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**Do elevated stream temperatures affect larval Pacific
Lamprey growth, burrowing behavior or physiology?**

Annual Report: 2022



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Executive Summary:

Lampreys are obligate ectotherms and directly influenced by the ambient water temperature. In species other than Pacific Lamprey, evidence exists that lethal water temperatures for larvae occur near 27.0-31.0°C (e.g., Potter and Beamish 1975; Arakawa and Yanai 2021). Although few studies have addressed Pacific Lamprey, recent investigations indicated the ultimate upper incipient lethal temperature (UUILT) for larvae exceeds 27.5°C (Whitesel and Uh, 2022) and provided preliminary evidence that larvae have the ability to occupy natural areas warmer than 27.5°C (Whitesel and Sankovich 2022; Sankovich and Whitesel 2022a, 2022b). How climate change will ultimately influence the status of Pacific Lamprey is uncertain (see Wang et al. 2020). Currently, many of the locations where Pacific Lamprey rear naturally experience maximum water temperatures near 26.0°C. With predictions that during the next 25-35 years air temperatures in the Pacific Northwest may rise 2-5°C (Wu et al. 2012), maximum water temperatures where some Pacific Lamprey exist now may exceed the lethal limit for larvae. Whether there are effects at warm but sublethal temperatures is not well understood. We propose to investigate the effects of sublethally warm stream temperatures on larval Pacific Lamprey, specifically with respect to their growth, burrowing behavior, and physiology. We evaluated sublethal effects in both field and laboratory settings.

The study area for the field component of this project was the Umatilla River, Oregon (henceforth, river). Based on historical data, we partitioned the river into four thermal zones (TZ1 - TZ4) (see Whitesel and Sankovich 2022). Thermal Zone 2 (TZ2) was characterized by summer maximum temperatures that were expected to approach 31.0°C and be relatively constant throughout the zone. Thermal Zone 4 (TZ4) was characterized by summer maximum temperatures that were expected to range from < 27.5°C at the downstream end to 19.9°C at the upstream end. We considered TZ4 a control area (temperatures < UUILT) and TZ2 as a treatment area (temperatures nearing or exceeding the UUILT).

To determine if growth differed between larval lamprey in TZ2 and TZ4 during the period of peak stream temperatures, we captured larval lamprey via electrofishing on 13 (TZ2) and 14 (TZ4) July 2022, placed each captured lamprey in an individual container, and partially buried the containers in the stream bed. Each larval lamprey was measured (nearest 1 mm in total length [TL]) and weighed (nearest 0.1 g) before being placed in the container. The containers were approximately 17 cm wide and long and 11 cm high and had screening material on the sides and top to allow stream flow to pass through them (Appendix Figure 1). We filled the containers with Type 1 habitat to a depth of approximately 3.5 cm to allow the larval lamprey to burrow.

We buried the containers (n = 24 and 18 in TZ2 and TZ4, respectively) at two sites within each thermal zone and deployed temperature recorders on the substrate surface and 4-7 mm below it at each of the sites. The containers and temperature recorders (recording hourly) were left in place until 23 (TZ2) and 24 (TZ4) August 2022 (i.e., for 40 days), when the larval lamprey were removed from their containers, measured, and weighed.

In TZ2, three of the containers contained no larval lamprey, and one of the containers contained a dead larval lamprey, so we measured 20 larval lamprey in that thermal zone. In TZ4, one of the containers contained no larva, so we measured 17 in that thermal zone. Maximum temperatures recorded on the substrate surface were 31.1 °C and 24.9 °C in TZ2 and TZ4, respectively, while maximum temperatures recorded below the substrate surface were 30.0 °C and 25.0 °C in those respective thermal zones (Appendix Table 1). Only two larval lamprey in each temperature zone increased in length while in the containers. Eighteen of the larval lamprey in TZ2 and fifteen of the larval lamprey in TZ4 either did not grow or decreased in length (median proportional change in length $[(\text{final TL} - \text{initial TL})/\text{initial TL}] = -0.08$ versus -0.02 ; Appendix Table 2). The larval lamprey in TZ2 decreased in length significantly more than those in TZ4 (Mann Whitney test; $p = 0.001$).

To determine if burrowing behavior differed between larval lamprey in TZ2 and TZ4, we electrofished in the two thermal zones to capture larval lamprey. Without removing them from water, we placed each larva in a 75 x 60 mm (radius x height), opaque container that was covered. Within 120 s of their capture, they were released from the container into a burrowing chamber outfitted with a GoPro (Appendix Figure 2) over Type 1 habitat, and a video recording was made of their behavior. The burrowing behavior trials were conducted on 27 and 28 July 2022, when maximum temperatures on and below the substrate surface in TZ2 were 31.1 °C and 30.0 °C and those in TZ4 were 24.8 °C and 24.9 °C.

We used six metrics to evaluate burrowing behavior: 1) exploration time, 2) active burrowing time, 3) inactive burrowing time, 4) total burrowing time, 5) number of stops while burrowing, and 6) number of burrowing attempts. We videotaped 23 larvae in TZ2 and 24 larvae in TZ4. Video quality was sufficient for evaluation of 17 of the larvae in TZ2 and 16 of the larvae in TZ4. Exploration time was the only metric that differed significantly between larval lamprey in TZ2 and TZ4 (Mann Whitney test; range in $p = 0.05 - 0.81$). Larvae in TZ2 tended to spend more time exploring than larvae in TZ4 (Appendix Table 3).

To compliment the information gained from larvae in the natural environment, we also conducted well-controlled laboratory experiments. The study area for the laboratory component of this project involved Cedar Creek (henceforth, creek), Washington, and the Columbia River Fish & Wildlife Conservation Office (CRFWCO) laboratory. On 5 August 2022, 36 larval lamprey were collected from the creek. These fish ranged from 61-115 mm and 0.44-2.90 g. The creek temperature at approximately 12:00 H was 17.5 °C. Larvae were transported to CRFWCO and randomly distributed into six, 37.9 L aquariums (6 larvae/aquarium) for rearing and experimentation. Rearing aquariums contained approximately 7 cm of river sand covered by 18 cm of well water being heated to 18 °C. Aquariums were randomly assigned to either an 18 °C (N=3) or 27 °C (N=3) treatment. Water temperature was increased an average of 1.5 °C /day in the 27 °C treatment aquariums until reaching the final temperature (over 7 days). Larvae were then reared at 18 °C or 27 °C for 32-33 days. As described in Jolley et al. (2015), each aquarium received 0.2 g of ground, BioVita Fry (Bio-Oregon) food/larvae twice each week (or 2.4 g/aquarium/week). Visible Elastomer Implant (VIE) tags and morphometric characteristics were used to identify individual larvae in each tank.

All of the larvae survived the rearing period. None of the larvae transformed to a juvenile during the experiment. On 14-15 September 2022, we evaluated burrowing behavior. This was done using the chamber and procedure described above, but placed in a 37.9 L aquarium containing 15 cm of river sand covered by 10 cm of well water at a temperature of 18°C or 27°C (corresponding to the treatment temperature of the larva being tested). Once introduced into the chamber, a larva was evaluated for up to 15 min then returned to its treatment tank. On 16 September 2022, following the burrowing evaluations, larvae were euthanized. The TL, total mass (TM) and liver mass (LM) of each larva were measured. Proportional change in length (PCL) was calculated as $(\text{final TL} - \text{initial TL}) / \text{initial TL}$. As a measure of larval lamprey physiology, we evaluated lipid levels and liver size. Hepatosomatic Index (HSI) was calculated as $(\text{LM}/\text{TM}) * 100$. A Mann Whitney test was used for all statistical comparisons. For future analysis of total lipid, liver and caudal muscle tissue were collected from each larva, rapidly frozen and stored at -80°C.

During burrowing experiments, larvae reared in 18°C and 27°C exhibited similar exploration times, number of attempts to burrow, total time to burrow, active time and inactive time during burrowing (range in $p = 0.17 - 0.77$). When compared to larvae reared in 18°C, those reared in 27°C tended to stop and rest more during burrowing ($p = 0.06$). Larvae reared in 18°C exhibited significant growth (median = 6.5 mm in TL; $p < 0.01$) whereas those reared in 27°C tended to become shorter (median = -1.0 mm in TL; $p = 0.05$). Proportional change in length for larvae reared in 18°C (median = 0.078) was significantly greater ($p < 0.01$) than for larvae reared in 27°C (median = -0.009). Change in condition for larvae reared in 18°C (median = -0.17) and 27°C (-0.11) were both negative as well as different from each other ($p < 0.01$). At the end of the rearing period, there were no differences ($p = 0.18$) in the HSI of larvae reared in 18°C (median = 0.79) and 27°C (median = 0.75).

In previous years of this study, larvae demonstrated the ability to occupy areas where the maximum water temperature above and below the substrate reached values of 33.6°C and 29.0°C, respectively, suggesting that their UUILT is at least 29.0°C (Whitesel and Sankovich 2022; Sankovich and Whitesel 2022a, 2022b). In general, our results from 2022 suggest elevated stream temperatures may affect growth but have minimal or no effects on burrowing behavior and hepatic physiology. However, in natural conditions, larvae may prefer to try and escape from (swim out of) rather than burrow in warm conditions. Furthermore, in natural and laboratory conditions, it is possible warmer temperatures were thermally stressful to larvae and resulted in impaired growth. Alternatively, it is possible that larvae reared in warmer temperatures were food limited or had a higher metabolic rate than those reared in cooler temperatures. The occupancy of larval Pacific Lamprey in rivers that reach temperatures exceeding 29.0°C, other than the Umatilla River, may not be uncommon (Reid and Goodman, personal communication). However, it is unclear whether sublethal effects exist outside of those we evaluated. How cooler temperatures in the substrate might serve as a thermal refuge and mitigate for elevated stream temperatures above the substrate is also unknown.

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Appendix Table 1. Minimum, maximum, and mean stream temperatures recorded on the surface of the stream bed and in the substrate at sites in relatively warm and cool temperature zones (2 and 4, respectively) where larval Pacific Lamprey were held in individual containers in the Umatilla River from 13 July to 24 August 2022.

Temperature zone	Reach	Location	Stream temperature (°C)			
			Minimum	Maximum	Mean	
	2	12	surface	18.3	30.8	24.3
			buried	18.7	30.0	24.1
	2	1	surface	17.8	31.1	24.0
			buried	18.0	29.7	23.8
	4	10	surface	16.8	24.6	20.5
			buried	16.0	22.9	18.6
	4	6	surface	15.7	24.9	19.9
			buried	15.7	25.0	19.9

Appendix Table 2. Median proportional change in length and minimum and maximum change in length of larval Pacific Lamprey held individually in containers buried in relatively warm and cool temperature zones (TZ2 and TZ4, respectively) in the Umatilla River from 13 July to 24 August 2022 or reared in the laboratory at 18°C or 27°C for 32-33 days.

Thermal conditions	Median proportional change in length	Minimum change in length (mm)	Maximum change in length (mm)
TZ2 (n = 20)	-0.08	-13	7
TZ4 (n = 20)	-0.02	-10	1
Lab 18°C (n=18)	0.01	0.08	0.25
Lab 27°C (n=18)	-0.06	-0.01	0.04

Appendix Table 3. Median exploration, active burrowing, and inactive burrowing times (in seconds), median total burrowing time (in seconds), and median number of stops and burrowing attempts for larval Pacific Lamprey released into burrowing chambers in relatively warm and cool temperature zones (TZ2 and TZ4, respectively) in the Umatilla River during 27 and 28 July 2022 or after being reared in laboratory conditions at 18°C or 27°C for 32-33 days.

Thermal zone	Median exploration time	Median active burrowing time	Median inactive burrowing time	Median total burrowing time	Median number of stops	Median number of burrowing attempts
2	2.1	14.8	0.0	15.3	0.0	1.0
4	0.8	19.1	0.0	17.5	0.0	1.0

Appendix Figure 1. Design of the containers in which larval lamprey were held during the growth experiment in the Umatilla River from 17 July to 24 August 2022.



Appendix Figure 2. Design of the plexiglass chamber and GoPro used in the burrowing experiment in the Umatilla River on 27 and 28 July 2022.



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