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
2001

DOE/BP-00004695-1

January 2002

This Document should be cited as follows:

Meeuwig, Michael, Jennifer Bayer, James Seelye, Rebecca Reiche, "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", Project No. 2000-02900, 54 electronic pages, (BPA Report DOE/BP-00004695-1)

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This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views in this report are the author's and do not necessarily represent the views of BPA.

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 2001

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Project Number 2000-029
Contract Number 00AI23249

January 2002

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EXECUTIVE SUMMARY

Two fundamental aspects of lamprey biology were examined in order 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 used for 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 development and survival 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 14 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 83% of sampling events and western brook lamprey 17% of sampling events) suggesting that pigmentation patterns do not change appreciably through time. Also, in 2001 we artificially spawned Pacific and western brook lampreys in the laboratory and collected 150 Pacific lamprey and 140 western brook lamprey embryos and 110 Pacific

lamprey and 70 western brook lamprey prolarvae/larvae for meristic and morphometric description.

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 had an overall significant effect on survival from fertilized egg to 50% hatch with increasing survival from 10° C to 18° C and decreased survival at 22° C for Pacific ($F_{3,28}$=74.10, P<0.0001) and western brook ($F_{2,24}$=66.50, P<0.0001) lampreys. Temperature had an overall significant effect on survival from fertilized egg to the time when prolarvae had assimilated 50% of their yolk reserves with increasing survival from 10° C to 18° C and decreased survival at 22° C for Pacific ($F_{2,21}$=53.00, P<0.0001) and western brook ($F_{2,24}$=70.16, P<0.0001) lampreys. Temperature had a significant effect on the occurrence of embryonic abnormalities prior to hatch for western brook lampreys ($F_{3,32}$=6.70, P=0.0012) with a greater percentage of embryonic abnormalities at 10° C and 22° C than at intermediate temperatures for both species. Temperature had an overall significant effect on the occurrence abnormalities at 50% yolk assimilation with a greater percentage of abnormalities at 22° C for Pacific ($F_{2,21}$=39.75, P<0.0001) and western brook ($F_{2,24}$=41.26, P<0.0001) lampreys. Based on survival data, the occurrence of embryonic abnormalities, and the occurrence of abnormalities at 50% yolk assimilation, the optimal temperature for development of early life stage Pacific and western brook lampreys appears to be approximately 18° C.

ACKNOWLEDGEMENTS

We thank individuals in the U.S. Fish and Wildlife Service, University of Idaho, Idaho Department of Fish and Game, Confederated Tribes of the Umatilla Indian Reservation, and the U.S. Geological Survey for their assistance with project activities. We also appreciate the assistance of Debbie Docherty, Project Manager, Bonneville Power Administration.

INTRODUCTION

Lampreys inhabit temperate regions in both the northern and southern hemispheres. Typically, lampreys spawn in fresh water 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 structure 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, parasitic 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 host animals. Lampreys exhibit rapid growth during the parasitic phase, which can last from less than one year to greater than two and a half years (Hardisty and Potter 1971b); however, there appears to be a high degree of variability in the duration of the parasitic phase 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, parasitic life history pattern and western brook lampreys (*L. richardsoni*) exhibit a non-migratory, non-parasitic life history pattern. Apart from their general feeding patterns, little is known about the biology of lamprey species found 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 1995), 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 stages is particularly important since this period is a critical determinant of recruitment for many fish populations (Houde 1987). Larval 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). Temperature is a pervasive component of the environment and its effects on survival have been studied for a broad range of taxa. For example, the range of optimal temperatures for survival of sea lamprey (*Petromyzon marinus*) embryos is narrow (Piavis 1961). Although similarities may exist, optimal temperatures for survival and development may differ among species of lampreys (Piavis 1961). Understanding how temperature affects survival of early life

history stages will help identify critical habitat needs that influence lamprey distribution and abundance (Holmes and Lin 1994). Information on the role of temperature on lamprey early life history 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 of embryo and larval stages of Pacific, western brook and river lampreys, and 2) examine the effects of temperature on development and survival of early life history stages of Pacific, western brook, and river lampreys.

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 the 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 project is currently in its second year and is scheduled for a third year. This document generally focuses on data collected since the production of the 2000 annual report of research to Bonneville Power Administration. For an overview of results from the first year of this study see Bayer et al. (2001). Due to equipment failure (see below), a third year of data collection is necessary to meet project goals. Experiments were not performed on river lampreys in 2000 or 2001 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

Adult Pacific lampreys were collected from the Columbia River at Bonneville Dam and adult 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 these experiments included six males (length = 508 ± 41 mm; mass = 287.5 ± 87.9 g) and six females (length = 494 ± 93 mm; mass = 352.7 ± 102.2 g). Western brook lampreys used for these experiments included 19 males (length = 127 ± 7 mm; mass = 3.938 ± 0.668 g) and 32 females (length = 122 ± 5 mm; mass = 4.206 ± 0.650 g). Mature lampreys were removed from holding tanks and anesthetized in 250 mg/L of tricaine methane sulfonate (MS-222) buffered with an equal concentration of sodium bicarbonate. Lampreys were rinsed in fresh water before spawning to remove traces of anesthetic. Female lampreys were positioned over a glass bowl filled with about 2 L of fresh water at approximately 16° C (temperature of water in 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 minutes and allowed to rest undisturbed for 0.5 h to allow fertilization to occur. After 0.5 h 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 0.5 h). 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 larvae

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 and mass 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 (laboratory analysis conducted by Dr. Matt Powell, University of Idaho). These samples will be used to genetically confirm species identification. Thirty-one larvae were individually marked with an injection of dyed elastomer and held at the Columbia River Research Laboratory. These larvae were sampled at approximately six-week intervals. At each sampling event lampreys were removed from aquaria, anesthetized in 250 mg/L of buffered MS-222,

measured for length (mm) and mass (g), 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 process will be repeated until: 1) individuals metamorphose, at which point species identification can be confirmed, or 2) individuals die, at which time genetic samples will be collected for analysis.

Morphometric and meristic description of laboratory spawned specimens

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 prolarvae (for definitions of early life stages see Piavis 1961) had assimilated approximately 50% of their yolk (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 will be 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. Digital images and preserved material will be used to produce morphometric and meristic descriptions of Pacific and western brook lampreys through a range of developmental stages.

Effects of temperature on early life history stages

Experimentation procedures were the same for both species spawned in 2001. Following fertilization (see above), zygotes were incubated at 10° C, 14° C, 18° C, and 22° C for 15 TU (Temperature Units) where:

$$TU = (\text{number of days}) \times (\text{degrees above } 0^\circ\text{ C})$$

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. Therefore, experimental individuals were selected that had reached the same developmental stage regardless of incubation 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. After 15 TU embryos were removed from hatching jars and 100 viable embryos were placed into each of ten rearing vessels (replicates) per temperature (treatment). Rearing vessels had a volume of approximately 60 ml and were constructed with a screen bottom to allow water to flow through. Rearing vessels were placed into an incubation bath of the appropriate temperature 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 0.5 h 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 (mg/L), pH, and total dissolved gasses (TDG) (Figure 2, Figure 3, and Figure 4, respectively).

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 prolarvae had hatched and had assimilated approximately 50% of their yolk reserves (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 prolarvae, 3) number of abnormal embryos, and 4) number of abnormalities at 50% yolk assimilation. Embryonic abnormalities and abnormalities at 50% yolk assimilation were traits considered to have a potential negative affect on survival, development, or fitness in conditions less favorable than a laboratory setting. These included traits such as fragmented embryonic material, malformed embryonic material, and various body malformations of prolarvae. For examples of normal and abnormal development see Figures 5 through 8. From the data collected we derived our response variables of: 1)

percent survival, 2) percent abnormal embryos, and 3) percent abnormalities at 50% yolk assimilation.

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, to compare the effects of temperature on survival and development of lampreys, we developed an adjusted temperature unit model (TU_a) specific to this experiment. This was done to allow comparisons to be made among lampreys exposed to different temperatures at similar developmental stages. Logistic regression (SAS v8.01) was used to predict the time to 50% hatch for each species held at each temperature, where 50% hatch was considered to be a discrete developmental event. It was assumed that all species by 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 temperature combinations. Therefore:

$$TU_a = (\text{number of day}) \times (\text{degrees above } 0^\circ \text{ C}) \times (\text{adjustment factor})$$

This adjusted temperature unit model provides a means to standardize time to specific developmental stages for making comparisons among lampreys exposed to different 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 $\alpha=0.05$ using SAS v8.01 software. For each species, analysis of variance (ANOVA) was used to make comparisons among

lampreys reared at different temperatures. Specifically, we examined the effects of temperature on: 1) percent survival to 50% hatch (280 TU_a), 2) percent survival to 550 TU_a (50% yolk assimilation), 3) percent of embryos exhibiting developmental abnormalities at 190 TU_a (late embryonic development prior to any hatching), and 4) percent of prolarvae exhibiting developmental abnormalities at 550 TU_a. When temperature had an overall significant effect, Bonferroni *t*-tests were used to make pairwise comparisons between temperatures.

Due to equipment failure, control of temperature was lost for the 14° C treatment midway through this experiment. Because of this, comparisons could not be made between lampreys reared at 14° C and at other temperatures for Pacific lampreys after 50% hatch (280 TU_a) or for western brook lampreys after 190 TU_a.

PRELIMINARY RESULTS AND DISCUSSION

Identification of larvae

Validation of current diagnostic characteristics

Fifty larvae were collected from the wild, identified to species (Richards et al. 1982), measured for length and mass, photographed, and sacrificed to provide genetic samples. Of the 50 individuals sampled in this manner, 42 were identified as Pacific lampreys and eight were identified as western brook lampreys based on current diagnostic characteristics (Appendix A). Researchers at the University of Idaho were unable to locate genetic sequences or loci suitable for differentiating Pacific and western brook lampreys. 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 genetic techniques for differentiating lampreys found in the Columbia River Basin.

The 31 larvae held at the Columbia River Research Laboratory for repeated identification have been sampled 14 times to date (some individuals less due to mortality) (Appendix B). In the case of mortality, genetic samples have been taken for later species confirmation. This sampling 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, specifically with regards to diagnostic characteristics, of these species over time.

Of the 31 larvae sampled in this manner, species identification has been confirmed for two Pacific lampreys that have metamorphosed. These individuals were identified as Pacific lampreys during 100% of the sampling events. Species identification has been consistent (100% of sampling events) for 28 of the un-metamorphosed lampreys. Only one individual has been identified inconsistently (Pacific lamprey in 83% of sampling events; western brook lamprey in 17% of 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

A total of 150 Pacific and 140 western brook lamprey embryos have been digitized and preserved for morphometric analysis (Appendix C). These individuals range in development from one-day post-fertilization to immediately pre-hatching.

Measurements have not yet been made on this material, but will likely consist of yolk diameter of individuals prior to formation of neural plate (non-spherical embryo) and chorion diameter of all individuals.

A total of 110 Pacific and 70 western brook lamprey prolarvae/larvae (Piavis 1961) have been digitized and preserved for morphometric and meristic analysis (Appendix C). For early prolarval stages, morphometrics will be limited to notochord length and possibly some measurement of depth, as distinct features are not distinguishable until later in development. Morphometrics for later stage prolarvae and larvae will likely consist of a series of linear measurements between homologous landmarks. These measurements will be analyzed using traditional multivariate techniques (Marcus 1990). The outcome of this analysis should provide information about what characteristics are important for identifying and differentiating Pacific and western brook lampreys. Meristic analyses 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 history stages

Cumulative survival for the duration of the experiment was high for Pacific and western brook lampreys 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 9 and Figure 10). This suggests a major change in the effects of temperature on survival based on

developmental stage and indicates that embryos may be more sensitive to the effects of temperature than early stage prolarvae.

To further investigate the effects of temperature on survival, comparisons among and between temperatures were made at discrete intervals. Overall, temperature had a significant effect on survival to 50% hatch (280 TU_a) for Pacific lampreys ($F_{3,28}$=74.10, P<0.0001) and western brook lampreys ($F_{2,24}$=66.50, P<0.0001). For both species there was a slight increase in survival from 10° C to 18° C followed by a decrease in survival at 22° C (Figure 11). There was a significant decrease in survival at 22° C when compared to other temperatures examined for Pacific and western brook lampreys (Table 1 and Table 2, respectively).

Temperature had a significant effect on survival to 550 TU_a for Pacific lampreys ($F_{2,21}$=53.00, P<0.0001) and western brook lampreys ($F_{2,24}$=70.16, P<0.0001). 550 TU_a corresponded to 50% yolk assimilation and the beginning of exogenous feeding. For both species there was a slight increase in survival from 10° C to 18° C followed by a decrease in survival at 22° C (Figure 12). There was a significant decrease in survival at 22° C when compared to other temperatures examined for Pacific and western brook lampreys (Table 3 and Table 4, respectively).

Among the temperatures examined, these data suggest that 18° C was the most beneficial temperature for survival of Pacific and western brook lampreys. This is similar to the thermal optima reported for survival of sea lampreys. Piavis (1961) and Rodriguez-Munoz et al. (2001) reported optimal survival temperatures from zygote to burrowing larvae (developmentally similar to individuals at 550 TU_a in this experiment)

for sea lampreys to be 18.4° C and 19° C, respectively. Although similarities in beneficial temperatures for survival exist among the species studied in this experiment and sea lampreys, the range of temperatures for survival of Pacific and western brook lampreys appears to be greater than that observed for sea lampreys. Data from this study suggest high survival rates for lampreys reared at 10° C, 14° C, and 18° C. However, Piavis (1961) observed no survival to the burrowing stage below 15.5° C or above 21.1° C and Rodriguez-Munoz et al. (2001) observed low survival from fertilization to hatching and no survival from hatching to burrowing for sea lampreys at 11° C.

Thermal requirements for survival provide a good indication of extreme temperature limits; however, sub-lethal effects of temperature on a species may play a role in long-term survival and fitness. It is likely that the occurrence of developmental abnormalities may decrease long-term survival, growth, and fitness. To provide a greater understanding of the effects of temperature on Columbia River Basin lampreys the effects of temperature on the occurrence of developmental abnormalities was examined at discrete intervals. The effect of temperature on the occurrence of embryonic abnormalities was examined at 190 TU_a, an interval prior to any lampreys hatching. Temperature had a slightly insignificant effect on the percent abnormal embryos for Pacific lampreys ($F_{3,28}$=2.73, P=0.0629); however, temperature had a significant effect on the percent abnormal embryos for western brook lampreys ($F_{3,32}$=6.70, P=0.0012). There were a greater percentage of embryonic abnormalities at 10° C and 22° C than at 14° C or 18° C for both species (Figure 13). For western brook lampreys there were significantly

more embryonic abnormalities at 22° C than at 14° C or 18° C and significantly more embryonic abnormalities at 10° C than at 18° C (Table 5).

The effect of temperature on the occurrence of abnormalities was examined at 550 TU_a, an interval after all lampreys had hatched, began exogenous feeding, and assimilated approximately 50% of their yolk reserves. Temperature had a significant effect on the occurrence of abnormalities at 50% yolk assimilation for Pacific lampreys ($F_{2,21}$=39.75, P<0.0001) and western brook lampreys ($F_{2,24}$=41.26, P<0.0001). For both species there was a greater percentage of abnormalities at 22° C than at 10° C or 18° C (Figure 14). For both species there were significantly more abnormalities at 22° C than at 10° C or 18° C (Table 6 and Table 7).

While differences in the percent of abnormal embryos were not consistently significant among and between treatments for both Pacific and western brook lampreys, certain trends similar to both species were observed. For both species there appears to be a higher percentage of embryonic abnormalities at 10° C and 22° C than at intermediate temperatures. It is difficult to speculate on the ultimate effect of the embryological abnormalities quantified in this experiment on survival, growth, and fitness; however, traits such as fragmented embryonic material may result in less material available for embryonic growth and malformed embryos may produce malformed larvae with decreased locomotor, feeding, or burrowing abilities. Abnormalities at 50% yolk assimilation were consistently higher at 22° C than at other temperatures examined for both species and included traits such as irregularly arched bodies, bulging body regions, and superfluous body parts (e.g., extra head or tail).

The developmental abnormalities observed in this experiment have the potential to significantly reduce larval viability through decreased locomotor, feeding, and burrowing performance. Therefore, it is important to synthesize the effects of temperature on survival and on development when considering the effects of temperature in this experiment. Considering information on both survival and the occurrence of developmental abnormalities, temperatures of approximately 18° C are most beneficial for viability of Pacific and western brook lampreys. These data should provide information necessary for the management of Columbia River Basin lampreys by indicating conditions necessary for spawning and rearing of Pacific and western brook lampreys.

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 1980 (Bond et al. 1983). Collection records indicate a known distribution from Sacramento, California to British Columbia, Canada. All of the specimens on record are adults that have been collected as bycatch from estuaries and bays along the northwest Pacific coastline. There are no known collections 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 the western brook lamprey and the river lamprey when compared to the Pacific lamprey. However, according to Docker (1999), river and western brook lampreys are genetically inseparable at this time.

Our search for river lampreys began in the fall of 1999 and is still an ongoing project. Originally, the pursuit was restricted to the Columbia River Basin, but has now expanded to include coastal rivers and estuaries from California to Canada. Within the Columbia River Basin we spoke with state, federal, tribal and private agencies and universities 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 of ammocoetes. 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 Marine Fisheries Service (NMFS), most of their recent sampling has 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 adult river lampreys in their collections, but none collected after 1983. In an effort to collect river lampreys from the Columbia River estuary we are currently working with the Association of Trawlers in Portland and are arranging to collect river lampreys during shrimp season.

In an attempt to locate a live river lamprey specimen we have broadened the search area to include all of Oregon and Washington. Both the Point No Point and Lower Elwha Tribes from the Puget Sound region were contacted. The Lower Elwha Tribe was

the most promising, with records indicating capture of river lampreys in the past. During the spring migration, traps on the Little Hoco River, Deep Creek and Leewaey Lee Creek are in operation. In the spring of 2002, we will attempt to confirm if river lampreys are captured at these sites and possibly obtain live specimens. In Oregon, the Siletz tribe 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 made collections of river lampreys, but will check the traps for them in the spring of 2002. The Hatfield Marine Science Center in Newport, OR was unable to provide us with new information on search locations. Previous collection trawls have not produced any lamprey and they currently have no future trawls planned.

Extending our search to include California and Canada has resulted in little success. In California, we have contacted both the Steinhart and the Monterey 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, CA. Currently, we are contacting the California Department of Fish and Wildlife to determine if they have any recent sightings on record.

Reports from Canada show the most recent records of river lamprey collections. River lampreys are reported to have been collected for several studies conducted by Richard Beamish within the Strait of Georgia in British Columbia, Canada. We have contacted staff members from the University of British Columbia and the University of

Windsor, Ontario, Canada. In the past, river lampreys were collected in the Fraser River Basin and off Victoria Island, but fewer river lampreys have been caught in recent years and they are unsure of population numbers. Researchers and managers are hesitant to remove any river lampreys until more accurate population data is available.

So far, we have been unable to obtain the live river lamprey specimens necessary for our research. For an overview of organizations contacted in our efforts to locate river lamprey specimens see Appendix D. Currently we are in the process of establishing contacts with Columbia River Basin trawlers to collect live specimens during shrimp season. In the spring we will contact the California Department of Fish and Wildlife and check back with ODFW, WDFW and the Lower Elwha Tribes to determine if any river lampreys were collected in their traps.

FUTURE GOALS

Identification of larvae

Validation of current diagnostic characteristics

Researchers at the University of Idaho were unable to provide us with genetic confirmation of species identification from tissue samples provided to them. Therefore, we are currently awaiting return of tissue samples and considering other techniques that may provide us with a means for positively identifying larvae of Columbia River Basin lampreys. We will also continue to sample larvae currently held at the Columbia River Research Laboratory at intervals of approximately six weeks. 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 begin compiling morphometric and meristic information of digitized/preserved material collected in 2000 and 2001. We will replicate this study in 2002 and continue sampling individuals reared at the Columbia River Research Laboratory to provide a larger sample size and to capture information on individuals as development progresses.

Effects of temperature on early life history stages

Due to equipment failure we were unable to collect a complete data set (from fertilization to 50% yolk assimilation) for Pacific and western brook lampreys reared at 14° C. We will repeat this experiment in 2002, which will not only fill in gaps in the data, but also provide a larger sample size and more replication.

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Table 1: Results of Bonferroni *t*-test on differences between sample means of survival to 50% hatch (280 TU_a) for Pacific lampreys reared at four temperatures. Sample 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
	Mean	93.50	95.66	97.11	54.11
Temperature					
10° C	93.50	-	2.16	3.61	39.39 *
14° C	95.66		-	1.45	41.55 *
18° C	97.11			-	43.00 *
22° C	54.11				-

Table 2: Results of Bonferroni *t*-test on differences between sample means of survival to 50% hatch (280 TU_a) for western brook lampreys reared at four temperatures. Sample 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
	Mean	94.75	N/A	96.50	76.11
Temperature					
10° C	94.75	-	N/A	1.75	18.64 *
14° C	N/A		-	N/A	N/A
18° C	96.50			-	20.39 *
22° C	76.11				-

Table 3: Results of Bonferroni *t*-test on differences between sample means of survival to 550 TU_a for Pacific lampreys reared at four temperatures. Sample 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
	Mean	89.67	N/A	96.11	53.44
Temperature					
10° C	89.67	-	N/A	6.44	36.23 *
14° C	N/A		-	N/A	N/A
18° C	96.11			-	42.67 *
22° C	53.44				-

Table 4: Results of Bonferroni *t*-test on differences between sample means of survival to 550 TU_a for western brook lampreys reared at four temperatures. Sample 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
	Mean	92.63	N/A	95.40	71.11
Temperature					
10° C	92.63	-	N/A	2.77	21.52 *
14° C	N/A		-	N/A	N/A
18° C	95.40			-	24.29 *
22° C	71.11				-

Table 5: Results of Bonferroni *t*-test on differences between sample means of percent abnormal embryos at 190 TU_a for western brook lampreys reared at four temperatures. Sample 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
	Mean	11.43	5.75	4.32	12.42
Temperature					
10° C	11.43	-	5.68	7.11 *	0.99
14° C	5.75		-	1.43	6.67 *
18° C	4.32			-	8.10 *
22° C	12.42				-

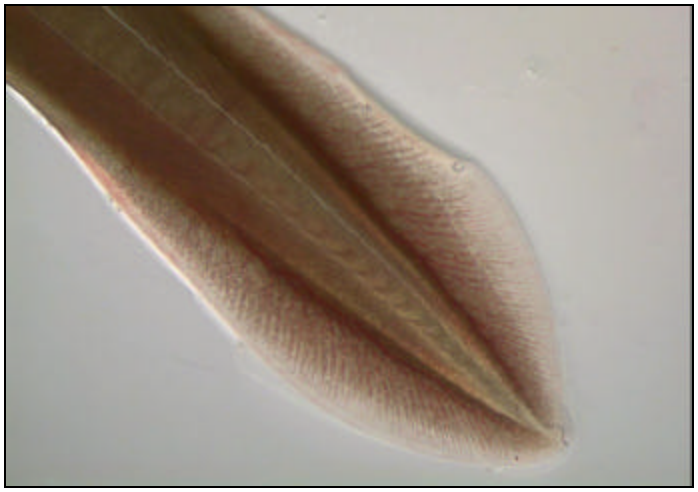
Table 6: Results of Bonferroni *t*-test on differences between sample means of percent abnormalities at 550 TU_a (50% yolk assimilation) for Pacific lampreys reared at four temperatures. Sample mean differences are represented as absolute values. Asterisk indicates a significant difference at α=0.05.

		Temperature			
		10° C	14° C	18° C	22° C
	Mean	1.42	N/A	3.56	17.54
Temperature					
10° C	1.42	-	N/A	2.14	16.12 *
14° C	N/A		-	N/A	N/A
18° C	3.56			-	13.98 *
22° C	17.54				-

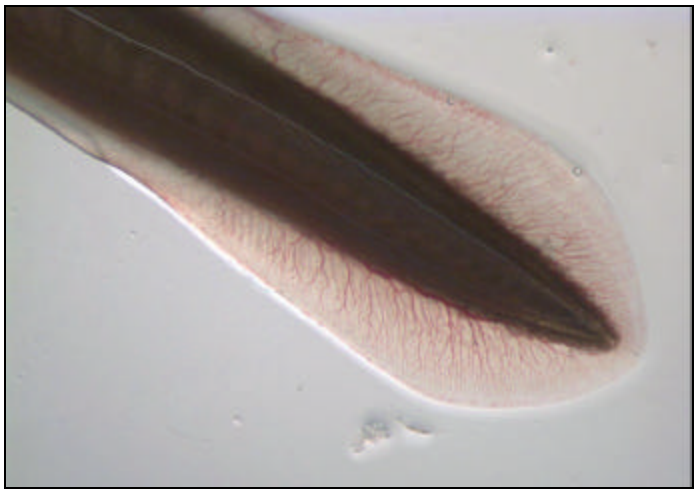
Table 7: Results of Bonferroni *t*-test on differences between sample means of percent abnormalities at 550 TU_a (50% yolk assimilation) for western brook lampreys reared at four temperatures. Sample mean differences are represented as absolute values. Asterisk indicates a significant difference at α=0.05.

		Temperature			
		10° C	14° C	18° C	22° C
	Mean	7.11	N/A	6.42	20.25
Temperature					
10° C	7.11	-	N/A	0.69	13.14 *
14° C	N/A		-	N/A	N/A
18° C	6.42			-	13.83 *
22° C	20.25				-

Figure 1: Examples of digitized 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).



1a



1b

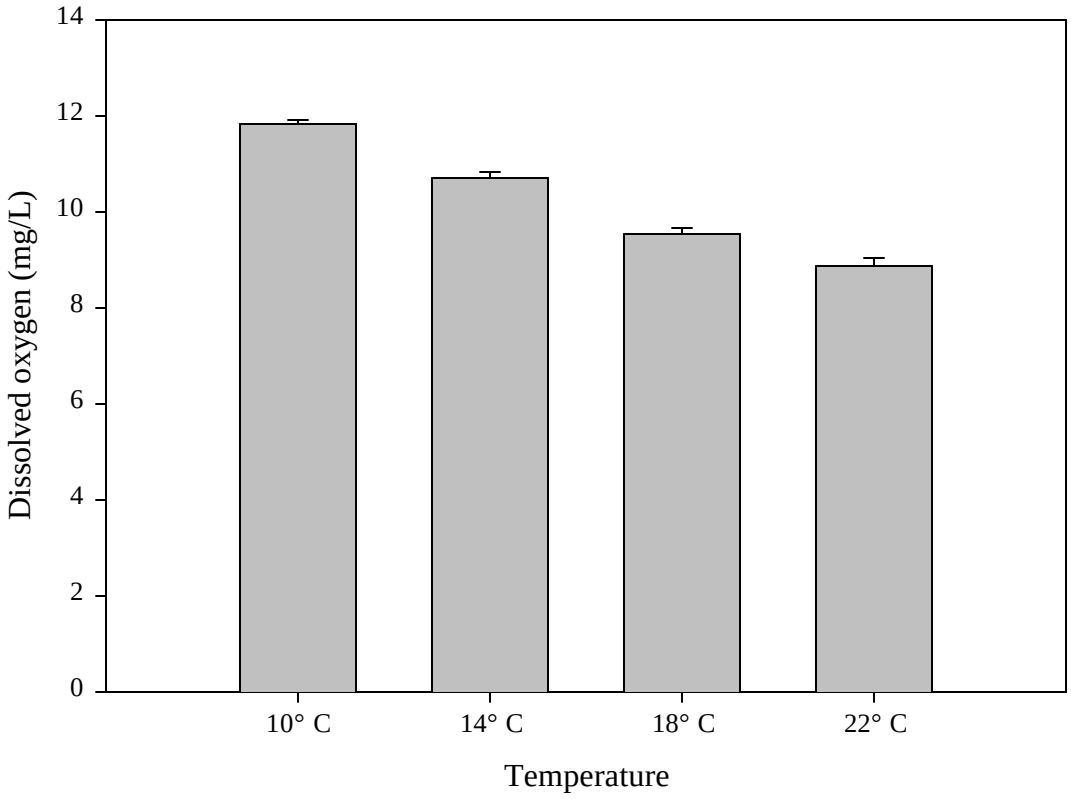


Figure 2: Dissolved oxygen content (mg/L) plus standard error of water baths at 10° C, 14° C, 18° C, and 22° C. Dissolved oxygen measurements taken daily for the duration of the experiment.

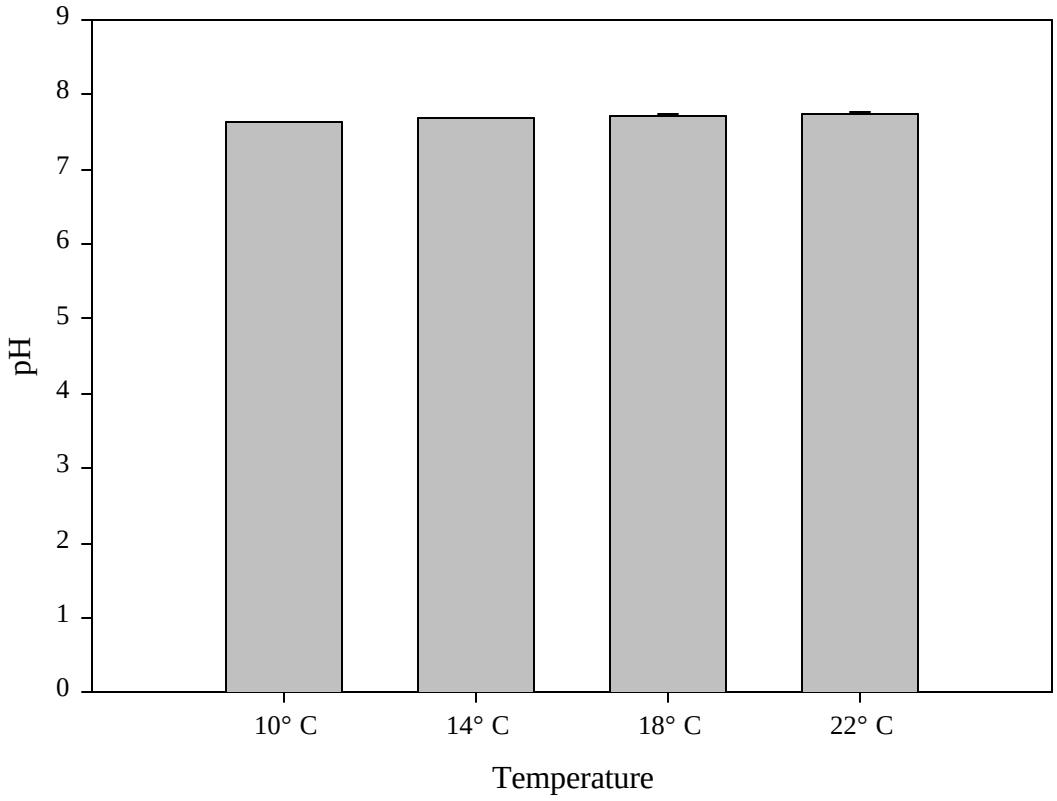


Figure 3: pH plus standard error of water baths at 10° C, 14° C, 18° C, and 22° C. pH measurements taken daily for the duration of the experiment.

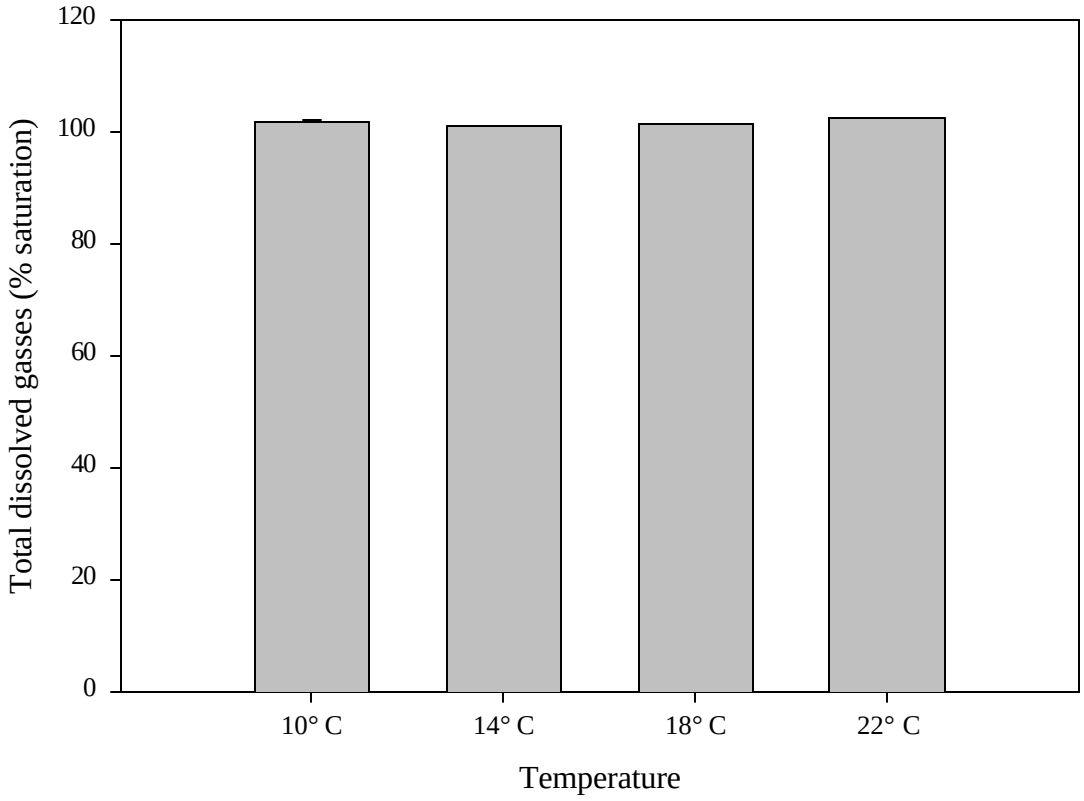
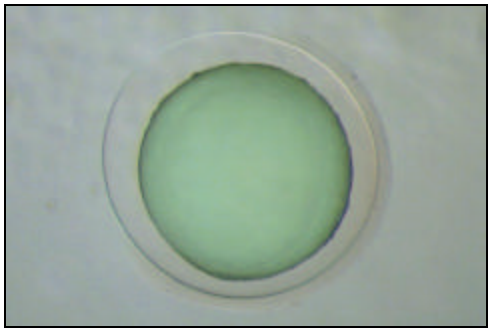
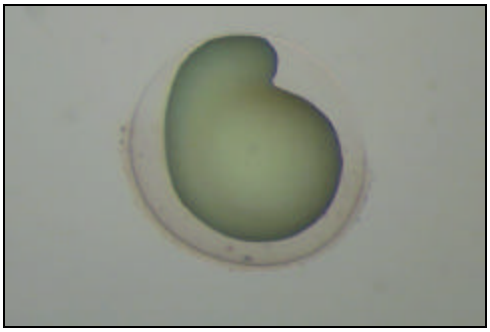


Figure 4: Total dissolved gasses (% saturation) plus standard error of water baths at 10° C, 14° C, 18° C, and 22° C. Total dissolved gas measurements taken daily for the duration of the experiment.

Figure 5: Time series of normal embryonic development of Pacific and western brook lamprey: 5a) relatively smooth and spherical embryo, 5b) differentiation of anterior and posterior ends of embryo, 5c) well-developed embryo exhibiting voluntary movement, and 5d) fully developed embryo prior to hatching.



5a



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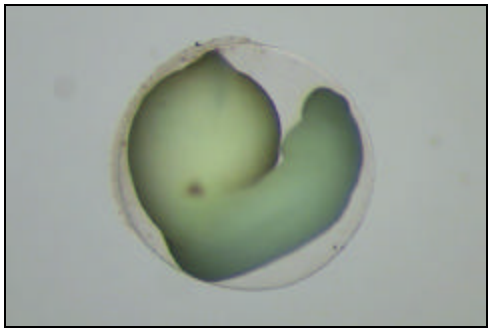

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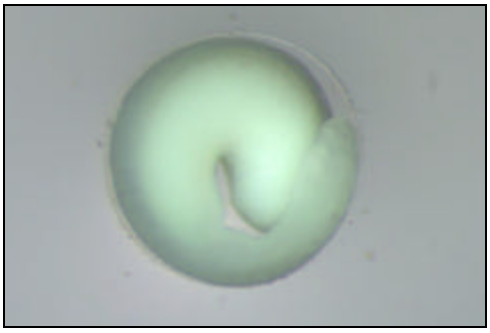
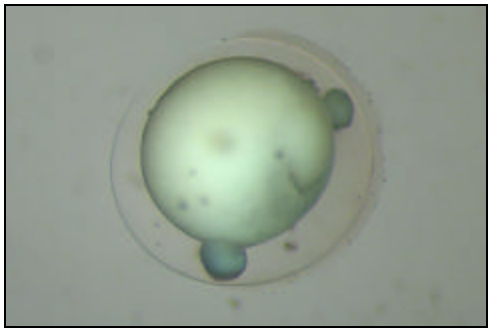


5d

Figure 6: Time series of abnormal embryonic development of Pacific and western brook lamprey: 6a) embryo with disconnected material, 6b) malformed embryonic material, 6c) disconnected embryonic material, and 6d) fully developed embryo with disconnected material.



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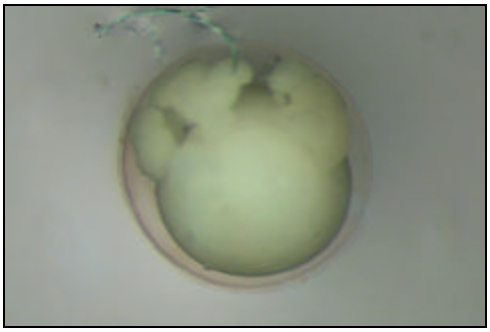

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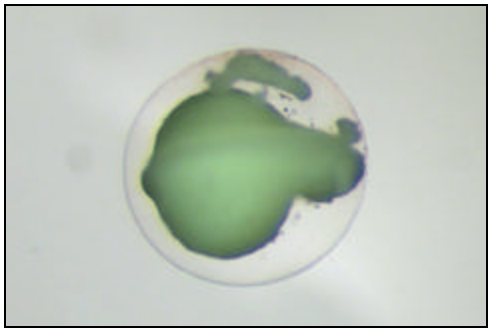

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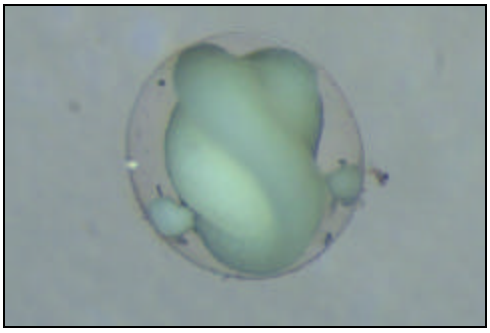


6d

Figure 7: Time series of normal prolarval development of Pacific and western brook lamprey: 7a) recently hatched prolarva exhibiting strong ventral flexion, 7b) less pronounced ventral flexion, 7c) slight ventral flexion in posterior region, and 7d) fully developed prolarva.

7a

7b

7c

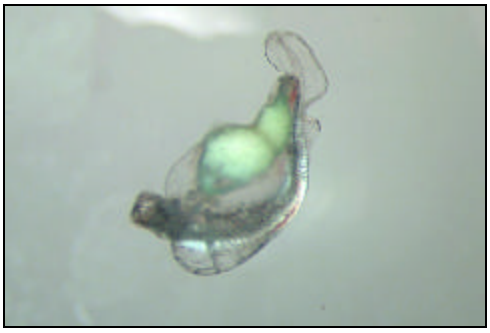
7d

Figure 8: Time series of abnormal prolarval development of Pacific and western brook lamprey: 8a) prolarva with malformed head, organ, and body regions, 8b) prolarva with malformed posterior region, 8c) prolarva with superfluous head and branchial region, and 8d) prolarva with extreme morphological malformations.

8a

8b

8c



8d

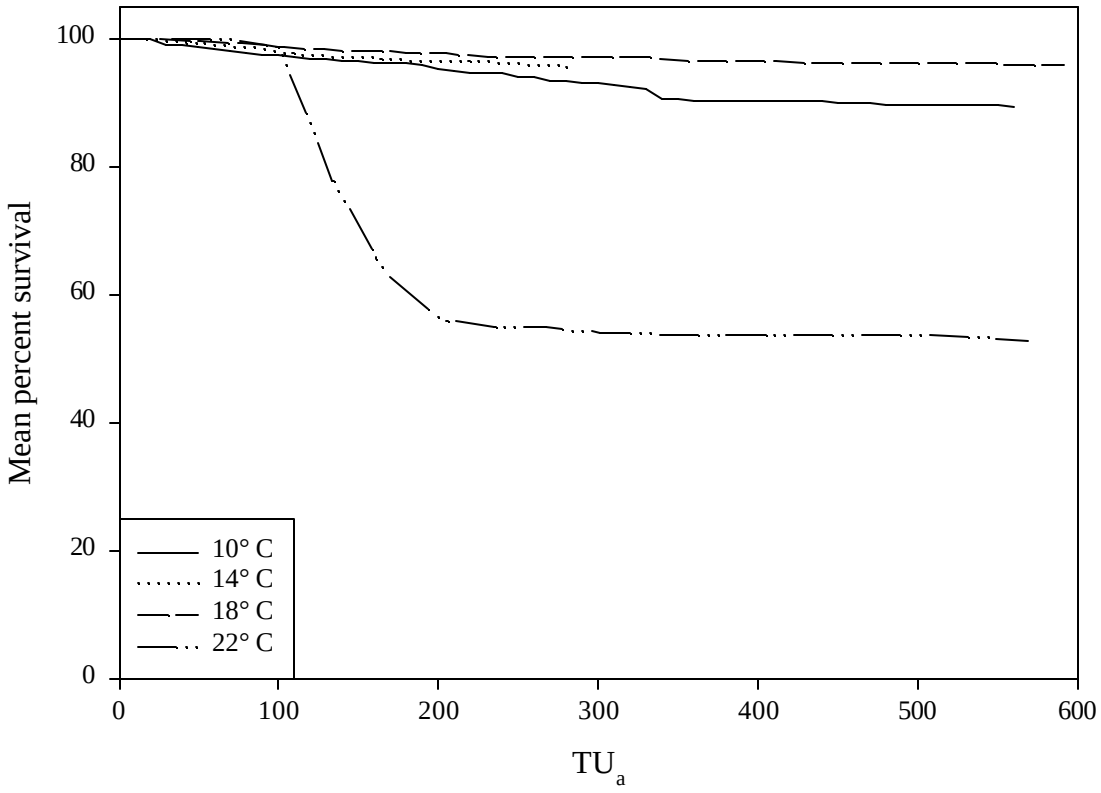


Figure 9: Cumulative sample mean percent survival for the duration of the experiment expressed as TU_a for Pacific lampreys. 50% hatch occurred at 280 TU_a.

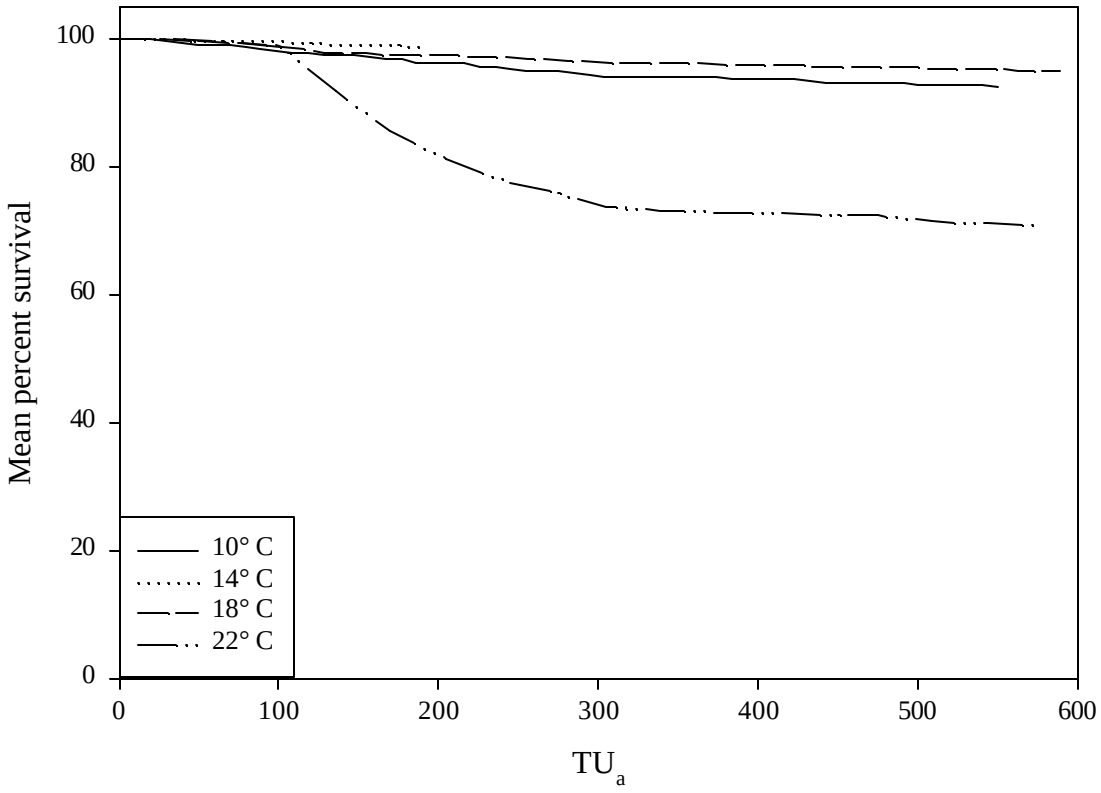


Figure 10: Cumulative sample mean percent survival for the duration of the experiment expressed as TU_a for western brook lampreys. 50% hatch occurred at 280 TU_a.

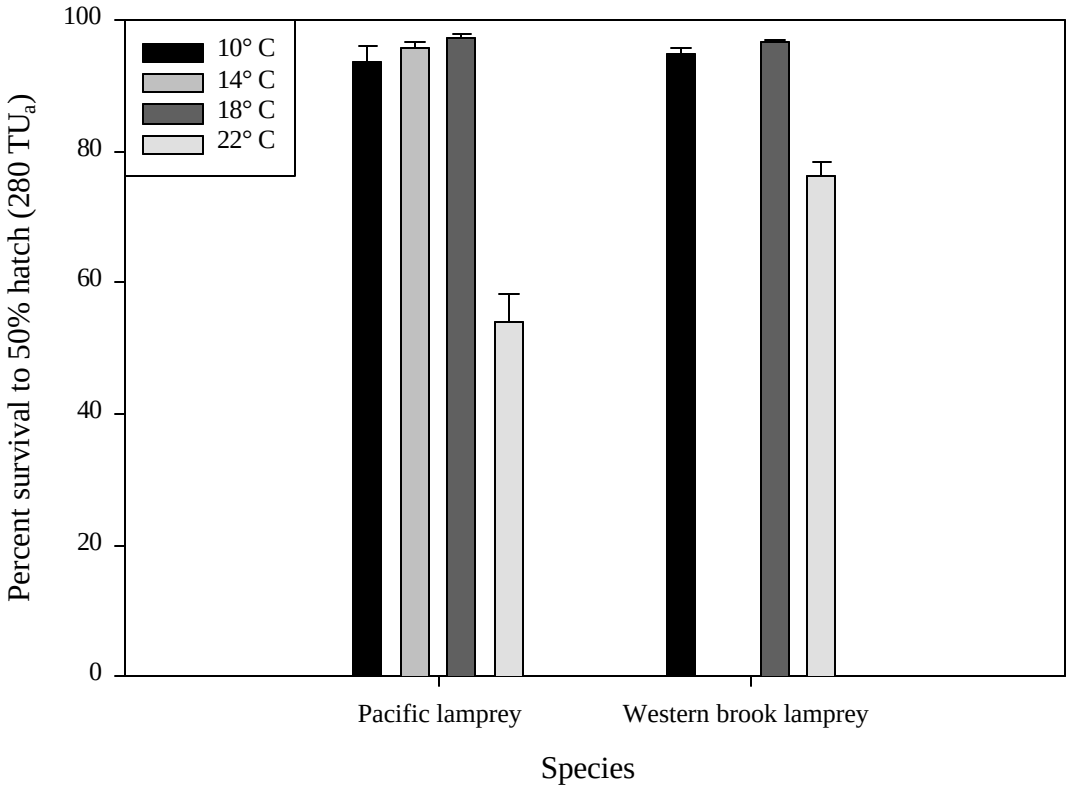


Figure 11: Percent survival to 50% hatch (280 TU_a) plus standard error for Pacific and western brook lampreys held at 10° C, 14° C, 18° C, and 22° C.

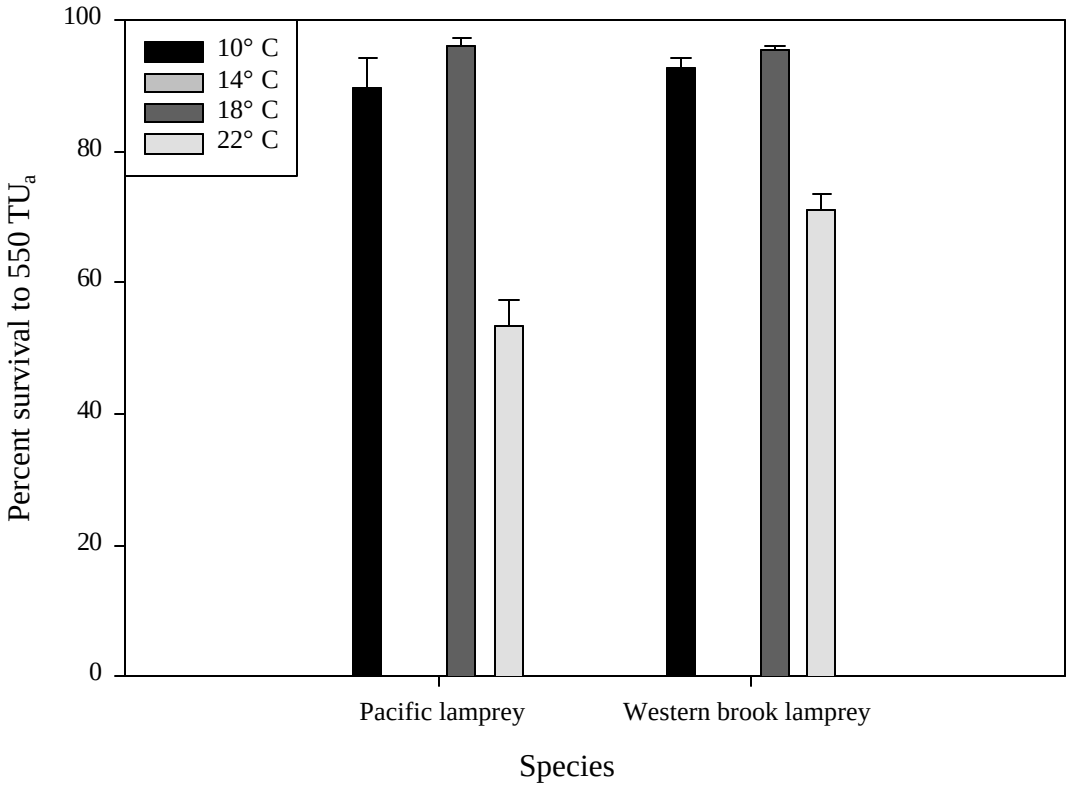


Figure 12: Percent survival to 550 TU_a plus standard error for Pacific and western brook lampreys held at 10° C, 14° C, 18° C, and 22° C.

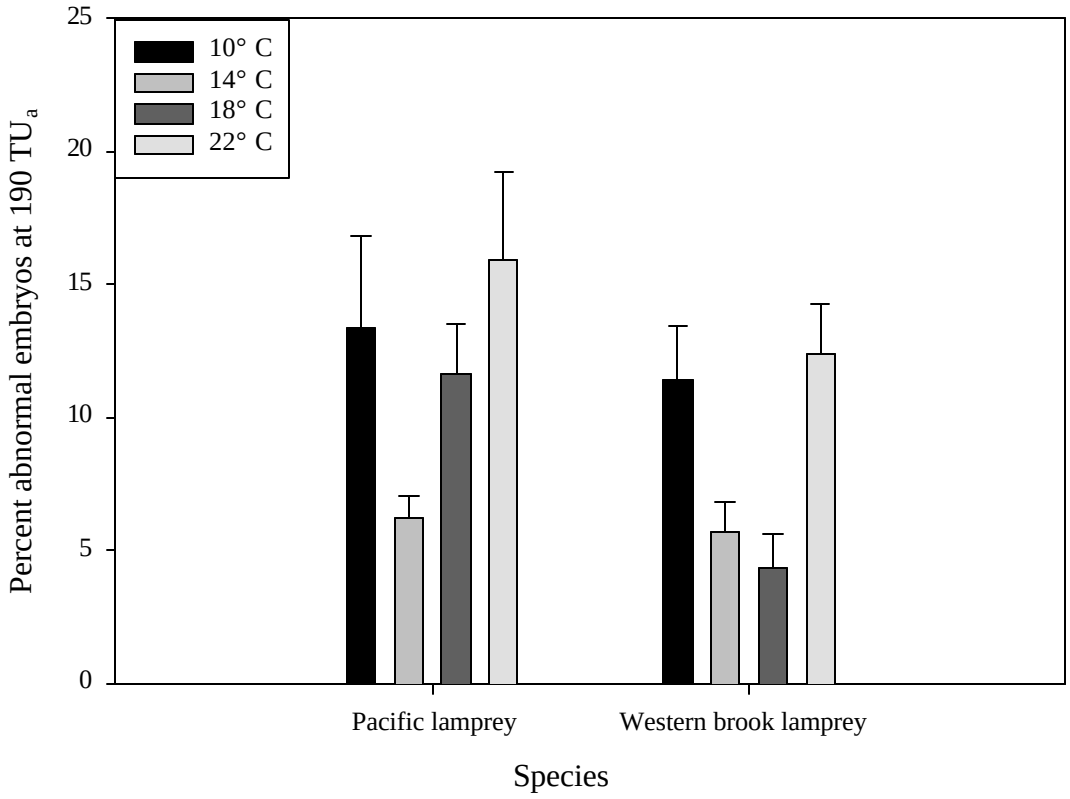


Figure 13: Percent abnormal embryos at 190 TU_a plus standard error for Pacific and western brook lampreys held at 10° C, 14° C, 18° C, and 22° C.

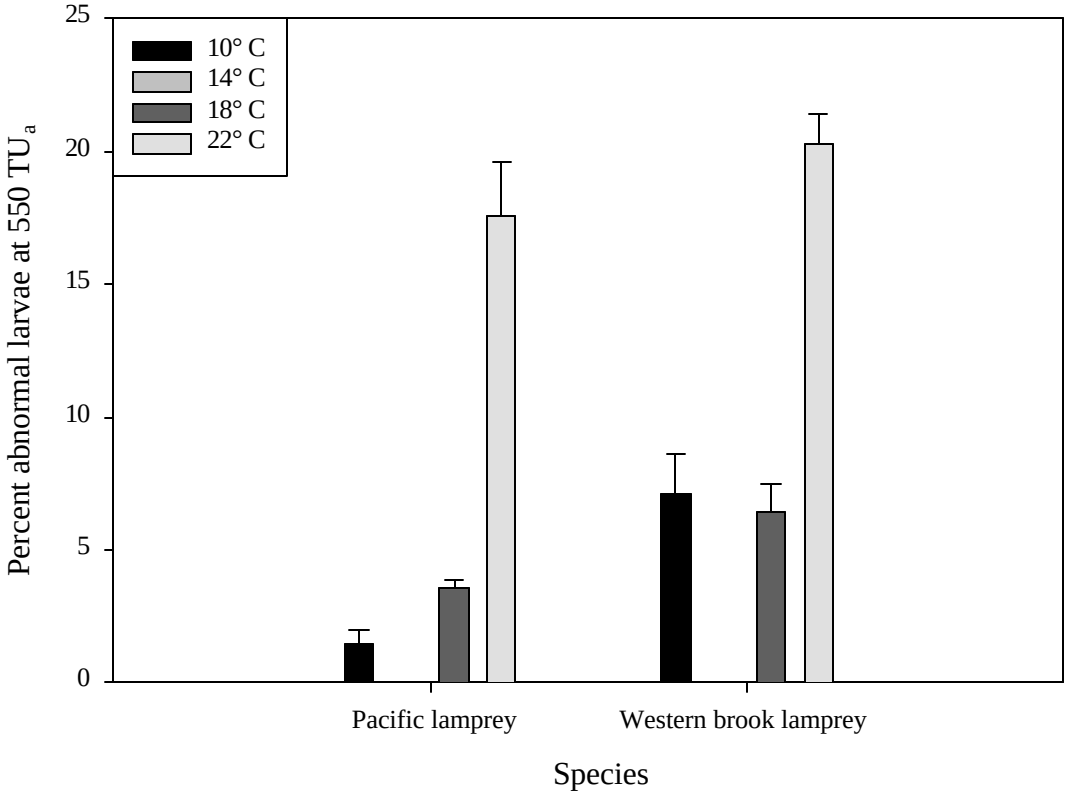


Figure 14: Percent abnormalities at 550 TU_a plus standard error for Pacific and western brook lampreys held at 10° C, 14° C, 18° C, and 22° C.

Appendix A: Sample number, collection location, length, mass, and preliminary species identification for current diagnostic characteristics for lamprey larvae identification. 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 B: 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	14	131	3.408	100	0	
RED	14	133	3.510	100	0	
RED	14	130	3.178	100	0	
RED	6	142	4.500	100	0	
RED	14	142	3.953	100	0	
ENT	14	130	3.275	100	0	
ENT	14	127	2.892	100	0	
ENT	13	108	1.713	100	0	
ENT	14	125	3.165	100	0	PCL
ENT	14	132	3.082	100	0	
ENT	14	137	3.915	100	0	
ENT	14	122	2.850	100	0	
ENT	14	127	2.876	100	0	
JDW	14	129	3.172	100	0	
JDW	14	123	2.469	0	100	
JDW	14	119	2.474	0	100	
JDW	14	116	1.996	0	100	
JDW	14	126	3.055	100	0	
JDW	14	123	2.657	100	0	
JDW	14	123	2.646	100	0	
JDW	14	114	2.065	0	100	

Appendix C: Number of days post fertilization that Pacific lamprey (PCL) and western brook lamprey (WBL) embryos and prolarvae/larvae were sampled for morphometric and meristic analysis. Sampling consisted of acquiring a digital image of each individual and preserving each individual in 10% formalin.

Days post fertilization	Number of PCL embryos sampled	Number of PCL larvae sampled	Number of WBL embryos sampled	Number of WBL larvae sampled
1	10		10	
2	10		10	
3	10		10	
4	10		10	
5	10		10	
6	10		10	
7	10		10	
8	10		10	
9	10		10	
10	10		10	
11	10		10	
12	10		10	
13	10			
14	10		10	
15	10		10	
16		10		
17		10		
18		10		
19		10		10
23		10		
26				10
30		10		
33				10
37		10		
47				10
51		10		
61				10
65		10		
88				10
92		10		
174				10
178		10		
Total	150	110	140	70

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

Contact name	Organization
Anderson, James	Washington State University
Bashman, Larry	Fish Passage Center- Portland
Beamish, Richard	Canadian Department of Fisheries and Oceans
Bond, Carl	Oregon State University - Retired
Crane, Pat	Lower Elwah Fisheries Office
Docker, Margret	University of Windsor Ontario
Elfonsen, Mel	Lower Elwah Fisheries Office
Goodwin, Kevin	Hatfield Marine Science Center
Haas, Gordon	University of British Columbia/ University of Alaska
Hinton, Sue	U.S. National Marine Fisheries Service
Jacobs, Steve	Oregon Department of Fish and Wildlife
Johnson, Thom	Point No Point Treaty
Loomis, Dave	Oregon Department of Fish and Wildlife
Mallat, Jon	Washington State University
Markle, Doug	University of Oregon
McCosker, John	Steinhart Aquarium
McRay, Gene	Hatfield Marine Science Center
Mongillo, Paul	Washington Department of Fish and Wildlife
Niemi, Dan	Toutle River Hatchery
Parkenson, Eric	University of British Columbia
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	Yakama Tribe
Tucker, Tom	Monterey Aquarium
Urbain, Brian	University of Washington
Vanderwetering, Stan	Siletz Tribe
Weinhimmer, John	Washington Department of Fish and Wildlife