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An evaluation of potential climate change impacts on the larval metamorphosis of Pacific Lamprey (*Entosphenus tridentatus*)

2021 Annual Report

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On the cover: *Larval Pacific Lamprey rearing tanks inside the Bonneville Dam Smolt Monitoring Facility on the Columbia River. (Photo Credit: Tim Blubaugh, U.S. Fish & Wildlife Service)*

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Abstract – Larval Pacific Lamprey must undergo metamorphosis to reproduce. Physiological and environmental factors appear to impact when this metamorphosis occurs. In other lamprey species, larvae that reach a certain threshold for energy storage appear to initiate metamorphosis when exposed to cold winter water temperatures followed by a rise in spring water temperatures. Since climate change driven increases in minimum winter water temperatures may affect the magnitude of this seasonal rise in temperatures, there is concern this might affect the rate of metamorphosis for larval Pacific Lamprey. Therefore, we conducted a laboratory study that examined rates of metamorphosis between lamprey experiencing natural winter water temperatures, and those who were exposed to minimum winter water temperatures that were truncated and prevented from falling below approximately 9°C. Larval Pacific Lamprey were collected (N = 90) and used for the experiment whose length and weight suggested that they were likely to transform in the autumn or winter of the following year. There was no significant difference between the rate of metamorphosis between fish exposed to natural water temperatures (37%) and warmed minimum water temperatures (43%), suggesting that changes in the magnitude of winter water temperatures may not have direct negative effects on the metamorphic rates of larval Pacific Lamprey. Our selective collection of larval Pacific Lamprey based on length and weight relationships resulted in relatively high metamorphic rates for reared fish compared to rates observed in other larval lamprey collections. Larvae that did not undergo metamorphosis in the autumn were of a size that models predict will undergo transformation in the following year. A new cohort of collected larvae, and fish from the original larval cohort that did not undergo metamorphosis, will be reared the following year to see if these results are repeatable and to examine if large larvae from the original cohort will transform.

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Introduction

All species of lamprey undergo some type of metamorphosis at the end of their larval life stage to advance to adulthood and reproduce. In anadromous Pacific Lamprey this transformation includes a juvenile life stage when individuals emigrate from freshwater to the ocean and begin parasitic feeding before returning as adults to spawn in fresh water. Pacific Lamprey typically complete larval metamorphosis between the months of August and December of a given year (McGree et al. 2008). Different environmental and physiological factors have been shown to influence if and when metamorphosis occurs each year. Some suggest that for larvae to initiate or complete metamorphosis, they may require a period of relatively cold water temperature in the winter, followed by a sharp rise in water temperature during the spring (similar to the requirement of many seeds for germination) (reviewed in Manzon et al. 2015). Previous studies of temperature effects on lamprey metamorphosis have used landlocked Great Lakes anadromous sea lamprey held in laboratory conditions with thermal regimes manipulated beyond what might occur naturally (constant high temperature, Holmes et al. 1994) or thermal regimes that wouldn't be expected as a result of climate change (i.e. using cool spring/summer thermal regimes resulting in low magnitude spring temperature rises, Holmes and Youson 1997; Youson et al. 1993). In many areas where larval Pacific Lamprey rear, climate change may result in warmer minimum temperatures in the winter and spring than larvae currently experience (Mote et al., 2005; Isaak et al. 2012; Abatzoglou et al., 2014; Yan et al. 2021). It is unclear if or how changes to minimum winter temperature patterns will affect metamorphosis of larval Pacific Lamprey. Therefore, we are conducting a laboratory-based investigation that evaluates the influence of winter water temperature on larval metamorphosis in Pacific Lamprey. We hypothesize that the incidence of metamorphosis for larvae reared under a current, natural winter temperature regime will be greater than the incidence of metamorphosis for larvae reared under an artificially warmed winter temperature regime meant to represent the potential increase of minimum winter temperatures resulting from climate change.

Methods

Larval Pacific Lamprey were collected during sampling events on October 22nd, October 29th, and November 9th 2020 in the Wind River, approximately 1 river kilometer (rkm) from its confluence with the Columbia River near Carson, WA. An AbP-2 backpack electrofisher (ETS Electrofishing Systems, LLC; Madison, WI) was used to capture lamprey embedded in sediment utilizing settings to bring lamprey out of the substrate (3:1 burst pattern, 3 pulse/sec, 25% duty cycle, 125 V). Captured larval lamprey were anesthetized with MS-222 in order to collect length and weight measurements and identify individuals to species (Docker et al. 2016). Only individuals were retained that, based on their length and weight relationship, were predicted to have a relatively high likelihood of completing metamorphosis the following summer (Whitesel et al. 2020). Retained larvae were transported to the U.S. Army Corps of Engineers (USACE) Smolt Monitoring Facility (SMF) at Bonneville Dam where 90 fish were randomly and evenly distributed among eight circular tanks with conical bottoms (76 cm diameter), resulting in each of the eight tanks receiving 11-12 individuals. The rearing tanks contained a sand substrate to enable burrowing (45kg, 3-12 cm depth range with sloped bottom) which was covered by water 7 cm deep. The rearing tanks used raw Columbia River water originating from the Bonneville Dam forebay that was passed through some combination of a degassing column and a sediment settling tank. Initial target flow rates of (1 l/min) were chosen to provide complete exchange approximately twice per hour within the rearing tanks. Lamprey were reared under a natural photoperiod provided by west facing windows until March 1st when work unrelated to this study required the room to be constantly illuminated. The addition of insulation to tanks on April 16th eliminated most light from entering the tanks. All larvae were fed twice a week with a slurry of active dry yeast and Otohime fish food (1.7-2.0 g/larvae and 0.4-0.5 g/larvae respectively) while water flow into the tank was usually shut off for one hour (range 0-5 hours). Alfalfa pellets (2.5-3.0 g/larvae) were initially added to the tanks during every feeding, but as the amount of organic material in the tanks accumulated this was reduced to biweekly in the middle of March. The organic matter and bacterial and fungal growth that accumulated on the sediment surface were broken up with gentle manual agitation at least every two weeks.

After fish were stocked in the tanks, four tanks were randomly assigned to the control group (natural water temperatures) and four were assigned to the treatment group (elevated or warmed water temperatures). Climate models suggest air temperature in the Columbia River basin could increase by at least 3 to 5°C under intermediate to extreme warming scenarios (Ahmadalipour et al. 2018), and current minimum water temperatures in Columbia River basin tributaries often reach 4°C. Therefore, we truncated low water temperatures at approximately 9°C in the warmed treatment tanks.

All fish were reared under a natural water temperature profile until the temperature decreased to approximately 9°C or below, when one or two submersible heaters (Finnex TH-0500S) in each treatment tank turned on to maintain minimum water temperatures at approximately 9°C using temperature controllers (Inkbird ITC-306T, resolution 0.1°C). Temperature loggers (HOBO® MX2201 or MX2202, Onset Computer Corporation, Bourn, MA) were imbedded under the sediment surface to monitor tank temperatures experienced by burrowed lamprey larvae.

Lamprey rearing habitat is both longitudinally distributed within streams and present in streams across a wide geographic area, so these fish can encounter a variety of thermal regimes across their geographic range. We compared tank temperatures to water temperatures in two nearby lower Columbia River tributaries to represent the conditions in rearing habitat near tributary confluences (Washougal River at 5.8 rkm, WA, https://fortress.wa.gov/ecy/eap/flows/station.asp?wria=28) and in upper tributaries (Wind River at 18.5 rkm, https://fortress.wa.gov/ecy/eap/flows/station.asp?wria=29). We also compared temperatures of the tanks to those measured in the Columbia River to better quantify how tank water sourced from the Bonneville Dam forebay diverged from river temperatures recorded at the closest continuously operating Columbia River gage (USGS gage # 14105700 at The Dalles, OR). Lamprey were removed from tanks by using electrofishing on August 23rd and October 27th 2021. Lamprey were anesthetized with MS-222 upon removal and fish were examined for weight, length, and external signs of metamorphosis. After examination in August, individuals still in the larval life stage were returned to their rearing tanks. We used changes in the distribution of fish size and condition as a proxy for lipid accumulation, thereby ensuring larval lamprey were expected to have the physiological capacity to transform based on reported values for Pacific Lamprey. Body condition was expressed as (Holmes et al. 1994):

$$\frac{weight\ (g)}{total\ length\ (mm)^3} \times 10^6$$

Finally, we compared the incidence of metamorphosis between replicates and treatment groups, and hypothesized that winter temperature differences would be great enough to see differences in metamorphic rates between treatments.

Results

Ideally water temperatures of the natural rearing tanks would match temperatures recorded near the water source in the Bonneville Dam forebay and would approximate temperatures in Columbia River tributaries where many larval Pacific Lamprey rear. Water temperatures in rearing tanks were strongly influenced by flow rates. We observed warming associated with warm natural air temperatures when flow was turned off for feeding. Initial efforts to maintain consistent temperatures among tanks were somewhat hampered by variable flow rates caused by frequent partial blockages in flow adjustment valves. As a result, the dates at which water temperatures first reached ≤9°C and heaters began operating varied among tanks over 11 days (Table 1). This temperature variability was reduced through target flow rate adjustments (1-2.5 L/min), modifications to the water supply plumbing, temperature controller recalibrations and probe repositioning, supplemental water heaters, disabling of room heaters, and tank insulation. As a result, the minimum mean daily temperature was similar among the 4 tanks of each treatment group and the last dates at which water temperatures were ≤9°C were similar among of all the tanks (Table 1). Although minimum winter water temperatures in treatment tanks were warmer than in control tanks during the treatment period (Table 1), a power outage caused all experimental group tanks to drop to the same temperature as control group tanks (range = 6.1-6.7°C and 6.3-6.6°C, respectively) for approximately 20 hours between January 11th and 12th, 2021.

Minimum daily water temperatures for the natural rearing tanks were slightly warmer than mainstem water temperatures for the 2021 water year (Figure 1). Natural rearing tanks remained above 9°C approximately 1 week later in the fall and exceed 9°C for a maximum of four days earlier in the spring compared to the 10 year median of daily mean temperatures recorded in the Columbia River at The Dalles (Table 1). Temperatures from downstream and upstream sections of nearby tributaries were cooler than mainstem river temperatures, typically dropping to ≤9°C three to six weeks earlier in autumn (Table 1; Figure 1) and usually rising above 9°C from nine days to five weeks later in the spring (Table 1; Figure 1). Cumulative temperatures (ATUs, Table 2), calculated while natural tank temperatures were diverting from the temperature of the warmed treatment tanks, shows that mean daily temperatures of the natural rearing tanks were closer to temperatures of the Washougal River and mainstem Columbia River compared to the warmed tanks and the cooler temperatures of the upper Wind River. Tributary temperatures were more variable than tank and mainstem Columbia River temperatures, so lamprey in both experimental and control group rearing tanks experienced less temperature fluctuations compared to any fish occupying tributary rearing habitat.

Overall, 369 larval Pacific Lamprey were collected near the mouth of the Wind River. We retained 90 fish that were most likely to metamorphose the following year based on a model of predictive length and weight thresholds. Among the individuals retained, lengths ranged from 91-135 mm (median = 117 mm) and weight ranged from 1.43-3.81 g (median = 2.59 g). Most retained fish (73 of 90, 81%) met or exceeded the minimum length, weight, and condition factor (CF) at which Whitesel et al. (2020) observed transformation for autumn-collected Pacific Lamprey larvae (102 mm, 2.0 g, 1.59 CF). All ninety fish were stocked into the experimental tanks. Known mortality of larval lamprey was low. One death occurred five days (November 14th 2020) after the last larvae were added to the rearing tanks and another was found in April 2021. Assuming one additional fish that was unable to be recovered at the end of the experiment died, we calculated 3% mortality of reared fish during the experiment (Table 3).

When we examined lamprey in August 2021, none were observed undergoing metamorphosis. There were no significant differences in size (length, weight) or condition among replicate tanks in each treatment group for the August examination, so individuals within replicate tanks were pooled to be visualized within treatment groups ($P > 0.05$, ANOVA). The size distribution of larval lamprey was approximately 40% longer than at their time of collection from the Wind River, and the weight distribution was approximately 300% greater. The distribution of fish condition during the August 2021 examination overlapped with, but skewed higher than, the distribution of fish condition observed upon collection (Figure 2).

When we examined lamprey in October 2021, 35 (40%) larvae had undergone metamorphosis and 52 (60%) had remained larvae among all tanks. Proportionately less larval Pacific Lamprey metamorphosed when reared on a natural temperature regime (37%, n=16) compared to those reared under the warm water treatment (43%, n=19). Lamprey were pooled across tanks for the statistical analysis, since tanks were not a significant factor in larval lamprey transformation within each treatment (Fisher's Exact Test, $p > 0.05$). Ultimately this difference in the proportion of lamprey larvae transformed between temperature treatments was not statistically significant (Fisher's Exact Test, $p = 0.66$). There were no significant differences in size (length, weight) or condition among replicate tanks in each treatment group for the October examination, so individuals within replicate tanks were pooled to be visualized within treatment groups ($P > 0.05$, ANOVA). In October, the size distribution for metamorphosed juveniles was shorter and of less weight compared to larvae that had not undergone metamorphosis (Figure 2). The distribution of fish condition was greater for juveniles that underwent metamorphosis compared to untransformed larvae.

Conclusion

Key factors regulating the initiation of larval lamprey metamorphosis in late summer and autumn include water temperatures in the winter and spring prior to transformation and lipid accumulation in the autumn of the previous year (Manzon et al. 2015). Fish that reach the appropriate amount of lipid accumulation, often measured by proxy as condition factor, can initiate metamorphosis when exposed to warming after fish experience low winter temperatures. For example, landlocked Sea Lamprey larvae of certain size thresholds (≥120 mm, 3.0 g, 1.45 CF) often transformed when held under natural winter and spring water temperatures (approximately 3 – 7°C [Holmes and Youson 1994], or 1 – 7°C [Holmes et al. 1994]; Youson et al. 1993) but did not transform under constant water temperatures (21°C) or when spring temperatures did not rise above 9°C (Holmes and Youson 1997). The temperature of both natural and warm tanks rose above 9°C by mid-April in our study, suggesting that temperatures were warm enough for metamorphosis. Similar rates of lamprey metamorphosis in tanks where cold winter temperatures were truncated suggests that the reduced magnitude of the temperature rise observed in this study is not an important factor in metamorphosis, similar to findings for Sea Lamprey (Holmes and Youson 1997), and that the warming of winter and spring water temperatures in the Columbia River basin may not have a direct adverse effect on the metamorphosis of larval Pacific Lamprey.

Larval Pacific Lamprey, like anadromous lamprey in general (Youson et al 1993), appear to have much lower size thresholds for metamorphosis (102 mm, 2.0 g, 1.59 CF; Whitesel et al. 2020) relative to other larval lamprey, and transformation rates of 2 – 21% have been reported for Pacific Lamprey ammocetes captive-reared under natural water temperatures (McGree et al. 2008; Whitesel et al. 2020). These fish were wild-caught and of an unknown age. Most fish we collected (73 of 90) exceeded the minimum length, weight, and condition factor at which Whitesel et al. (2020) observed transformation for autumn-collected Pacific Lamprey larvae. The percentage of fish that underwent metamorphosis in this study was higher than observed elsewhere (40%), suggesting the predictive power of the length weight relationship in autumn for the metamorphosis of larval Pacific Lamprey (Whitesel et al. 2020) is repeatable and useful.

Most of the larval lamprey that did not undergo metamorphosis by autumn of the study were large relative to the size at which lamprey would be expected to metamorphose the following year. Only one of the 52 fish that did not transform is under the 145 mm body length threshold at which the Whitesel et al. (2020) model would predict all larval lamprey to metamorphose the following year. These larvae that failed to metamorphose were retained and will be reared for another year to observe their adherence to the model. We are unaware of other reports of captured and reared larvae with similar size and condition characteristics failing to metamorphosis, but this could be due to reporting bias. Most Pacific Lamprey ammocetes transform between the ages of 5 and 7 (Dawson et al. 2015). However, fish reaching 100mm of total length are likely a minimum of 3 years old (Meeuwig and Bayer 2005), suggesting that many of these fish are old enough to transform. Low rates of metamorphosis for these fish the following year would suggest that other external factors necessary for larval metamorphosis are not present.

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Table 1. Winter water temperatures from lamprey rearing tanks and nearby representative stream gages. The stream gages monitored include the mainstem Columbia River USGS gage below The Dalles Dam, and two Columbia River tributary monitoring stations operated by Washington Department of Ecology. The treatment period for warmed rearing tanks is defined by the date range that heaters were operating (identified from patterns in hourly data readings). The earliest and latest dates that mean daily water temperature was ≤9°C represents the treatment periods for the natural rearing tanks and river gage temperatures. Minimum mean daily temperatures exclude data from the warmed treatment tanks during the power outage on January 11th and 12th 2021.

Tank	Treatment	Treatment Period			Minimum Mean Daily Temperature	
					°C	Date
1	Warmed	4 Dec	-	11 Apr	8.35	14 Feb
3	Warmed	13 Dec	-	11 Apr	8.01	9 Mar
5	Warmed	3 Dec	-	11 Apr	7.88	22 Feb
8	Warmed	5 Dec	-	11 Apr	8.22	22 Feb
2	Natural	6 Dec	-	8 Apr	4.47	14 Feb
4	Natural	5 Dec	-	7 Apr	4.46	15 Feb
6	Natural	11 Dec	-	11 Apr	4.46	14 Feb
7	Natural	5 Dec	-	11 Apr	4.39	14 Feb
Columbia River	2021 Water Year	30 Nov	-	11 Apr	3.2	19 Feb
Columbia River	Median Daily (2011-2020)	28 Nov	-	11 Apr	3.9	9 Feb
Washougal River	2021 Water Year	22 Oct	-	14 Apr	1.7	13 Feb
Washougal River	Median Daily (2011-2020)	8 Nov	-	20 Apr	4.3	2 Jan
Wind River	2021 Water Year	22 Oct	-	20 May	0.4	13 Feb
Wind River	Median Daily (2011-2020)	12 Oct	-	25 May	3.9	2 Jan

Table 2. Accumulated thermal units (ATU) experienced by larval Pacific Lamprey during the treatment period. The treatment period (December 3rd 2020 to April 11th 2021) encompasses the earliest and latest dates that heaters were operating in any lamprey rearing tank (identified from patterns in hourly data readings). ATUs were calculated by summing the mean temperature (°C) of days during the treatment period. ATUs were also calculated for a mainstem Columbia River USGS gage below The Dalles Dam, and two Columbia River tributary monitoring stations operated by Washington Department of Ecology.

Tank	Treatment	ATU
1	Warmed	1175
3	Warmed	1155
5	Warmed	1180
8	Warmed	1156
2	Natural	912
4	Natural	899
6	Natural	907
7	Natural	892
Columbia River	2021 Water Year	773
Columbia River	Median Daily (2011-2020)	723
Washougal River	2021 Water Year	852
Washougal River	Median Daily (2011-2020)	835
Wind River	2021 Water Year	698
Wind River	Median Daily (2011-2020)	618

Table 3. Fate of larval Pacific Lamprey stocked in circular tanks. Fish were collected between October 22nd and November 9th 2020, and tanks were checked for fish that had undergone metamorphosis on August 23rd and October 22nd 2021.

Tank	Treatment(s)	# stocked	Larvae recovered (August)	Larvae recovered (October)	Juveniles recovered (October)	# mortality	# missing
1	Warmed	11	11	6	5	0	0
3	Warmed	12	12	6	6	0	0
5	Warmed	11	10	7	3	1	0
8	Warmed	11	11	6	5	0	0
Total	Warmed	45	44	25	19	1	0
2	Natural	11	11	6	5	0	0
4	Natural	12	11	8	3	0	1
6	Natural	11	11	8	3	0	0
7	Natural	11	10	5	5	1	0
Total	Natural	45	43	27	16	1	1
Total	Both	90	87	52	35	2	1

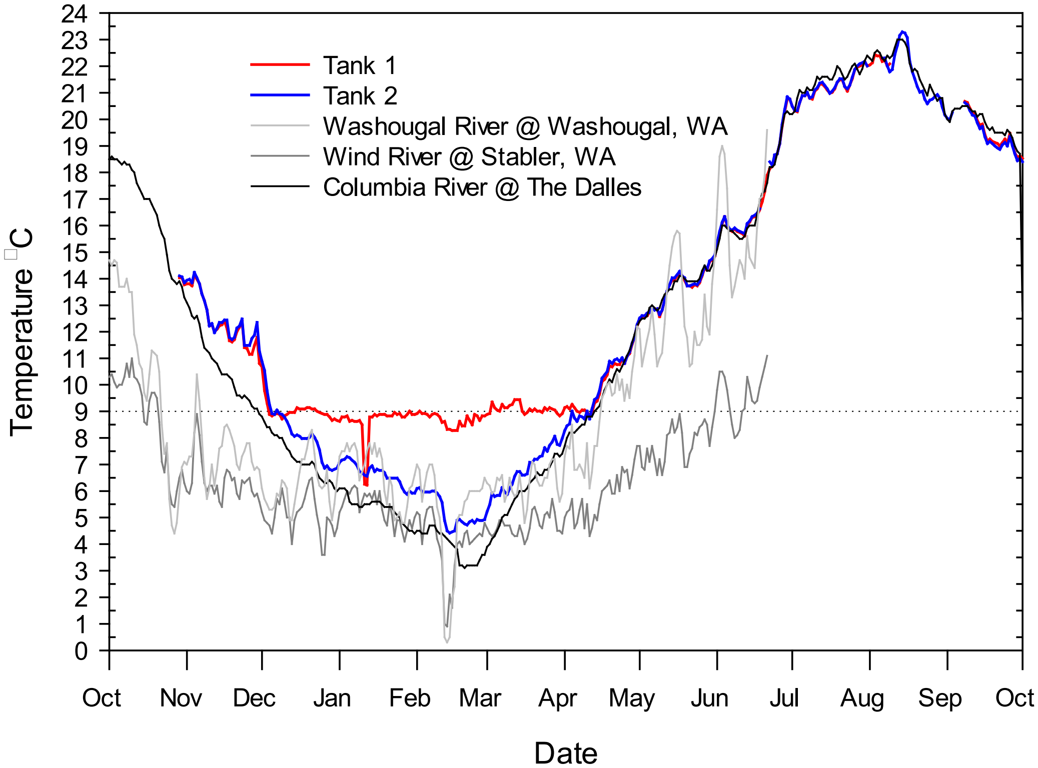

Figure 1. Minimum daily water temperatures for lamprey rearing tanks and nearby representative stream gages. Example tanks from the warm (Tank 1) and natural (Tank 2) experimental treatments are included, along with the minimum daily water temperatures (2021 water year) collected from the Columbia River below The Dalles Dam (USGS), and Washington Department of Ecology monitoring stations in the Washougal River and the Wind River. The dotted line references the 9°C target temperature threshold in this study. Tributary data was unavailable after June.

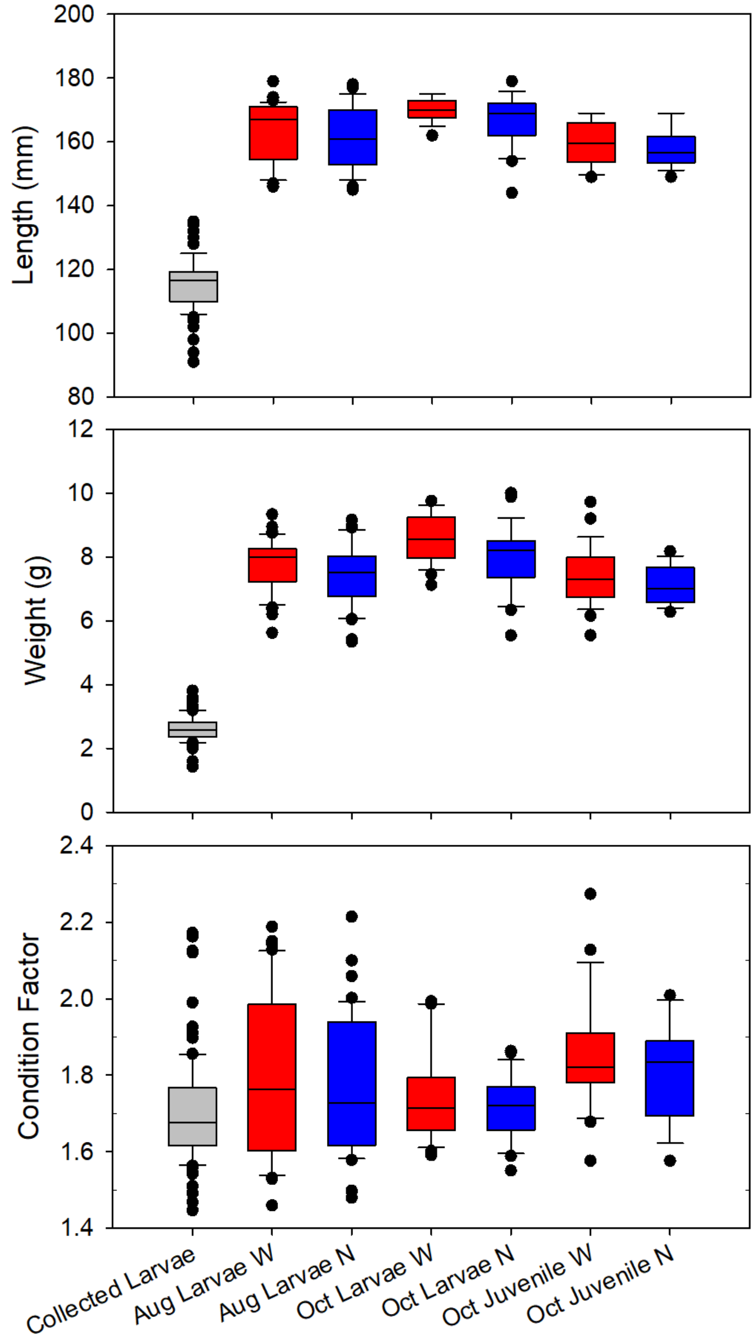

Figure 2. Distribution of the length, weight, and condition factor of larval Pacific Lamprey. Size and condition distributions are displayed at fish collection (collected larvae, grey boxes), and at two examinations for fish transformation (August [Aug] and October [Oct]). Fish are pooled across tanks by tank treatment (warm [W] = red, natural [N] = blue) and metamorphic status during the October check (larvae, juvenile). The box encompasses the median (represented by a horizontal line within the box), 25th percentile, and 75th percentile. Below and above the boxes are lines (whiskers) representing values within the 10^{th} and 90^{th} percentiles.

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