



**U.S. Fish and Wildlife Service**  
**Columbia River Fish & Wildlife Conservation Office**



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

*Annual Report: 2021*



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

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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, *in preparation*) 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 in both zones on 19-21 July 2021, 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 = 18 per thermal zone) at two sites each within TZ2 and TZ4 and deployed temperature recorders on the substrate surface and 4-7 mm below the substrate surface at each of the sites. The containers and temperature recorders were left in place until 30 August 2021 (i.e., for 40 – 42 days), when the larval lamprey were removed from their containers and measured.

Two of the containers, one each in TZ2 and TZ4, contained no larval lamprey, so we measured 17 in each thermal zone. Maximum temperatures recorded on the substrate surface were 30.5 and 27.8°C in TZ2 and TZ4, respectively, while maximum temperatures recorded below the substrate were 29.0 and 25.1°C in those respective thermal zones (Appendix Table 1). Larval lamprey in TZ2 grew slightly more than those in TZ4 (median proportional change in length  $[(\text{final TL} - \text{initial TL})/\text{initial TL}] = 0.04$  versus 0.03; Appendix Table 2) but the difference was not significant (Mann Whitney test;  $p = 0.59$ ).

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. They were then released from the container within 120 s of their capture (again, without being removed from water) over Type 1 habitat and videotaped. We used two techniques to video tape the larval lamprey. One involved allowing the larvae to swim freely while being videotaped from above the water's surface, and the other involved placing them in a burrowing chamber outfitted with a GoPro (Appendix Figure 2). The burrowing behavior trials were conducted on 4 and 5 August 2021.

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 24 larvae in each thermal zone and 12 larvae using each videotaping technique (freely swimming versus chambered) within each thermal zone. Video quality was sufficient for evaluation of 10 of the freely swimming larvae and 11 of the chambered larvae in TZ2, and for 10 of the freely swimming larvae and 10 of the chambered larvae in TZ4.

Based on the six metrics we evaluated, there was no difference in burrowing behavior between larval lamprey in TZ2 and TZ4. Larvae in the chamber and released to swim freely in TZ2 generally did not perform as well as those in the chamber and released to swim freely in TZ4 (Appendix Table 3); however, there were no significant differences within the six metrics (Mann Whitney test; range in  $p = 0.07 - 0.64$  for larva in the burrowing chamber and  $p = 0.44 - 1.00$  for freely swimming larvae).

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 24 August 2021, 36 larval lamprey were collected from the creek. These fish ranged from 63-119 mm and 0.40-3.10 g. The creek temperature was 14.5°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 10 cm of river sand covered by 15 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 29-31 days. As described in Jolley et al. (2015), each aquarium received 0.2 g salmon carcass analog pellets/larvae twice each week (or

2.4 g/aquarium/week). Morphometric characteristics were used to identify individual larvae in each tank.

All of the larvae survived the rearing period. Six of the larvae transformed to a juvenile and were excluded from further analysis. On 28-29 September 2021, 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 30 September 2021, 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 (final TL – initial TL)/initial TL. Hepatosomatic Index (HSI) was calculated as (LM/TM)\*100. For future analysis of total lipid, liver and caudal muscle tissue were collected from each larva, frozen and stored at -80°C.

During burrowing, larvae reared in 18°C and 27°C exhibited similar exploration times, total time to burrow and active time burrowing (Mann Whitney test; range in p = 0.165 - 0.950). Inactive time (median) during burrowing of larvae reared in 27°C (17.3 sec) tended to be greater (Mann Whitney test; p = 0.101) than that of larvae reared in 18°C (4.8 sec). The number of stops (median) during burrowing of larvae reared in 27°C (2.0) was significantly greater (Mann Whitney test; p = 0.049) than that of larvae reared in 18°C (1.0). Larvae reared in 18°C exhibited significant growth (median = 9.0 mm) whereas those reared in 27°C did not (median = 0.5 mm). Proportional change in length for larvae reared in 18°C (11.9%) was also significantly greater (Mann Whitney test; p < 0.001) than for larvae reared in 27°C (0.8%). Change in condition for larvae reared in 18°C (-0.23) and 27°C (-0.20) were both negative but not different from each other (Mann Whitney test; p = 0.350). At the end of the rearing period, there were no differences (Mann Whitney test; p = 0.696) in the HSI of larvae reared in 18°C (0.82) and 27°C (0.80).

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). Our results from 2021 suggest there may be minimal or no sublethal effects, in terms of growth and burrowing behavior, of the elevated temperatures. In natural conditions of the Umatilla River on larval lamprey, no effects were observed. In laboratory conditions, it is possible that 27°C was thermally stressful to larvae and resulted in impaired growth. Alternatively, it is possible that larvae reared in 27°C were food limited or had a higher metabolic rate than those reared in 18°C. It is unclear whether occupancy is common in other rivers that reach temperatures exceeding 29.0°C, such as the Umatilla River, or 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 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 between 19 July and 30 August 2021.

Temperature zone	Reach	Location	Stream temperature (°C)		
			Minimum	Maximum	Mean
2	1a	surface	14.6	30.1	22.4
		buried	17.8	29.0	23.2
2	1b	surface	14.7	30.5	22.8
		buried	14.7	30.4	22.9
4	10	surface	15.1	26.9	20.5
		buried	15.9	25.1	20.6
4	6	surface	14.1	27.8	19.5
		buried	13.6	25.9	19.4



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 (2 and 4, respectively) in the Umatilla River from 19 July to 30 August 2021.

Temperature zone	Median proportional change in length	Minimum change in length (mm)	Maximum change in length (mm)
2 (n = 17)	0.04	-8	13
4 (n = 17)	0.03	-8	15

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 (C) or into the water column to swim freely (F) in relatively warm and cool temperature zones (2 and 4, respectively) in the Umatilla River during 4 and 5 August 2021.

Thermal zone	Treatment	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	C (n = 11)	4.0	19.0	1.0	20.0	1.0	1.0
	F (n = 10)	2.9	9.6	0.0	9.6	0.0	1.0
4	C (n = 10)	2.0	17.0	0.0	19.5	0.0	1.0
	F (n = 10)	2.1	7.4	0.0	7.4	0.0	1.0

Appendix Figure 1. Design of the containers in which larval lamprey were held during the rowth experiment in the Umatilla River from 19 July to 30 August 2021.



Appendix Figure 2. Design of the plexiglass chamber and GoPro used in the burrowing experiment in the Umatilla River on 4 and 5 August 2021.



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