Identification of Larval Pacific Lampreys (Lampetra tridentata), River Lampreys (L. ayresi), and Western Brook Lampreys (L. richardsoni) and Thermal Requirements of Early Life History Stages of Lampreys

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IDENTIFICATION OF LARVAL PACIFIC LAMPREYS (*LAMPETRA TRIDENTATA*), RIVER LAMPREYS (*L. AYRESI*), AND WESTERN BROOK LAMPREYS (*L. RICHARDSONI*) AND THERMAL REQUIREMENTS OF EARLY LIFE HISTORY STAGES OF LAMPREYS

ANNUAL REPORT 2002

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Executive summary

Two fundamental aspects of lamprey biology were examined to provide tools for population assessment and determination of critical habitat needs of Columbia River Basin lampreys (the Pacific lamprey, *Lampetra tridentata*, and the western brook lamprey, *L. richardsoni*). In particular: 1) we examined the usefulness of current diagnostic characteristics in identification of larval lampreys, specifically pigmentation patterns, and collected material for development of meristic and morphometric descriptions of early life stages of lampreys, and 2) we examined the effects of temperature on survival and development of early life stages of Columbia River Basin lampreys.

In 1999 thirty-one larval lampreys (ammocoetes) were collected from locations throughout the Columbia River Basin and transported to the Columbia River Research Laboratory. They are being examined and identified to species at approximately six-week intervals until they metamorphose and their identity can be confirmed by dentition patterns. Currently, these lampreys have been sampled 21 times, and two individuals have metamorphosed allowing confirmation of species identification. Of the lampreys that have not metamorphosed, only one has been inconsistently identified (Pacific lamprey in 83% of the sampling events and western brook lamprey in 17% of the sampling events), suggesting that pigmentation patterns do not change appreciably through time.

In 2001 and 2002 we artificially spawned Pacific and western brook lampreys in the laboratory to provide material for meristic and morphometric descriptions. We have

collected, digitized, preserved, and measured the mean chorion diameter of 320 fertilized Pacific lamprey eggs and 280 fertilized western brook lamprey eggs. Pacific lamprey eggs were significantly larger than western brook lamprey eggs ($t = 32.758$, df = 528.62, $P < 0.0001$) with a mean difference of 0.231 mm. We have collected, digitized, and preserved 156 Pacific lamprey larvae (standard length: 7.20 - 31.79 mm) and 146 western brook lamprey larvae (standard length: 6.64 - 25.90 mm). For morphometric descriptions of these individuals we have selected eight homologous landmarks that define a two-cell truss network with two appended triangles. Truss element lengths have been summarized based on the original measurement scale and on a scale proportional to each individual's standard length.

In 2001 and 2002 Pacific and western brook lampreys were artificially spawned and resulting progeny were reared at the Columbia River Research Laboratory at 10° C, 14° C, 18° C, and 22° C. Temperature significantly affected survival of early life stage Columbia River Basin lampreys ($F_{3, 124} = 208.42$, $P < 0.0001$) with a significant decrease in survival at 22° C when compared to other rearing temperatures. A significant difference in survival over the course of the experiment was observed between Pacific (83.8%) and western brook (84.8%) lampreys ($F_{1, 124} = 6.20$, $P = 0.0141$); however, the biological significance of this difference is questionable. A significant difference in survival between the time of 50% hatch and the time of 50% yolk assimilation was also observed ($F_{1, 125} = 81.46$, $P < 0.0001$). Temperature significantly affected the occurrence of larval abnormalities at 50% yolk assimilation ($F_{3, 111} = 130.49$, $P < 0.0001$) with a greater percentage of abnormalities occurring at 22° C than at other rearing temperatures.

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Introduction

Lampreys inhabit temperate regions in both the northern and southern hemispheres. Typically, lampreys spawn in freshwater streams where, after hatching, larval lampreys (ammocoetes) burrow into soft substrate and spend an extended larval period filtering particulate matter from the water column. During this larval period, lampreys are characterized by greatly reduced subcutaneous eyes, reduced fins, unidirectional flow of water from the mouth through the gill pores for filter feeding, and the absence of tooth-like keratin plates (the structures most often used to differentiate lamprey species). After approximately three to seven years (Hardisty and Potter 1971a), lampreys go through a metamorphosis marked by drastic physiological and morphological changes. The resulting juvenile lampreys exhibit fully developed eyes, fins, and characteristic dentition patterns.

Once metamorphosis is complete, lampreys adopt one of two species-specific life history phases. Non-migratory, non-feeding species reside in streams until sexually mature, at which time they spawn and die. Migratory, predatory species move from natal streams into large bodies of freshwater (landlocked) or into marine habitats (anadromous). Both landlocked and anadromous forms use their oral disc to attach to and feed on animals. Lampreys exhibit rapid growth during the predatory phase, which can last from less than one year to greater than two and a half years (Hardisty and Potter 1971b), with the duration ranging greatly among geographical locations and species. Once lampreys have reached an adequate size they cease feeding, migrate into freshwater streams, spawn, and die.

Within the Columbia River Basin the occurrence of three native species of lampreys has been documented. Of these species, Pacific lampreys (*Lampetra tridentata*) and river lampreys (*L. ayresi*) exhibit a migratory, predatory life history pattern, while the life history of western brook lampreys (*L. richardsoni*) is non-migratory and non-predatory. Apart from their general feeding patterns, little is known about the biology of lamprey species in the Columbia River Basin (Kan 1975; Hammond 1979), and what information is available for these species is from work conducted in Canada (Pletcher 1963; Beamish 1980; Richards 1980; Beamish and Levings 1991). Due to the lack of information on lamprey habitat requirements, population sizes, and community structure, relatively little is known about the status of lamprey species within the Columbia River Basin. Dam passage data and anecdotal information indicate that Pacific lampreys are in decline in the Columbia River Basin (Close et al. 1995). The declining trend of Pacific lampreys, along with the ecological, economic, and cultural significance of Pacific lampreys (Kan 1975; Close et al. 1995; NPPC 1994), has stimulated interest in recovery actions in the Columbia River Basin.

Documenting the distribution and relative abundance of lampreys in streams and rivers tributary to the Columbia River will help identify factors limiting lamprey populations, identify areas in need of rehabilitation, and help assess the efficacy of management actions. Surveys of larval lampreys may provide an effective means of determining the distribution and abundance of lampreys since larvae are readily collected from rearing areas by electrofishing. However, within the Columbia River Basin, larvae of different species often have sympatric or partially overlapping distributions.

Therefore, to accurately estimate lamprey distribution and abundance it is necessary to be able to positively identify larvae to the species level. Richards et al. (1982) developed descriptive keys for identifying larvae of lampreys found in British Columbia, Canada. Their study indicates that pigmentation patterns of the tail, head, and tongue precursor can be used to separate Pacific, river, and western brook lampreys. However, use of these identification techniques has proven less diagnostic for larval lampreys in the Columbia River Basin (USGS, unpublished data), which may be due to geographic variability in pigmentation patterns within and among species.

Along with the ability to distinguish lamprey species in the field, identification of ecological factors limiting lampreys in the Columbia River Basin is critical to population assessment and recovery efforts. Understanding factors influencing survival during early life stages is particularly important since this period is a critical determinant of recruitment for many fish populations (Houde 1987). Larval fish abundance may be determined by a number of habitat characteristics, including water temperature during early development (Potter and Beamish 1975; Young et al. 1990; Youson et al. 1993). Optimal temperatures for survival and development of sea lampreys (*Petromyzon marinus*) have been studied extensively (Piavis 1961; McCauley 1963; Holmes and Lin 1994; Rodriguez-Munoz et al. 2001); however, little information is available for other lamprey species. Knowledge of the role of temperature in lamprey early life development will provide managers with a means to assess the suitability of available spawning and rearing habitats, which may be sub-optimal due to alterations in thermal regimes of the Columbia River and its tributaries (Quinn and Adams 1996).

The goal of this project is to address two fundamental aspects of lamprey biology in order to provide tools for population assessment and determination of critical habitat needs of Columbia River Basin lampreys. In particular, our objectives are to: 1) determine diagnostic characteristics for species identification of embryo and larval stage Pacific, western brook, and river lampreys, and 2) examine the effects of temperature on survival and development of early life stages of these three species.

This work will answer questions about lampreys posed by regional fishery managers. Specifically, providing tools for population assessment and the quantification of habitat needs will help managers in developing strategies to assure the long-term stability of lamprey populations and reduce the likelihood that management will be handled through the regulatory process. Accurate identification techniques will allow managers to conduct larval surveys and thus determine the relative abundance of each species in various habitats. Knowledge of early life history characteristics and ecological requirements of these species will aid in future research and management of lampreys in the Columbia River Basin.

This document presents preliminary analyses of data collected in 2000, 2001, and 2002 for the purpose of validating current diagnostic characteristics of larval lampreys, and preliminary analyses of data collected in 2001 and 2002 for the purpose of providing morphometric descriptions of embryonic and larval lampreys and defining their thermal requirements. Experiments were not performed on river lampreys in 2000, 2001, or 2002 due to our inability to locate live specimens within the Columbia River Basin (see:

Preliminary results and discussion - River lampreys in the Columbia River Basin; this report).

Methods

Artificial spawning

Pacific and western brook lampreys were artificially spawned at the Columbia River Research laboratory to provide material for morphometric and meristic descriptions and for examining the effects of temperature on survival and development of early life stage lampreys. Pacific lampreys were collected from the Columbia River at Bonneville Dam, and western brook lampreys were collected from Gibbons Creek, WA, and Yellowhawk Creek, WA. Both species were transported to the Columbia River Research Laboratory and held until sexually mature. At the Columbia River Research Laboratory Pacific lampreys were held in 1400 L circular tanks provided with a continuous inflow of water (ca. 0.3 L/min/kg). Western brook lampreys were held in 38 L aquaria provided with burrowing substrate and a continuous inflow of water (ca. 0.3 L/min). Water provided to all lampreys was from the Little White Salmon River. Water was filtered with a sand filter and heated to simulate seasonal thermal trends within the Columbia River Basin. All lampreys were exposed to a simulated photoperiod provided by 25-watt incandescent lights on timers with 0.5 h of increasing and decreasing illumination at the beginning and ending of each light phase, respectively.

Pacific lampreys used for experiments in 2001 were six males (mean ± SD: length = 508 ± 41 mm; mass = 287.5 ± 87.9 g) and five females (length = 459 ± 42 mm; mass = 318.4 ± 65.4 g), and in 2002 were six males (length = 480 ± 37 mm; mass = 267.6

± 69.5 g) and six females (length = 446 ± 28 mm; mass = 292.0 ± 42.0 g). Western brook lampreys used for experiments in 2001 were 19 males (length = 127 ± 7 mm; mass = 3.938 ± 0.668 g) and 31 females (length = 122 ± 5 mm; mass = 4.236 ± 0.656 g), and in 2002 were 28 males (length = 124 ± 9 mm; mass = 3.758 ± 0.995 g) and 29 females (length = 122 ± 10 mm; mass = 4.545 ± 1.130 g). Prior to spawning, mature lampreys were anesthetized in 250 mg/L of tricaine methane sulfonate (MS-222) buffered with an equal concentration of sodium bicarbonate and rinsed in fresh water to remove traces of anesthetic. Female lampreys were positioned over a glass bowl filled with about 2 L of fresh water at the approximate temperature of holding tanks and aquaria. Eggs were forced out the urogenital opening by squeezing the abdomen in a downward motion. This was repeated until blood appeared with the gametes. Sperm was removed from males in a similar fashion.

Gametes were mixed with a gentle flow of water from a large pipette for 5 min and allowed to rest undisturbed for 30 min to allow fertilization to occur. After 30 min the fertilized eggs were divided into four glass bowls and the water temperature of each bowl was gradually adjusted through the addition of cool or warm water until the target temperatures of 10° C, 14° C, 18° C, and 22° C were reached (approximately 30 min). Once target temperatures were reached, fertilized eggs were transferred to flow-through hatching jars (6.86 L McDonald type) of the appropriate temperature (one hatching jar per temperature).

Identification of early life stage lampreys

Validation of current diagnostic characteristics

In the fall of 1999 larval lampreys were collected from five locations in the Columbia River Basin: Red River (Clearwater River sub-basin), Entiat River (Snake River sub-basin), John Day River (John Day River sub-basin), Walla Walla River (Walla Walla River sub-basin), and Cedar Creek (Lewis River sub-basin). Ten to 25 larvae from each location were collected by cooperators and transported to the Columbia River Research Laboratory. Lampreys were divided among four 19 L aquaria such that individuals were separated based on collection location: 1) Red River, 2) Entiat River, 3) John Day/Walla Walla Rivers, and 4) Cedar Creek. Each aquarium was provided with burrowing substrate, a continuous inflow of water (ca. 0.3 L/min), and aeration. Filtered river water (sand filter) was provided from the Little White Salmon River and was heated to simulate seasonal thermal trends within the Columbia River Basin. Lampreys were fed a suspension of active yeast and commercial fry feed two to three times per week.

In February 2000 each larva was measured for length (mm) and mass (g) and identified to species based on existing diagnostic characteristics (Richards et al. 1982). Fifty larvae were photographed and sampled to provide tissue for genetic testing (Appendix 1). Mitochondrial DNA will be examined in an effort to genetically confirm species identification (conducted by Dr. Matt Powell, University of Idaho). Thirty-one larvae were uniquely marked with an injection of dyed elastomer, held at the Columbia River Research Laboratory, and sampled at intervals of approximately six-weeks (Appendix 2). At each sampling event lampreys were removed from aquaria,

anesthetized in 250 mg/L buffered MS-222, measured for length and mass, identified to species, and digital images of their caudal region were taken (Figure 1). Digital images of individuals were captured using a high-resolution, color digital camera mounted to a dissecting microscope. This procedure is being performed to: 1) determine if it is possible to separate these species based on pigmentation patterns (Richards et al. 1982), and 2) determine if there is a change in pigmentation patterns over time, specifically with regards to diagnostic characteristics of these species. This process will be repeated until individuals metamorphose, at which point species identification can be confirmed based on dentition, or individuals die, at which point genetic samples will be collected for analysis.

Morphometric and meristic description of laboratory spawned specimens

The following methods were performed on the progeny of individuals spawned in 2001 and 2002. Following fertilization (see above), Pacific and western brook lampreys were sampled periodically to provide a time series of embryological and larval development. Individuals were held in flow-through hatching jars at 14° C (see above) from the time of fertilization until larvae had assimilated approximately 50% of the yolk in their gut (referred to as 50% yolk assimilation). After 50% yolk assimilation, lampreys were transferred to 19 L aquaria. Each aquarium was provided with burrowing substrate, a continuous inflow of water (ca. 0.3 L/min), and aeration. Filtered river water (sand filter) was provided from the Little White Salmon River and was heated to simulate seasonal thermal trends within the Columbia River Basin. Lampreys were fed a suspension of active yeast and commercial fry feed two to three times per week.

Pacific and western brook lampreys were sampled to provide morphometric and meristic information and to determine if morphometric or meristic traits exist that are useful in describing species differences. At each sampling event ten individuals were removed from their holding vessel (flow-through hatching jar or aquaria), anesthetized in 250 mg/L buffered MS-222, digitized, and preserved in 10% formalin. Digital images of individuals were captured using a high-resolution, color digital camera mounted to a dissecting microscope.

We have digitized and preserved 320 fertilized Pacific lamprey eggs (2001 cohort, $N = 150$; 2002 cohort, $N = 170$) and 280 fertilized western brook lamprey eggs (2001 cohort, $N = 140$; 2002 cohort, $N = 140$). Using image analysis software, the chorion circumference of each digitized egg was traced and the mean chorion diameter (mm) was calculated from diameter measurements made through the centroid at 2-degree intervals. Differences in mean chorion diameter between species were compared using an unpaired *t*-test.

We have digitized and preserved 350 Pacific lamprey larvae (2001 cohort, $N = 120$; 2002 cohort, $N = 230$) and 300 western brook lamprey larvae (2001 cohort, $N = 80$; 2002 cohort, $N = 220$) for morphometric and meristic descriptions of early stage larvae. A set of eight homologous landmarks has been identified for preliminary analysis. The locations of these landmarks define a two-cell truss network with two appended triangles (Bookstein et al. 1985) (Figure 2; Appendix 3). Image analysis software was used to plot the coordinates of each landmark, and truss element lengths have been calculated.

Effects of temperature on early life stages

The following methods were performed on Pacific and western brook lampreys and were replicated in 2001 and 2002. Following fertilization (see above), zygotes were incubated at 10° C, 14° C, 18° C, and 22° C for 15 Temperature Units (TU), where:

TU = (number of days) x (degrees above 0° C).

Temperature units combine the effects of time and temperature on development so that individuals exposed to similar temperature units should have reached approximately the same developmental stage. After 15 TU, individuals were removed from hatching jars and 100 viable embryos were placed into each of 10 rearing vessels per temperature. A lag of 15 TU between the time of fertilization and the time of selecting experimental individuals was used to allow development to reach a point where fertilization could be confirmed. Each rearing vessel had a volume of approximately 60 ml and was constructed with a screen bottom to allow water to flow through. Rearing vessels were placed into an incubation bath of the appropriate rearing temperature (10° C, 14° C, 18° C, and 22° C), and each vessel was supplied with freshwater inflow at a rate of 0.05 L/min and subjected to a simulated natural photoperiod provided by 25-watt incandescent lights on timers with 30 min of increasing and decreasing illumination at the beginning and ending of each light phase, respectively. Water was supplied from the Little White Salmon River, WA, and was treated with sand filters and ultraviolet sterilizers prior to use. Water supplied to rearing vessels was monitored daily for dissolved oxygen content, pH, and total dissolved gasses (Figure 3).

Individuals in each rearing vessel were examined daily for the duration of the experiment. The duration of the experiment was from the time that individuals were assigned to a rearing vessel until individuals had hatched and assimilated approximately 50% of the yolk in their gut (referred to as 50% yolk assimilation), at which time exogenous feeding had begun. For daily examinations, each rearing vessel was removed from the incubation bath, placed in a petri dish with water of the appropriate temperature, and examined under a dissecting microscope at 10X to 40X. Data recorded for each rearing vessel consisted of: 1) number of dead embryos, 2) number of dead larvae, 3) number of larvae exhibiting abnormalities, and 4) number of individuals hatched/not hatched. Larval abnormalities were traits considered to have a potential negative affect on survival, development, or fitness in conditions less favorable than a laboratory setting, such as malformations of the body. For examples of normal and abnormal development see Figure 4 and Figure 5, respectively. From the data collected we derived the response variables: 1) percent survival, and 2) percent abnormal larvae.

Although temperature units are often used to predict the onset of particular developmental events, time and temperature are not the only factors that may affect developmental rates. Therefore, we developed an adjusted temperature unit model (TU_a) specific to this experiment. This was done to allow comparisons to be made among lampreys at similar developmental stages, regardless of which rearing temperature they had been exposed to. Logistic regression was used to predict the time to 50% hatch for each species held at each rearing temperature, where 50% hatch was considered to be a discrete developmental event. It was assumed that all species by rearing temperature

combinations had been exposed to the same number of adjusted temperature units (TU_a) at the occurrence of 50% hatch. The time required for Pacific lampreys reared at 10° C to reach 50% hatch was used as a reference point to derive an adjustment factor specific to each species by rearing temperature combinations. Therefore:

$$TU_a = (\text{number of days}) \times (\text{degrees above } 0° \text{ C}) \times (\text{adjustment factor}).$$

This adjusted temperature unit model provides a means to standardize time to specific developmental stages for making comparisons among lampreys exposed to different rearing temperatures in this experiment; however, it should not be used to generate predictions for conditions outside the scope of this experiment.

All statistical analyses were performed at $\alpha = 0.05$. A repeated measures factorial analysis of variance was used to examine differences in survival of individuals at discrete time intervals among species and rearing temperatures. Specifically, we examined the effects of species and rearing temperature on: 1) percent survival to 260 TU_a (50% hatch), and 2) percent survival to 540 TU_a (50% yolk assimilation). Variance in the response variable percent survival was stabilized using an arcsine transformation. A factorial analysis of variance was used to examine differences in percent abnormal larvae at 540 TU_a among species and rearing temperatures. Variance in the response variable percent abnormal larvae was stabilized using a square root transformation. For both analyses a blocking factor (Sokal and Rohlf 1995) was used to account for systematic variation associated with the year in which the experiment was performed (2001 and 2002) and interactions between species and rearing temperature were examined. When

main factors had an overall significant effect, Bonferroni *t*-tests were used to make pairwise comparisons between treatment combinations.

Preliminary results and discussion

Identification of early life stage lampreys

Validation of current diagnostic characteristics

In February 2000, 50 larval lampreys were sacrificed to provide genetic material for mitochondrial DNA analysis. Of these individuals, 42 were identified as Pacific lampreys and eight were identified as western brook lampreys, based on caudal region pigmentation (Appendix 1). Researchers at the University of Idaho were unable to locate genetic sequences or loci suitable for differentiating Pacific and western brook lampreys. This inability to separate Columbia River Basin lampreys may be a result of the molecular techniques used. Docker et al. (1999) was able to separate Pacific lampreys from western brook and river lampreys; however, they were unable to separate western brook and river lampreys. Based on these data, Docker et al. (1999) suggests western brook and river lampreys diverged within the past 70,000 years. Due to this recent divergence, mitochondrial DNA, which is highly conserved, may not be suitable for separation of lamprey species found in the Columbia River Basin; therefore, other techniques, such as microsatellite analysis, may merit investigation. The ability to accurately identify Columbia River Basin lampreys is essential to productive management actions; therefore, samples provided to the University of Idaho are being returned so that we may archive them for later analysis and development of suitable molecular techniques for differentiating lampreys found in the Columbia River Basin.

The 31 larvae held at the Columbia River Research Laboratory for repeated examination have been sampled 21 times to date (some individuals less due to mortality) (Appendix 2). In the case of mortality, genetic samples were taken for later species confirmation. Of these larvae, species identification was confirmed, based on dentition patterns, for two Pacific lampreys that metamorphosed. These individuals were consistently identified as Pacific lampreys (100% of the sampling events). Species identification was also consistent for 28 of the un-metamorphosed lampreys. Only one individual was identified inconsistently (Pacific lamprey in 83% of the sampling events; western brook lamprey in 17% of the sampling events). Preliminary results indicate that over time there is not a significant change in pigmentation patterns associated with species identification.

Morphometric and meristic description of laboratory spawned specimens

Mean chorion diameter was greater and more variable for Pacific lampreys (1.468 ± 0.107 mm, $N = 320$) than for western brook lampreys (1.237 ± 0.064 mm, $N = 280$). Due to unequal variance an approximate t-statistic was calculated, using estimated degrees of freedom based on the Satterthwaite approximation (Satterthwaite 1946), to examine differences between the mean chorion diameter of Pacific and western brook lamprey eggs. Pacific lamprey eggs were significantly larger than western brook lamprey eggs ($t = 32.788$, df = 528.62, $P < 0.0001$) with a mean difference of 0.231 mm. Although a highly significant difference was observed, the magnitude of the difference was at a scale that would be difficult to measure under field conditions. Chorion diameter was chosen as a potential measurement for distinguishing between lamprey

species because it remains relatively constant throughout development from fertilization to hatching. Also, because of the chorion's spherical shape, diameter measurements should be roughly equivalent regardless of egg orientation. Other measurements related to the size and shape of developing embryos may be useful for species identification; however, the dynamic nature and 3-dimentional complexity of developing embryos would make this a daunting task even under the most controlled conditions.

Of the larvae collected for morphometric and meristic descriptions, 156 Pacific lampreys and 156 western brook lampreys were developmentally advanced enough for landmark locations to be quantified. Pacific lampreys ranged in size from 7.20 mm to 31.79 mm standard length (SL), and western brook lampreys ranged in size from 6.64 mm to 25.90 mm SL. Truss element lengths have been calculated and summarized for each species based on the original measurement scale (mm) (Table 1) and on a scale proportional to the individual's standard length (Table 2). The relationship of these measurements will be examined and analyzed using traditional multivariate techniques (Marcus 1990). These analyses will be performed in an attempt to separate Columbia River Basin lampreys based on morphometric characteristics; however, qualitative examination of these measurements (Table 2) suggests that if separation of these species can be achieved through the selected measurements, the scale at which the differences occur may be difficult to achieve under field conditions. Along with morphometric descriptions of these species, meristic analyses be will performed, but these will likely be limited to myomere counts as lampreys do not possess most other structures commonly used in meristic analyses (e.g., fin rays and spines, scale rows, and lateral line pores).

Effects of temperature on early life stages

Cumulative survival for the duration of the experiment was high for Pacific and western brook lampreys reared at 10° C, 14° C, and 18° C with a decrease in cumulative survival at 22° C. At 22° C, survival decreased early and consistently until individuals began to hatch, at which time cumulative survival began to stagnate (Figure 6; Figure 7). This suggests that the affect of temperature on survival may be dependent on developmental stage and indicates that embryos may be more sensitive to the effects of temperature than early stage larvae.

Percent survival was significantly affected by rearing temperature ($F_{3, 124} = 208.42$, $P < 0.0001$) and by species ($F_{1, 124} = 6.20$, $P = 0.0141$). Repeated measures indicated a significant difference in percent survival among hatching (260 TU_a) and larval (540 TU_a) developmental stages ($F_{1, 125} = 81.46$, $P < 0.0001$). Systematic variation was not observed among years ($F_{1, 125} = 0.34$, $P = 0.5599$) and there was no interaction between treatment temperature and species ($F_{3, 124} = 1.37$, $P = 0.2560$). Because no interaction was observed, mean comparisons were made between levels of main effects. No significant difference was observed in percent survival between Columbia River Basin lampreys reared at 10° C, 14° C, and 18° C, but significantly lower survival was observed at 22° C when compared to other rearing temperatures (Table 3; Figure 8). Mean percent survival of western brook lampreys (84.82%, SE = 2.09) was statistically greater than mean percent survival of Pacific lampreys (83.77%, SE = 1.69); however, the biological significance of the observed 1.05% difference in mean survival is questionable. Although cumulative survival remained relatively constant after 260 TU_a

(Figure 6; Figure 7), the difference in survival from 260 TU_a (86.35%, SE = 1.62) to 540 TU_a (82.05%, SE = 2.11) was statistically significant, suggesting significant mortality occurred after 50% hatch.

Thermal requirements for survival provide a good indication of extreme temperature limits; however, sub-lethal effects of temperature on a species, such as developmental abnormalities, may decrease long-term survival, growth, and fitness. Thus, we examined the effects of temperature and lamprey species on the occurrence of developmental abnormalities. Percent abnormal larvae at 540 TU_a was significantly affected by rearing temperature ($F_{3, 111} = 130.49$, $P < 0.0001$), but there was no difference among species ($F_{1, 111} = 0.65$, $P = 0.4214$) and there was not a significant interaction between rearing temperature and species ($F_{3, 111} = 2.40$, $P = 0.0720$). Variation among years was observed and accounted for with a blocking factor ($F_{1, 111} = 17.04$, $P < 0.0001$). Abnormalities at 540 TU_a were significantly higher at 22° C than at other temperatures examined (Figure 9), and included traits such as irregularly arched bodies, bulging body regions, and superfluous body parts (Figure 5).

Information about both the effects of temperature on survival and on development suggest that early life stage Columbia River Basin lampreys have a relatively broad thermal niche. We observed consistently high survival and low occurrence of developmental abnormalities at 10° C, 14° C, and 18° C, with a significant decrease in survival and increase in developmental abnormalities at 22° C. Comparatively, Piavis (1961) reported the optimal temperature for survival from zygote to burrowing larvae (developmentally similar to individuals at 540 TU_a in this experiment) of sea lampreys to

be 18.3° C, and observed no survival to the burrowing stage at temperatures below 15.6° C or above 21.1° C. This corresponds to a range of temperatures in which early life stage sea lampreys can survive of less than 4.5° C. Rodriguez-Munoz et al. (2001) observed a similar optimal temperature for early life stage sea lampreys (19° C), but observed a broader range of temperatures in which sea lampreys survived to the burrowing stage, with similar survival rates at 16° C, 19° C, and 23° C. The results of our experiments suggest that, for the effective time from fertilization to burrowing, early life stage Columbia River Basin lampreys have a lower maximum incipient lethal temperature (significant decrease in survival at 22° C) and a lower minimum incipient lethal temperature (high survival rates at 10° C) than sea lampreys (definitions follow the terminology of Fry 1971). Unfortunately, it is difficult to speculate on the thermal tolerance zone of these species, as we did not directly observe the lower incipient lethal temperature of Columbia River Basin lampreys. However, these data do suggest differences in the thermal requirements of Columbia River Basin lampreys as compared to other lamprey species and provide useful information about the suitability of potential lamprey spawning and rearing habitats within the Columbia River Basin.

River lampreys in the Columbia River Basin

The river lamprey (*Lampetra ayresi*) is an anadromous species that has been found in the Columbia River Basin as recently as 1983 (Bond et al. 1983). Collection records indicate a known distribution from Sacramento, California, to British Columbia, Canada. All of the specimens on record are predatory phase individuals that have been collected as bycatch from estuaries and bays along the northwest Pacific coastline. There

is no known collection of river lamprey larvae, which has been attributed to the difficulty in distinguishing between the three species of lampreys found in the Pacific Northwest (Pacific, western brook, and river lampreys). Presently, genetic testing indicates a distinct difference between Pacific lampreys when compared to western brook and river lampreys. However, based on analysis of mitochondrial DNA (Docker et al. 1999), river and western brook lampreys are genetically inseparable, which suggest a divergence time of less than 70,000 years ago.

Our search for live river lamprey specimens began in the fall of 1999 and was originally restricted to the Columbia River Basin, but in 2001 was expanded to include coastal rivers and estuaries from California to Canada (for an overview of agencies and organizations contacted see Appendix 4). Within the Columbia River Basin we spoke with personnel from universities and state, federal, tribal, and private agencies in an attempt to collect river lampreys. Initially, the Oregon Department of Fish and Wildlife (ODFW) and Washington Department of Fish and Wildlife (WDFW) were contacted to establish a list of possible collection locations. Individuals contacted within these agencies stated that there have been no sightings of adult river lampreys and that they have no way of distinguishing between the three species during larval life stages. Individuals contacted at both the Fish Passage Center for the Columbia River and the Lower Columbia River Estuary Program reported no sightings. According to the National Oceanic and Atmospheric Administration Fisheries Service (NOAA Fisheries Service), most of their recent sampling had been conducted in the Columbia River estuary where they were performing bottom and mid-water column trawls that were not

conducive to lamprey collection. The Yakama Nation reported that they had no sightings of river lampreys on the Klickitat River. Both Oregon State University and the University of Washington currently have predatory phase river lampreys in their collections, but none collected after 1983.

We broadened our search to areas outside of the Columbia River Basin, including portions of California and Canada and all of Oregon and Washington. In California, we have contacted both the Steinhart and the Monterey Bay Aquariums, neither of which have live lampreys on site. The Steinhart Aquarium has preserved specimens of river lampreys in their ichthyology collection; the most recent of which, collected in 1984, was found in the stomach contents of a sea bass in the San Francisco Bay, California. In Canada, river lampreys have been collected for research purposes within the Strait of Georgia and in the Fraser River Basin; however, few have been collected in recent years and their population status is unknown. Because of this, researchers and managers are hesitant to remove any river lampreys until more accurate population data are available.

In Washington, both the Point No Point Treaty Council and the Lower Elwha Klallam Tribe from the Puget Sound region were contacted. The Lower Elwha Klallam Tribe was the most promising, with records indicating capture of river lampreys in the past, but nothing currently. In Oregon, the Confederated Tribes of the Siletz Indian Reservation has collected river lampreys in the past, but has not had any recent sightings. Local WDFW offices were contacted for the Puget Sound, the Klickitat River Basin, Willamette River Basin, Umpqua River Basin, and the Smith River Basin. None of these offices have recorded sightings or collections of river lampreys. The Hatfield Marine

Science Center in Newport, Oregon, was unable to provide us with new information on search locations.

In May 2002 predatory phase river lampreys were located in Skagit Bay, Washington. Working in conjunction with NOAA Fisheries Service, 36 river lampreys were collected during regularly scheduled trawling. Unfortunately, these individuals were not sexually mature, and therefore were not directly useful for completion of this project's objectives. However, due to the difficulty we encountered in locating and collecting live river lamprey specimens, their presence in Skagit Bay is of interest and we are exploring the feasibility of maintaining river lampreys in a laboratory setting. Currently 14 of the 36 river lampreys collected by NOAA Fisheries Service are being held at the U.S. Geological Survey, Marrowstone Marine Field Station, Marrowstone Island, Washington, in seawater at ambient temperature.

FUTURE GOALS

Identification of early life stage lampreys

Validation of current diagnostic characteristics

We will continue to sample larvae currently held at the Columbia River Research Laboratory at intervals of approximately six weeks until: 1) metamorphosis occurs, at which point positive identification may be made, 2) mortality, at which point tissue samples will be collected, or 3) project termination, at which point tissue samples will be collected. This will allow us to follow known individuals through time and stages of metamorphosis. We will potentially be able to distinguish morphological changes and characteristics associated with various stages of metamorphosis for different species of

lampreys, providing us with information to determine the validity of current diagnostic characteristics.

Morphometric and meristic description of laboratory spawned specimens

We will continue sampling individuals reared at the Columbia River Research Laboratory to provide more material for morphometric and meristic analyses. Meristic data will be gathered during the fall/winter of 2002/2003 for inclusion in the final report of research for this project. Various multivariate techniques are currently being explored for their appropriateness in analyzing the morphometric data we have gathered, with sequential one-way discriminant function analysis a likely candidate (Tabachnick and Fidell 1996).

Effects of temperature on early life stages

We are currently exploring a number of temperature models for predicting rate processes in hope that these will give us the flexibility to provide more information on the role of temperature in development of Columbia River Basin Lampreys, including the ability to predict the zero development temperature (as in Rodriguez-Munoz et al. 2001). We are also finalizing data analyses in a way that will allow these data to be more directly compared to other research conducted on various lamprey species.

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Table 1: Descriptive statistics of truss elements for Pacific and western brook lampreys based on the original measurement scale (mm).

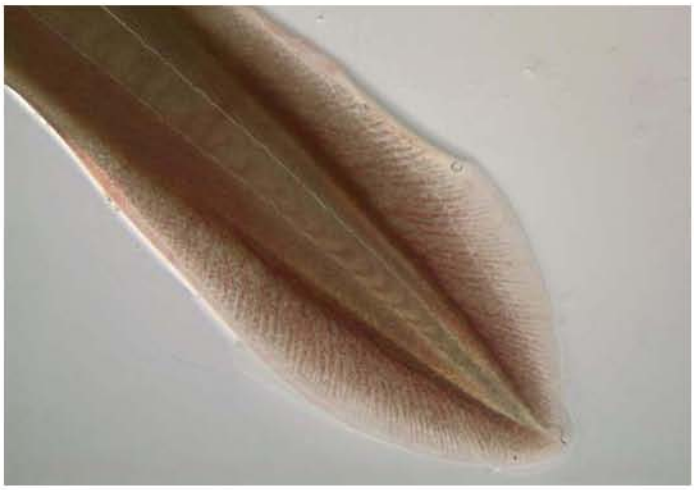
Species	Truss element	Mean length (mm)	Minimum length (mm)	Maximum length (mm)
Pacific lamprey	A	0.498	0.195	1.297
	B	2.525	0.630	9.556
N = 156	C	0.706	0.408	1.866
Mean SL (mm) = 10.41	D	2.797	1.639	7.956
Minimum SL (mm) = 7.20	E	2.623	1.423	7.506
Maximum SL (mm) = 31.79	F	2.791	1.625	7.850
	G	2.865	1.654	8.128
	H	0.947	0.650	2.209
	I	5.924	4.496	16.153
	J	6.132	4.414	16.163
	K	6.092	4.482	16.270
	L	6.087	4.526	16.260
	M	0.812	0.590	1.574
	N	1.365	0.529	6.823
	O	1.275	0.494	6.724
Western brook lamprey	A	0.452	0.199	1.185
	B	1.991	0.311	6.647
N = 156	C	0.653	0.371	1.499
Mean SL (mm) = 9.67	D	2.622	1.655	6.464
Minimum SL (mm) = 6.64	E	2.453	1.396	6.494
Maximum SL (mm) = 25.90	F	2.614	1.629	6.512
	G	2.673	1.578	6.815
	H	0.868	0.572	1.788
	I	5.487	3.784	13.871
	J	5.608	3.674	14.014
	K	5.598	3.798	14.048
	L	5.600	3.749	13.957
	M	0.663	0.377	1.271
	N	1.306	0.480	4.470
	O	1.196	0.445	4.534

Table 2: Mean length and standard deviation of truss elements, proportional to individual's standard length, for Pacific and western brook lampreys.

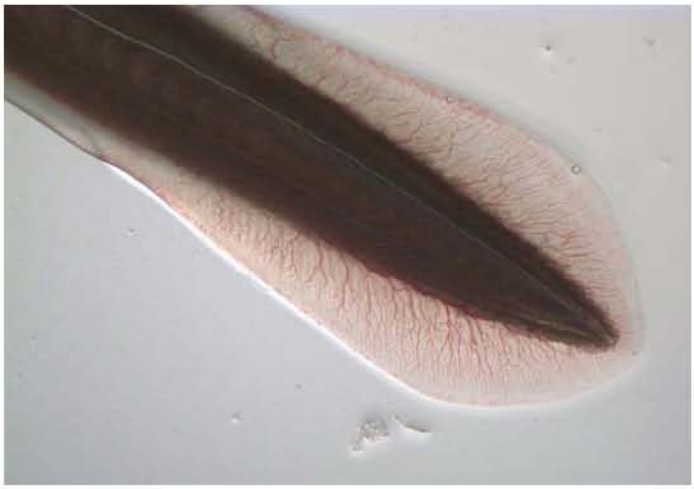
	Pacific lamprey		Western brook lamprey	
Truss element	Mean length proportional to SL	Standard deviation	Mean length proportional to SL	Standard deviation
A	0.048	0.007	0.046	0.008
B	0.250	0.076	0.213	0.083
C	0.068	0.008	0.068	0.007
D	0.269	0.022	0.272	0.016
E	0.250	0.032	0.250	0.027
F	0.268	0.024	0.271	0.019
G	0.274	0.030	0.274	0.024
H	0.093	0.008	0.093	0.008
I	0.579	0.051	0.577	0.042
J	0.601	0.051	0.592	0.045
K	0.597	0.053	0.590	0.044
L	0.595	0.048	0.590	0.043
M	0.082	0.013	0.073	0.011
N	0.123	0.024	0.128	0.020
O	0.113	0.027	0.114	0.026

Table 3: Results of Bonferroni *t*-test on differences between mean survival at four rearing temperatures for Columbia River Basin lampreys. Mean differences are represented as absolute values. Asterisk indicates a significant difference at α=0.05.

		Temperature			
		10° C	14° C	18° C	22° C
Temperature	Mean	95.29	95.93	96.49	54.14
10° C	95.29	-	0.64	1.20	41.15 *
14° C	95.93		-	0.56	41.79 *
18° C	96.49			-	42.35 *
22° C	54.14				-

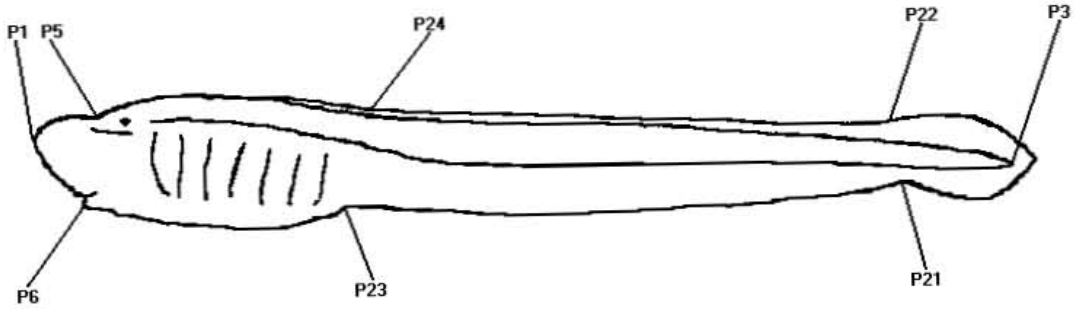


1a

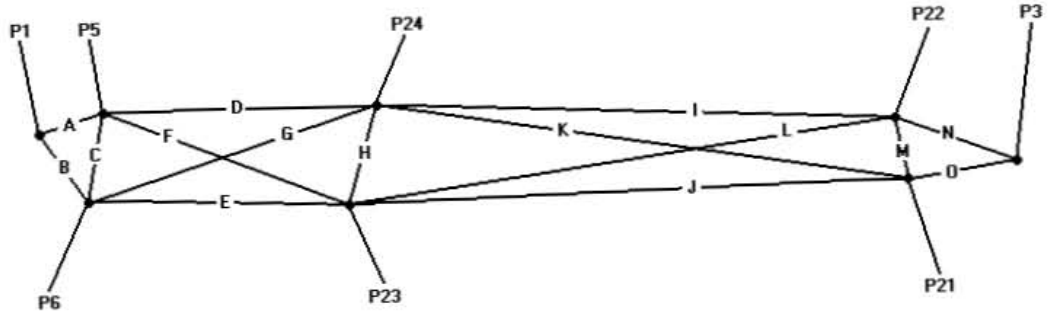


1b

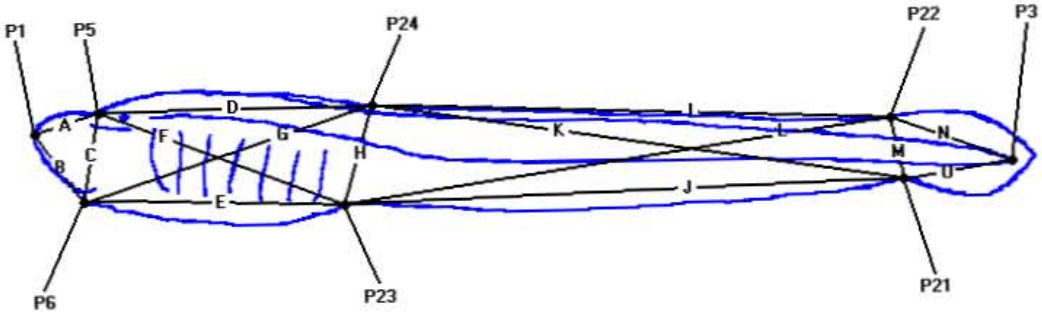
Figure 1: Examples of digital images of caudal region of: 1a) Pacific lamprey; characterized by light pigmentation along the caudal ridge, and 1b) western brook lamprey; characterized by dark, even pigmentation along the caudal ridge (Richards et al. 1982).



2a



2b



2c

Figure 2: 2a) Representation of a larval lamprey indicating the location of the eight homologous landmarks, 2b) representation of the structure of the two-cell truss network with two appended triangles, and 2c) representation of a larval lamprey with the truss network overlaid.

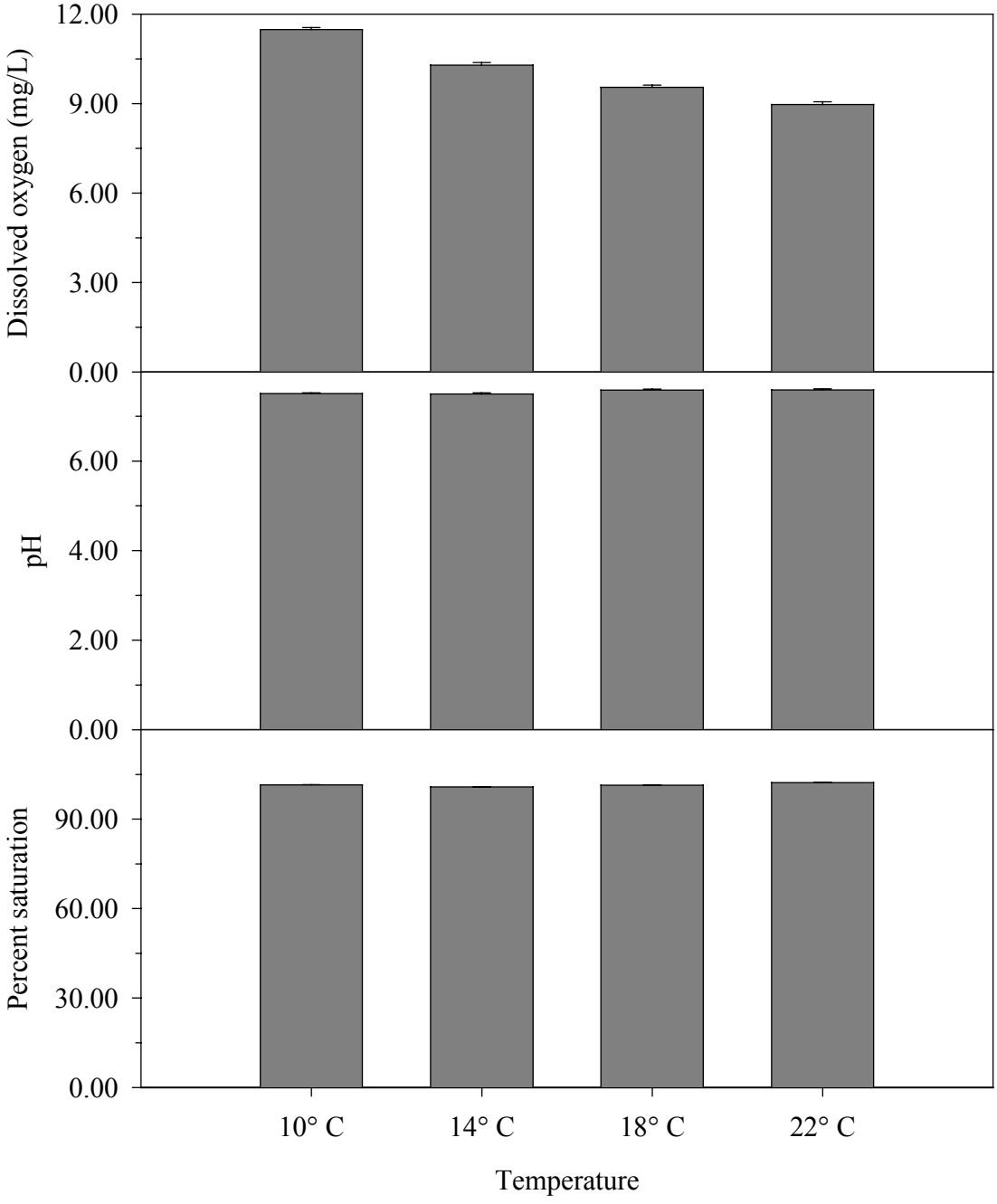


Figure 3: Mean dissolved oxygen content (mg/L), pH, and total dissolved gasses (% saturation) (plus SE) of water baths at 10° C, 14° C, 18° C, and 22° C. Measurements were taken daily for the duration of the experiment.

4a

4b

4c

4d

Figure 4: Time series of normal larval development of Pacific and western brook lampreys: 4a) recently hatched larva exhibiting well-pronounced ventral flexion, 4b) less pronounced ventral flexion, 4c) slight ventral flexion in posterior region, and 4d) fully developed larva.

5a

5b

5c

5d

Figure 5: Time series of abnormal larval development of Pacific and western brook lampreys: 5a) larva with malformed head, branchial, and trunk regions, 5b) larva with malformed trunk region, 5c) larva with superfluous head and branchial region, and 5d) larva with extreme morphological malformations.

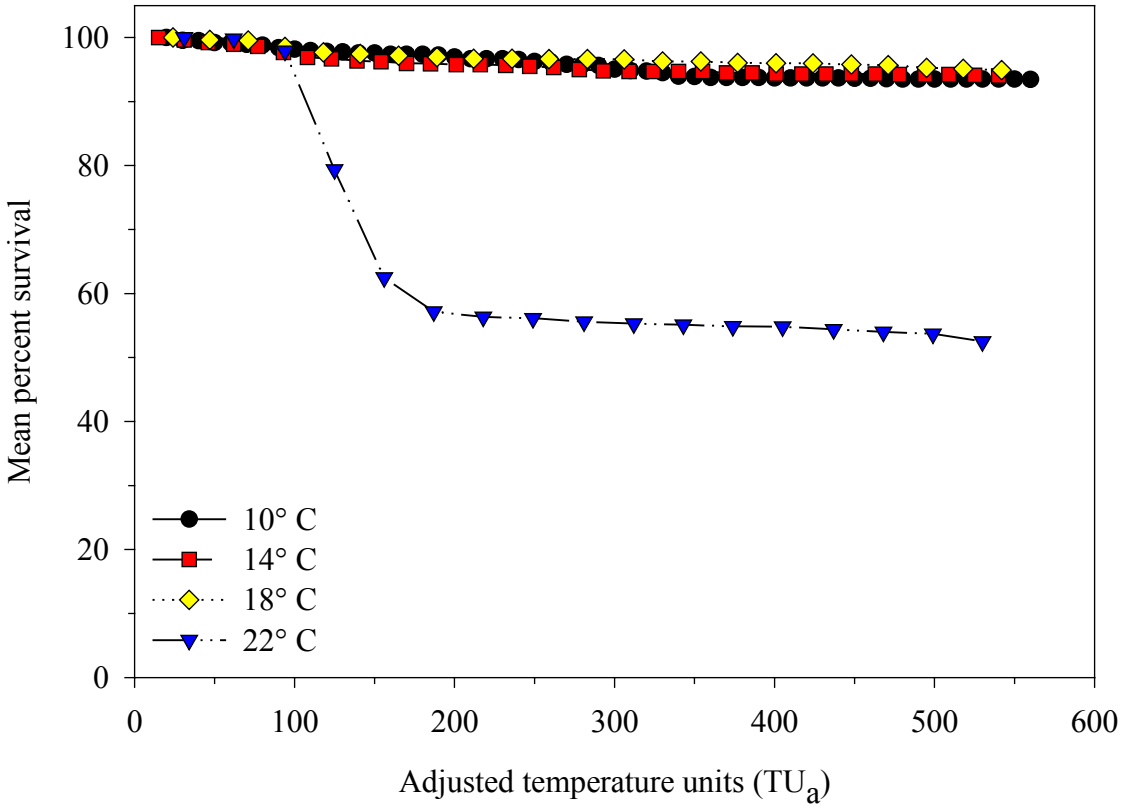


Figure 6: Cumulative mean percent survival for the duration of the experiment expressed as TU_a for Pacific lampreys. 50% hatch occurred at 260 TU_a.

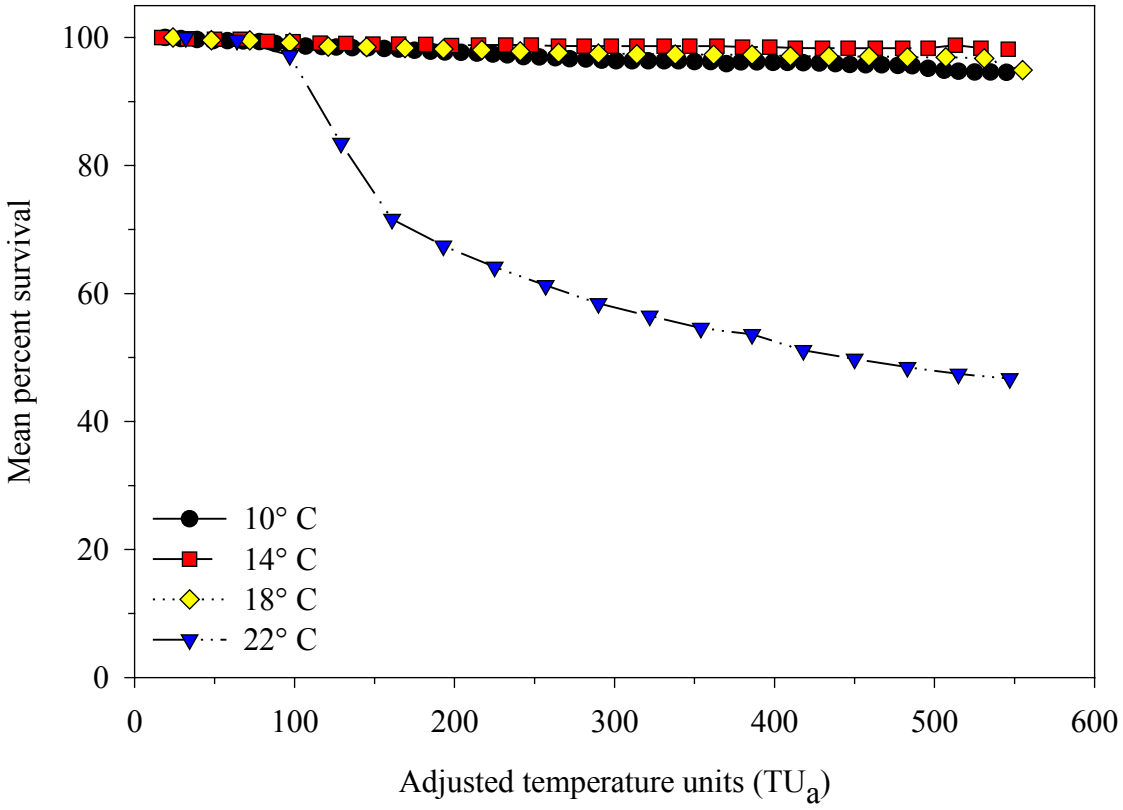


Figure 7: Cumulative mean percent survival for the duration of the experiment expressed as TU_a for western brook lampreys. 50% hatch occurred at 260 TU_a.

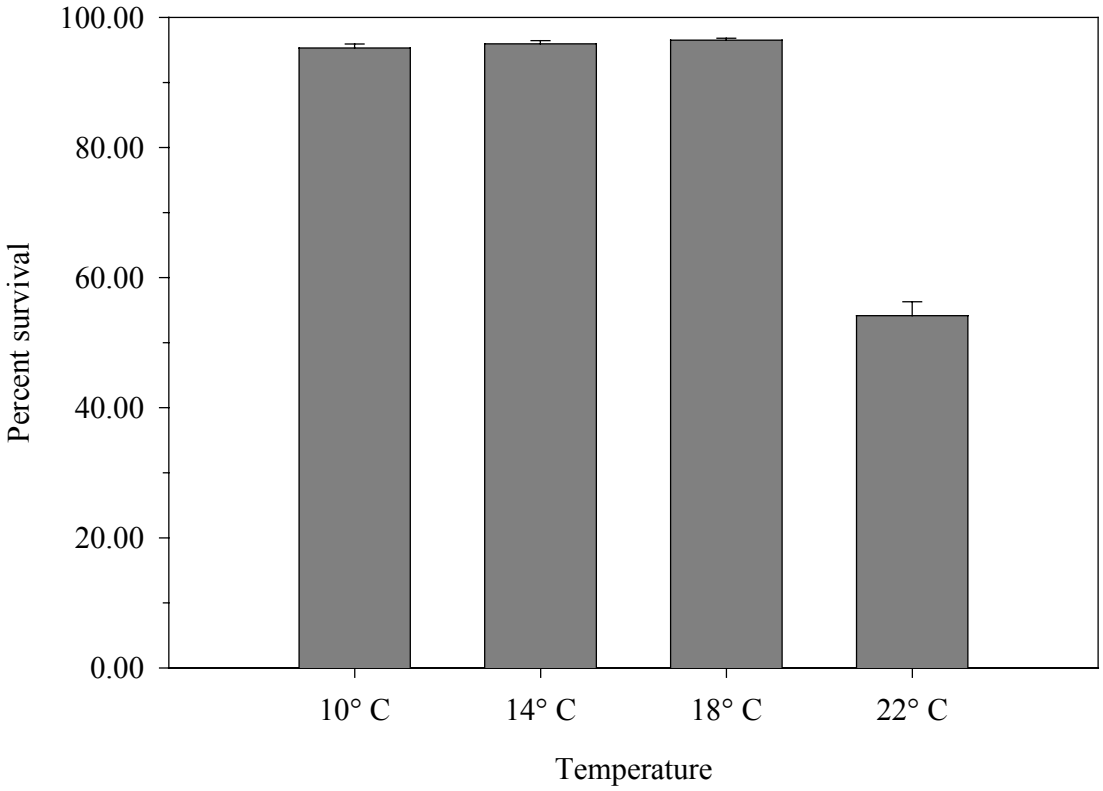


Figure 8: Mean percent survival (plus SE) for Columbia River Basin lampreys reared at four temperatures (10° C, 14° C, 18° C, and 22° C). A significant decrease in percent survival was observed at 22° C when compared to lampreys reared at other temperatures.

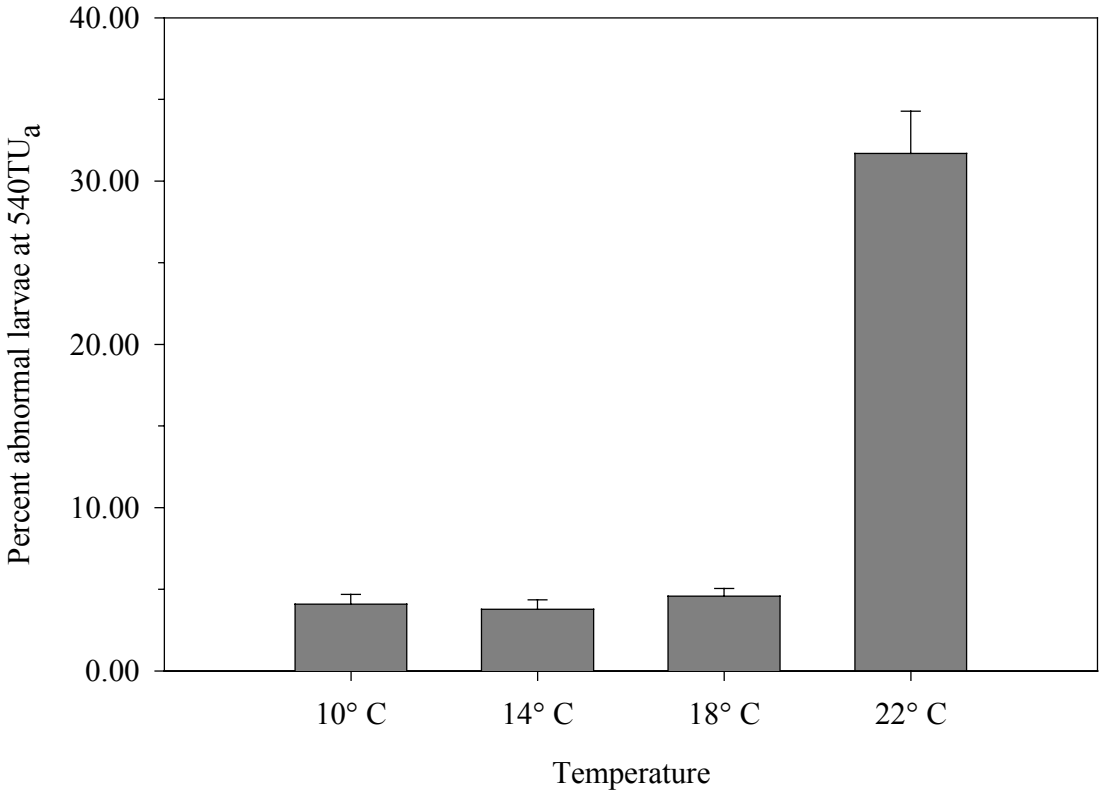


Figure 9: Mean percent abnormal larvae at 540 TU_a (plus SE) of Columbia River Basin lampreys. A significant increase in percent abnormal larvae at 540 TU_a was observed at 22° C when compared to other rearing temperatures.

Appendix 1: Sample number, collection location, length (mm), mass (g), and preliminary species identification based on current diagnostic characteristics for lamprey larvae sacrificed for genetic analyses. Genetic confirmation of identification is not yet available (NYA). Collection location: ENT=Entiat River, JDW=John Day/Walla Walla Rivers, RED=Red River, and CED=Cedar Creek. Preliminary species identification: PCL=Pacific lamprey and WBL=western brook lamprey.

Sample number	Collection location	Length (mm)	Mass (g)	Preliminary species identification	Genetic confirmation
1	ENT	130	3.481	PCL	NYA
2	ENT	126	2.824	PCL	NYA
3	ENT	134	3.555	PCL	NYA
4	ENT	133	3.631	PCL	NYA
5	ENT	137	3.997	PCL	NYA
6	ENT	123	3.125	PCL	NYA
7	ENT	127	3.427	PCL	NYA
8	ENT	145	4.277	PCL	NYA
9	ENT	134	3.955	PCL	NYA
10	ENT	141	3.593	PCL	NYA
11	ENT	143	4.161	PCL	NYA
12	ENT	130	3.441	PCL	NYA
13	JDW	148	4.840	WBL	NYA
14	JDW	131	3.501	WBL	NYA
15	JDW	124	2.950	PCL	NYA
16	JDW	126	3.086	WBL	NYA
17	JDW	146	4.765	WBL	NYA
18	JDW	143	4.337	WBL	NYA
19	JDW	127	3.136	PCL	NYA
20	JDW	138	3.089	WBL	NYA
21	JDW	130	3.858	PCL	NYA
22	JDW	129	3.471	PCL	NYA
23	JDW	128	3.280	PCL	NYA
24	JDW	132	3.567	WBL	NYA
25	JDW	132	3.521	WBL	NYA
26	JDW	115	2.507	PCL	NYA
27	RED	141	4.560	PCL	NYA
28	RED	152	5.551	PCL	NYA
29	RED	141	4.543	PCL	NYA
30	RED	122	2.772	PCL	NYA
31	RED	111	2.190	PCL	NYA
32	RED	137	4.084	PCL	NYA
33	CED	117	2.280	PCL	NYA
34	CED	111	1.985	PCL	NYA
35	CED	104	1.587	PCL	NYA
36	CED	107	1.877	PCL	NYA
37	CED	108	1.749	PCL	NYA
38	CED	86	1.038	PCL	NYA
39	CED	119	2.474	PCL	NYA
40	CED	120	2.576	PCL	NYA
41	CED	119	2.439	PCL	NYA
42	CED	113	2.062	PCL	NYA
43	CED	97	1.201	PCL	NYA
44	CED	122	2.752	PCL	NYA
45	CED	116	2.595	PCL	NYA
46	CED	115	2.158	PCL	NYA
47	CED	107	1.768	PCL	NYA
48	CED	95	1.330	PCL	NYA
49	CED	96	1.316	PCL	NYA
50	CED	94	1.440	PCL	NYA

Appendix 2: Number of sampling events (at approximately six week intervals), mean length (mm), mean mass (g), percent of sampling events where individual was identified as PCL (Pacific lamprey), percent of sampling events where individual was identified as WBL (western brook lamprey), and species identification, if confirmation was possible, for 31 individuals from four collection sites (CED = Cedar Creek, WA; ENT = Entiat River, WA; RED = Red River, WA; JDW = John Day River, OR/Walla Walla River, WA).

Collection site	Number of sampling events	Mean length (mm)	Mean mass (g)	Percent of events identified as PCL	Percent of events identified as WBL	Confirmed species identification
CED	7	90	0.901	100	0	
CED	11	109	1.560	100	0	
CED	4	93	1.047	100	0	
CED	11	85	0.849	100	0	
CED	5	82	0.792	100	0	
CED	13	85	0.859	100	0	
CED	12	84	0.956	83	17	
CED	14	94	1.108	100	0	
CED	12	91	0.876	100	0	
RED	11	135	3.589	100	0	PCL
RED	21	130	3.162	100	0	
RED	21	134	3.445	100	0	
RED	21	129	2.941	100	0	
RED	6	142	4.500	100	0	
RED	21	140	3.727	100	0	
ENT	21	129	3.147	100	0	
ENT	21	125	2.667	100	0	
ENT	13	108	1.713	100	0	
ENT	18	124	2.919	100	0	PCL
ENT	21	129	2.768	100	0	
ENT	21	135	3.634	100	0	
ENT	21	121	2.736	100	0	
ENT	21	123	2.538	100	0	
JDW	20	125	2.752	100	0	
JDW	21	122	2.312	0	100	
JDW	21	117	2.276	0	100	
JDW	15	114	1.921	0	100	
JDW	21	124	2.752	100	0	
JDW	21	117	2.202	100	0	
JDW	21	120	2.368	100	0	
JDW	20	111	1.841	0	100	

Appendix 3: Landmark and truss element reference labels (Figure 2) and descriptions.

Feature	Label	Description
Landmark	P1	Anterior most portion of the larva (snout)
Landmark	P3	Posterior most portion of the notochord
Landmark	P5	Margin of the oral hood and the head
Landmark	P6	Anterior most portion of the transverse lip of the oral hood
Landmark	P21	Anterior most portion of the vent
Landmark	P22	Point at the termination of a line drawn from P21 to, and perpendicular to, the dorsal surface of the larva
Landmark	P23	Ventral margin of the branchial region and the trunk region
Landmark	P24	Point at the termination of a line drawn from P23 to, and perpendicular to, the dorsal surface of the larva
Truss element	A	Line connecting P1 and P5
Truss element	B	Line connecting P1 and P6
Truss element	C	Line connecting P5 and P6
Truss element	D	Line connecting P5 and P24
Truss element	E	Line connecting P6 and P23
Truss element	F	Line connecting P5 and P23
Truss element	G	Line connecting P6 and P24
Truss element	H	Line connecting P23 and P24
Truss element	I	Line connecting P24 and P22
Truss element	J	Line connecting P23 and P21
Truss element	K	Line connecting P24 and P21
Truss element	L	Line connecting P23 and P22
Truss element	M	Line connecting P21 and P22
Truss element	N	Line connecting P22 and P3
Truss element	O	Line connecting P21 and P3

Appendix 4: Contact name and affiliation of organizations contacted during investigation for potential sources of river lamprey specimens.

Contact name	Organization
Bashman, Larry	Fish Passage Center, Portland, Oregon
Beamish, Richard	Canadian Department of Fisheries and Oceans
Bond, Carl	Oregon State University
Crane, Pat	Lower Elwha Klallam Tribe
Docker, Margret	University of Windsor, Ontario, Canada
Goodwin, Kevin	Oregon State University, Hatfield Marine Science Center
Haas, Gordon	University of Alaska Fairbanks
Hinton, Sue	National Oceanic and Atmospheric Administration Fisheries Service
Jacobs, Steve	Oregon Department of Fish and Wildlife, Corvallis
Johnson, Thom	Point No Point Treaty Council
Loomis, Dave	Oregon Department of Fish and Wildlife, Roseburg
Mallat, Jon	Washington State University
Markle, Doug	Oregon State University
McCosker, John	Steinhart Aquarium
McRay, Gene	Oregon State University, Hatfield Marine Science Center
Mongillo, Paul	Washington Department of Fish and Wildlife
Niemi, Dan	Washington Department of Fish and Wildlife, Fish Collection Facility
Parkenson, Eric	University of British Columbia
Rice, Casey	National Oceanic and Atmospheric Administration Fisheries Service
Rien, Tom	Oregon Department of Fish and Wildlife
Smith, Mysi	Steinhart Aquarium
Sutherland, Bruce	Lower Columbia River Estuary Program
Thompson, Terry	Association of Trawlers
Tinus, Eric	Oregon Department of Fish and Wildlife
Tucker, Tom	Monterey Bay Aquarium
Urbain, Brian	University of Washington
Van der Wetering, Stan	Confederated Tribes of the Siletz Indian Reservation
Weinheimer, John	Washington Department of Fish and Wildlife