larval Pacific lamprey (*Lampetra tridentata*) at two spatial scales," *Freshwater Biology*, vol. 49, no. 5, pp. 614–630, 2004.

[46] B. Hayden, M. Ferron, R. A. Cunjak, and K. Samways, "Fruit of the forest—larval sea lamprey *Petromyzon marinus* are fueled by allochthonous resources," *Journal of Fish Biology*, vol. 95, no. 3, pp. 781–792, 2019.

[47] T. M. Evans and J. E. Bauer, "Identification of the nutritional resources of larval sea lamprey in two Great lakes tributaries using stable isotopes," *Journal of Great Lakes Research*, vol. 42, no. 1, pp. 99–107, 2016.

[48] T. M. Evans, A. R. Bellamy, and J. E. Bauer, "Radioisotope and stable isotope ratios (Δ14C, $\int$15N) suggest lamprey growth is dependent on both fresh and aged organic matter in streams," *Ecology of Freshwater Fish*, vol. 28, no. 3, pp. 365–375, 2018.

[49] J. W. Moore and J. M. Mallatt, "Feeding of larval lamprey," *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 37, pp. 1658–1664, 1980.

[50] S. Bowen and M. R. Yap, "Crowding reduces feeding rate, effectiveness of diet selection, and efficiency of digestion by northern brook lamprey ammocetes (*Ichthyomyzon fossor*)," *Environmental Biology of Fishes*, vol. 101, pp. 1385–1394, 2018.

[51] R. Rodríguez-Muñoz, A. G. Nicieza, and F. Braña, "Density-dependent growth of sea lamprey larvae: evidence for chemical interference," *Functional Ecology*, vol. 17, pp. 403–408, 2003.

[52] P. Carilli, *The Physiological Response of Larval Pacific Lamprey to Chronic Heat Stress*, Masters Thesis, Oregon State University, p. 32, 2020.

[53] S. B. Morkert, W. D. Swink, and J. G. Seelye, "Evidence for early metamorphosis of sea lampreys in the Chippewa river, Michigan," *North American Journal of Fisheries Management*, vol. 18, pp. 966–971, 1998.

[54] E. S. Dunlop, M. R. Christie, R. McLaughlin, and T. B. Steeves, "Life history evolution of sea lamprey is predicted to reduce the effectiveness of pesticide control," *Journal of Great Lakes Research*, vol. 47, no. Supplement 1, pp. S259–S271, 2021.

[55] N. S. Johnson, W. D. Swink, and T. O. Brenden, "Field study suggests that sex determination in sea lamprey is directly influenced by larval growth rate," *Proceedings of the Royal Society B*, vol. 284, no. 1851, Article ID 20170262, 2017.

[56] M. F. Docker and I. C. Potter, "Life history evolution in lampreys: alternative migratory and feeding types," in *Lampreys: Biology, Conservation and Control*, M. F. Docker, Ed., vol. 37 of *Fish & Fisheries Series*, pp. 287–409, Springer, Dordrecht, 2019.