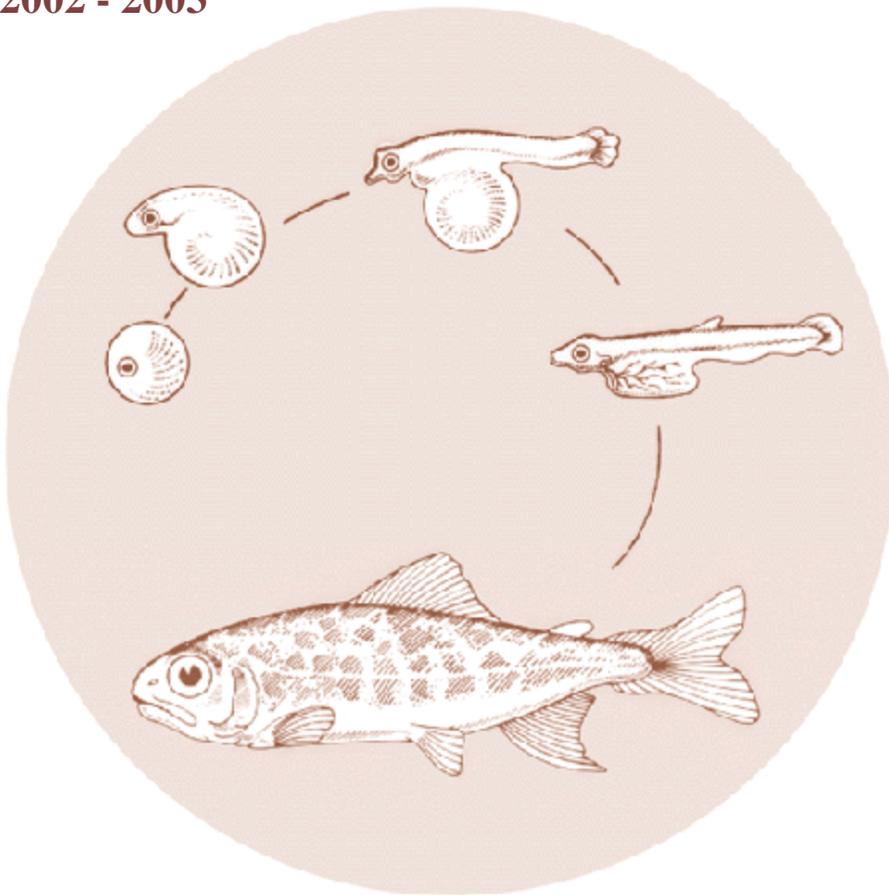


# Identification of Larval Pacific Lampreys, River Lampreys, and Western Brook Lampreys, and Thermal Requirements of Early Life History Stages of Lampreys

Annual Report  
2002 - 2003



This Document should be cited as follows:

*Meeuwig, Michael, Jennifer Bayer, Rebecca Reiche, "Identification of Larval Pacific Lampreys, River Lampreys, and Western Brook Lampreys, and Thermal Requirements of Early Life History Stages of Lampreys", Project No. 2000-02900, 62 electronic pages, (BPA Report DOE/BP-00004695-3)*

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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 of research 2003

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Project Number 2000-029  
Contract Number 00AI23249

January 2004

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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 (CRB) lampreys (the Pacific lamprey, *Lampetra tridentata*, and the western brook lamprey, *L. richardsoni*). We evaluated the usefulness of current diagnostic characteristics for identification of larval lampreys (i.e., pigment patterns) and collected material for development of meristic and morphometric descriptions of early life stage CRB lampreys, and we determined the effects of temperature on survival and development of early life stage CRB lampreys.

Thirty-one larval lampreys were collected from locations throughout the CRB and transported to the Columbia River Research Laboratory. Lampreys were sampled at six-week intervals at which time they were identified to the species level based on current diagnostic characteristics. Sampling was repeated until lampreys metamorphosed, at which time species identification was validated based on dentition, or until they died, at which time they were preserved for genetic examination. These lampreys were sampled 30 times with two individuals metamorphosing, both of which were consistently identified, and subsequently validated, as Pacific lampreys. Of the remaining lampreys, only one was inconsistently identified (Pacific lamprey in 83% of the sampling events and western brook lamprey in 17% of the sampling events). These data suggest 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 collected, digitized, preserved, and measured the mean chorion diameter of Pacific and western brook lamprey embryos. Embryos ranged in development from 1 d post fertilization to just prior to hatch, and were incubated at 14° C. Mean chorion diameter was greater and more variable for

Pacific lampreys (mean  $\pm$  SD;  $1.468 \pm 0.107$  mm,  $N = 320$ ) than for western brook lampreys ( $1.237 \pm 0.064$  mm,  $N = 280$ ). An unpaired  $t$ -test showed that the difference in mean chorion diameter between species was highly significant ( $t = 32.788$ ,  $df = 528.62$ ,  $P < 0.0001$ ). For larvae, we collected, digitized, and preserved 156 individuals from each species. Eight homologous landmarks defining a two-cell truss network with two appended triangles were selected for morphometric analyses and species discrimination. A full model discriminant analysis correctly classified 92% of the Pacific lampreys and 93% of the western brook lampreys in a classification data set. When applied to a test data set, the classification functions correctly classified 91% of the Pacific lampreys and 85% of the western brook lampreys. A backward elimination discriminant analysis removed four variables from the full model, and the reduced model correctly classified 91% of the Pacific lampreys and 93% of the western brook lampreys in a classification data set. The reduced model classification functions correctly classified 91% of the Pacific lampreys and 85% of the western brook lampreys in a test data set.

In 2001 and 2002 Pacific and western brook lampreys were artificially spawned and resulting progeny were reared in the laboratory at 10° C, 14° C, 18° C, and 22° C. The estimated temperature for zero development was 4.85° C for Pacific and 4.97° C for western brook lampreys. Survival was greatest at 18° C followed by 14° C, 10° C, and 22° C, with significant differences observed between 22° C and other temperatures. Overall survival was significantly greater for western brook than for Pacific lampreys, although the difference in proportion of individuals surviving was only 0.02. Survival to hatch was significantly greater than survival to the larval stage with a difference of only 0.03. The proportion of individuals exhibiting abnormalities at the larval stage was greatest at 22° C followed by 18° C, 10° C, and 14° C, with significant differences observed between 22° C and other temperatures.

### **Acknowledgements**

Bonneville Power Administration provided funding for this project (Project #2000-029). We thank individuals at the U.S. Fish and Wildlife Service, National Oceanic and Atmospheric Administration Fisheries, University of Idaho, Idaho Department of Fish and Game, Confederated Tribes of the Umatilla Indian Reservation, and U.S. Geological Survey for their assistance with project activities. We also appreciate the assistance of Debbie Docherty, Project Manager, Bonneville Power Administration. Use of trade or firm names in this document is for reader information only and does not constitute endorsement of a product or service by the U.S. Government.

## 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 patterns. Resident species remain in streams until sexually mature, at which time they spawn and die. Migratory 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 a variety of aquatic species (Hardisty and Potter 1971b). Lampreys exhibit rapid growth during their 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 (CRB) 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 life history pattern, while the western brook

lamprey (*L. richardsoni*) exhibits a resident life history pattern. Apart from these generalities, little is known about the biology of lamprey species in the CRB (Kan 1975; Hammond 1979), and what information is available for these species is from work conducted in Canada (Pletcher 1963; Beamish 1980a; 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 CRB. Dam passage data and anecdotal information indicate that Pacific lampreys are in decline in the CRB (Close et al. 1995). The declining trend of Pacific lampreys, along with the ecological, economic, and cultural significance of Pacific lampreys (Kan 1975; NPPC 1994; Close et al. 1995), has stimulated interest in recovery actions within the CRB.

Documenting the distribution and relative abundance of lampreys in tributaries of 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 (Richards et al. 1982). However, within the CRB, 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 CRB (USGS, unpublished data), which may be due to the effects of environmental conditions and age on

pigmentation patterns within and among species (Moyle and Cech 1996). Also, Richards et al. (1982) found that discriminatory pigmentation patterns were not fully developed in the first year of larval life, and were unable to document the timing at which these patterns did develop.

Along with the ability to distinguish among lamprey species, identification of ecological factors limiting lampreys in the CRB 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 lamprey population assessment and determination of critical habitat needs within the CRB. 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 ensure the long-term stability of lamprey populations. Accurate identification techniques will allow managers to conduct larval lamprey 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 CRB.

This document presents preliminary analyses of data collected in 2000, 2001, 2002, and 2003 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 early larval stage lampreys and for defining their thermal requirements. Information for river lampreys is not included in these analyses due to our inability to locate live specimens within the CRB during the timeline in which we were conducting experiments; however, information that we have gathered regarding this species is presented (River lampreys in the Columbia River Basin; pages 23-29).

## **Methods**

### **Laboratory environment**

Unless otherwise noted, animals were held under the following conditions at the U.S. Geological Survey - Columbia River Research Laboratory (CRRL). Water for all research was supplied from the Little White Salmon River, Skamania County, WA. Water was treated using sand filters and temperature was controlled to mimic seasonal thermal trends within the CRB. Temperatures followed ambient Columbia River water temperatures ( $\pm 0.5^\circ \text{C}$ ) at Bonneville Dam (University of Washington 2003) with the exception that they never exceeded  $15^\circ \text{C}$  ( $\pm 0.5^\circ \text{C}$ ). All tanks and aquaria were supplied with flow through water (larvae, sub-adult, and adult western brook lampreys at 0.3 L/min; sub-adult and adult Pacific lampreys at  $0.3 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ ), a

source of aeration, suitable burrowing substrate (larvae, sub-adult, and adult western brook lampreys), and were exposed to a simulated natural photoperiod provided by 25 W incandescent lights on timers with 0.5 h of increasing and decreasing illumination at the beginning and ending of each light phase.

### **Pigmentation based identification of larval lampreys**

In the fall of 1999, larval lampreys were collected from five locations in the CRB: 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 CRRL. 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. Lampreys were fed a suspension of active yeast and commercial fry feed two or three times per week.

In February 2000, each larva was anesthetized using 250 mg/L MS-222 (tricaine methane sulfonate) buffered with an equal concentration of sodium bicarbonate, measured for total length (mm) and wet mass (g), and identified to the species level based on existing diagnostic characteristics (Richards et al. 1982). Approximately 50% of the larvae from each species by collection location were terminally sampled (overdose of buffered MS-222; 750 mg/L) to provide tissue for genetic testing (Appendix 1). Mitochondrial DNA was 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 CRRL, and sampled at intervals of approximately six-weeks (Appendix 2). At each sampling event, lampreys were removed from aquaria, anesthetized using buffered MS-222 (250 mg/L),

measured for length and mass, identified to the species level (Richards et al. 1982), and digital images of their caudal region were taken (Figure 1). Digital images were captured using a Spot Insight digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI) mounted to a stereomicroscope (Wild M3Z, Leica AG, Heerbrugg, Switzerland). This procedure was performed to determine 1) if it is possible to separate these species based on pigmentation patterns (Richards et al. 1982), and 2) if there is a change in pigmentation patterns over time, specifically with regards to diagnostic characteristics of these species. This process was repeated until individuals metamorphosed, at which point species identification could be confirmed based on dentition (Vladykov and Follett 1965; Eddy and Underhill 1978), or individuals died, at which point genetic samples were collected for analysis.

### **Brood stock collection and holding conditions**

Lamprey embryos and larvae used in this project were the result of lampreys artificially spawned in the laboratory. Brood stock sample sizes and animal size measurements are summarized in Table 1. In the springs of 2001 and 2002, sub-adult Pacific lampreys were collected from the Columbia River at the Bonneville Dam north shore fish ladder (Skamania County, WA), and sub-adult western brook lampreys were collected from Gibbons Creek (Clark County, WA) and Yellowhawk Creek (Walla Walla County, WA). Both species were transported to the CRRL and held until sexually mature. At the CRRL, Pacific lampreys were held in 1400 L circular tanks and western brook lampreys were held in 38 L aquaria. Once lampreys reached sexual maturity, individuals were anesthetized using buffered MS-222 (250 mg/L) and rinsed in fresh water to remove traces of anesthetic. Female lampreys were positioned over a glass bowl filled with approximately 2 L of fresh water at the same temperature as the 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.

### **Morphometric identification of early life stage lampreys**

The following procedures were performed in 2001 and 2002 on both Pacific and western brook lampreys. Following artificial spawning (see above) the temperature of the fertilized eggs was adjusted through the addition of cool water until the target temperature of 14° C was reached (approximately 30 min), and the fertilized eggs were transferred to flow-through hatching jars (6.86 L McDonald type). Hatching jars were provided with a continuous inflow (approximately 1.5 L/min) of aerated, filtered (sand filter), and sterilized (ultraviolet sterilizer) river water maintained at a temperature of 14° C. Individuals were held in hatching jars from fertilization until animals had hatched and reached stage 17 (burrowing; Piavis 1961), at which point lampreys were transferred to 19 L aquaria. Larvae were fed a suspension of active yeast and commercial fry feed three times per week. Hatching jar temperature control was lost for a portion of the incubation period in 2001 due to equipment failure, during which time temperature decreased to approximately 10° C. Temperatures remained at approximately 10° C from 17 days post-fertilization for Pacific lampreys and 12 days post-fertilization for western brook lampreys until lampreys were transferred to aquaria.

Lampreys were sampled periodically to provide morphometric and meristic information. Embryos were sampled daily from the time of fertilization until just prior to hatching (approximately 15 days; Figure 2). Larvae were sampled from the time that they reached stage 17 and at various times during the first year of their larval life stage (stage 18; Piavis 1961;

Figure 3). At each sampling event, ten individuals were removed from their holding vessel (flow-through hatching jar or aquaria), anesthetized with buffered MS-222 (250 mg/L), digitized, and preserved in 10% formalin (formaldehyde solution). Digital images were captured using a Spot Insight digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI) mounted to a stereomicroscope (Wild M3Z, Leica AG, Heerbrugg, Switzerland). Image analysis software (Image Pro Plus, Media Cybernetics, Silver Spring, MD) was used to measure digitized specimens. For embryos, measurements consisted of the mean chorion diameter (Figure 4), which was determined by tracing the chorion circumference and making 180 diameter measurements through the embryo's centroid at 2-degree intervals. For larvae, a set of eight homologous landmarks were established (Figure 5a; Appendix 3) and their coordinates were quantified. The locations of the landmarks defined a two-cell truss network with two appended triangles (Bookstein et al. 1985) for which the truss element lengths were calculated (Figure 5b; Appendix 3). Length measurements (i.e., truss element lengths and standard length) were log transformed (base  $e$ ) to stabilize variance caused by variability in size (Jolicoeur 1963; Humphries et al. 1981), and used for morphometric descriptions of larval lampreys.

All analyses were performed using SAS software (SAS version 8.1, SAS Institute Inc., Cary, NC) with statistical significance set at  $\alpha = 0.05$  unless noted otherwise. For embryos, the difference in mean chorion diameter between species was analyzed using an unpaired  $t$ -test. Due to unequal variance, an approximate  $t$ -statistic was calculated using estimated degrees of freedom based on the Satterthwaite approximation (Satterthwaite 1946). For larvae, principle components analysis was used to examine the relationship among the log transformed length measurements. Discriminant analysis was performed on the log transformed length measurements. Both full model and reduced model discriminant analyses were examined for

their ability to accurately predict group membership. The reduced model was produced by a stepwise, backward elimination procedure with the probability to stay in the model set at  $\alpha = 0.15$ . For each analysis a classification dataset composed of a random sample of 90% of the individuals was used to produce classification functions (Tabachnick and Fidell 1996). A test dataset composed of the remaining lampreys was used to test the classification functions.

### **Effects of temperature on early life stage lampreys**

The following procedures were performed in 2001 and 2002 on both Pacific and western brook lampreys. Following artificial spawning (see above) fertilized eggs were divided into four glass bowls and the temperature of the water in 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).

Following fertilization, zygotes were incubated at 10° C, 14° C, 18° C, and 22° C for 15 temperature units (degrees above 0° C · days), after which 100 viable embryos were placed into each of 10 rearing vessels per temperature. A lag of 15 temperature units 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 a water 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. Water supplied to rearing vessels and illumination was similar to above (i.e., Laboratory environment) with the addition of water treatment by ultraviolet sterilizers. Water supplied to

rearing vessels was monitored daily for dissolved oxygen concentration, pH, and total dissolved gasses (Figure 6, 7, 8).

Individuals in each rearing vessel were examined daily for the duration of the experiment, which lasted from the time that individuals were assigned to a rearing vessel until individuals had reached the larval stage (i.e., stage 18; Piavis 1961; Figure 3). The larval stage is marked by differentiation of all systems (except genital) and the extrusion of yolk from the gut (Piavis 1961). 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 stereomicroscope at 10X to 40X (Wild M3Z, Leica AG, Heerbrugg, Switzerland). The number of individuals hatched/not hatched, the number of surviving individuals, and the number of abnormal larvae were recorded. Dead individuals were removed from rearing vessels daily. Larval abnormalities were traits considered to have a potential negative effect on survival or fitness in conditions less favorable than a laboratory setting, such as malformations of the body (Piavis 1961). For examples of normal and abnormal larvae see Figure 9 and Figure 10.

All statistical analyses were performed at  $\alpha = 0.05$  using SAS software (SAS version 8.01, SAS Institute Inc., Cary, NC). Less than 20 replicates (10 replicates  $\cdot$  2 years) were available for some treatment combinations due to mechanical trauma resulting from improperly adjusted freshwater inflow. Due to unbalanced data, degrees of freedom were estimated following Satterthwaite (1946). Using the number of individuals hatched/not hatched for each rearing vessel, logistic regression was used to estimate the number of days to 50% hatch ( $D_{H50}$ ) and the number of days to 95% hatch ( $D_{H95}$ ) (terminology follows Rodríguez-Muñoz et al. 2001). For each species, a linear regression model was fit to describe the effects of temperature on the development rate to 50% hatch. We then estimated the temperature for zero development

( $T_0$ ), the effective temperature ( $E_T = T - T_0$ ), and the accumulated degree-days to which individuals were exposed ( $DD = E_T \cdot \text{Days}$ ). Degree-days were calculated in order to provide a standardized measure for the effects of time and temperature on development. The number of days individuals were held under experimental conditions (see above) was the days required to reach the larval stage ( $D_L$ ). A repeated measures factorial analysis of variance was used to examine the effects of species and rearing temperature on the proportion of individuals surviving to hatch ( $S_H = \text{proportion of individuals surviving to } D_{H95}$ ) and the proportion of individuals surviving to the larval stage ( $S_L = \text{proportion of individuals surviving to } D_L$ ). A factorial analysis of variance was used to examine the effects of species and rearing temperature on the proportion of abnormal individuals at the larval stage ( $A_L = \text{proportion of abnormal larvae at } D_L$ ). Year (2001 and 2002) was included as a blocking factor in all analyses to account for systematic variation associated with the time the experiment was performed (Sokal and Rohlf 1995). Variance in response variables was stabilized using an arcsin transformation for  $S_H$  and  $S_L$ , and a square-root transformation for  $A_L$ . When main factors had an overall significant effect, Bonferroni  $t$ -tests were used to make pairwise comparisons between treatment combinations. Statistical comparisons are based on transformed data; however, reported mean values are based on the original measurement scale (Kuehl 1994).

### **Preliminary results and discussion**

#### **Pigmentation based identification of larval lampreys**

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 (Richards et al. 1982; 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 CRB lampreys may be a result of the molecular techniques used. Docker et al. (1999) were able to separate Pacific lampreys from the composite group of western brook and river lampreys; however, they were unable to genetically distinguish western brook and river lampreys from each other. 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 may not be suitable for separation of lamprey species found in the CRB; therefore, other techniques, such as microsatellite analysis, may merit investigation. The ability to accurately identify CRB 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 CRB.

The 31 larvae held at the CRRL for repeated examination were sampled up to 30 times (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 (Vladykov and Follett 1965; Eddy and Underhill 1978), 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 identification of early life stage lampreys**

Mean chorion diameter was greater and more variable for Pacific lampreys ( $1.468 \pm 0.107$  mm,  $N = 320$ ) than for western brook lampreys ( $1.237 \pm 0.064$  mm,  $N = 280$ ). An unpaired  $t$ -test showed that the difference in mean chorion diameter between species was highly significant ( $t = 32.788$ ,  $df = 528.62$ ,  $P < 0.0001$ ) 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-dimensional complexity of developing embryos (Figure 2) would make this a daunting task even under the most controlled conditions.

For early life stage larvae, the first two principal components accounted for 97% of the variability in the length measurements, with 93% of the variability accounted for by PC1. Positive loadings on PC1 were observed for all variables (Table 2; Figure 11) indicating a positive relationship among all measured dimensions and suggesting PC1 may be a good descriptor of the general size of the animals examined (Bookstein et al. 1985; Bookstein 1991). While relatively similar in magnitude, slight differences in loadings indicate allometric growth (Bookstein et al. 1985). For PC2, truss elements defining body regions grouped together well and suggest differential rates of growth associated with body region or changes in animal shape associated with general size over the size range of lampreys examined. Large negative loadings were observed for truss elements A, B, C, E, and G, associated with the preorbital and branchial

regions, and large positive loadings were observed for truss elements I, J, K, L, and M, associated with the trunk region (Table 2; Figure 11).

The full model discriminant analysis correctly classified 92% of the Pacific lampreys and 93% of the western brook lampreys in the classification data set (see Table 3 for parameter estimates for the classification functions). When applied to the test data set, the classification functions correctly classified 91% of the Pacific lampreys and 85% of the western brook lampreys. The backward elimination procedure removed the variables standard length, truss element A, truss element I, and truss element K from the model. The reduced model correctly classified 91% of the Pacific lampreys and 93% of the western brook lampreys in the classification data set. When applied to the test data set, the classification functions correctly classified 91% of the Pacific lampreys and 85% of the western brook lampreys.

We found that a high percentage of Pacific and western brook lampreys could be classified using landmark based morphometrics. This technique may have practical applications when species identity must be determined for individuals that have not yet fully developed characteristic pigment patterns. However, the scale at which measurements must be taken will require specialized equipment and training and may not be applicable to field situations.

### **Effects of temperature on early life stage lampreys**

Mean  $D_{H50}$  and  $D_{H95}$  (Table 4) varied greatly among temperatures, and temperature accounted for a large proportion of the observed variance in developmental rate ( $1/D_{H50}$ ) for Pacific ( $r^2 = 0.9864$ ) and western brook ( $r^2 = 0.9828$ ) lampreys.  $T_0$  estimates from linear regression models were 4.85° C for Pacific and 4.97° C for western brook lampreys, and were used to calculate effective temperatures (Table 4).

Prior to examining the effects of temperature and species on  $S_H$  and  $S_L$ , interactions among species, temperature, and development stage (hatch and larva) were examined. There was not a significant interaction between species and temperature ( $F_{3,116} = 1.20, P = 0.31$ ), species and development stage ( $F_{1,120} = 1.90, P = 0.17$ ), or temperature and development stage ( $F_{3,120} = 1.56, P = 0.20$ ); therefore, data were combined to examine the effects of main factors on survival. There was a significant difference in survival among temperatures ( $F_{3,116} = 198.47, P < 0.0001$ ) and species ( $F_{1,116} = 5.22, P = 0.02$ ), and there was a significant difference in survival at hatching and at the larval stage ( $F_{1,120} = 53.77, P < 0.0001$ ). Survival was greatest at 18° C followed by 14° C, 10° C, and 22° C (Figure 12), and mean comparisons indicated that survival was significantly reduced at 22° C when compared to 10° C ( $t = 19.38, df = 116, P < 0.0001$ ), 14° C ( $t = 15.82, df = 116, P < 0.0001$ ), or 18° C ( $t = 21.40, df = 116, P < 0.0001$ ). Significant differences were not observed between other temperatures ( $P > 0.05$ ).

Survival was significantly greater for western brook lampreys than for Pacific lampreys ( $t = -2.28, df = 116, P = 0.02$ ); however, this difference may be due to the small degree of variability in the transformed data, as the difference in the proportion of individuals surviving between the species was only 0.02. Similarly, a significant decrease in survival occurred after hatch ( $t = 7.33, df = 120, P < 0.0001$ ), although the difference between  $S_H$  and  $S_L$  was only 0.03 (Figure 13).

There was not a significant interaction between species and temperature on the proportion of individuals exhibiting abnormalities at the larval stage ( $F_{3,111} = 2.33, P = 0.08$ ). There was a significant difference in the occurrence of abnormalities among temperatures ( $F_{3,111} = 127.49, P < 0.0001$ ), but not among species ( $F_{1,111} = 0.33, P = 0.56$ ). The occurrence of abnormalities was greatest at 22° C followed by 18° C, 10° C, and 14° C (Figure 14). Significant differences in the

occurrence of abnormalities were observed between 22° C and 18° C ( $t = -16.36$ ,  $df = 111$ ,  $P < 0.0001$ ), 14° C ( $t = -13.61$ ,  $df = 111$ ,  $P < 0.0001$ ), and 10° C ( $t = -15.38$ ,  $df = 111$ ,  $P < 0.0001$ ); however, significant differences were not observed between other temperatures ( $P > 0.05$ ).

Overall, Pacific and western brook lampreys responded similarly to temperature. Estimated days to 50% and 95% hatch were very consistent among species (Table 4), as were the predicted temperatures for zero development. Although a systematic difference was observed in the overall proportion of individuals surviving between the two species, the biological significance associated with the small magnitude of the difference is questionable. The slight increase in survival from 10° C to 18° C followed by a sharp decline in survival at 22° C suggests that over the range of temperatures examined, survival was optimum in the range of 10° C to 18° C. This is supported by the low occurrence of abnormalities at 10° C, 14° C, and 18° C, and the significant increase in abnormalities at 22° C.

The similarity in response to temperature by Pacific and western brook lampreys in this experiment suggests similar reproductive timing and thermal habitat requirements for early life stage development. Under conditions of sympatric distributions this may result in interspecific competition and partitioning of thermal resources for spawning and rearing habitats (Magnuson et al. 1979). While anecdotal data are abundant, quantitative distribution data for Pacific lampreys within the Columbia River Basin are limited to fish passage data collected at hydroelectric projects along the mainstem Columbia and Snake Rivers and a small number of localized studies (e.g., Cochnauer and Claire 2001; Close 2002). Distribution data for western brook lampreys within the CRB are essentially nonexistent. Therefore, the degree to which these species exhibit a sympatric distribution is unknown; however, both species have been observed

concurrently within the same Columbia River tributary (Gibbons Creek, WA; personal observation).

The relationship between temperature and reproductive timing may also have an effect on growth and long-term survival of fish. In general, the thermal tolerance zone for embryological development of fish is believed to be narrow; however, there is less agreement on the temperature sensitivity of other life stages and fish sizes (Brett 1970; Elliott 1981; Rombough 1988). For example, the most stenothermic life stage for sea lampreys appears to be the embryo, with a broader and more variable range of thermal tolerance for larvae, juveniles, and adults (see Rodríguez-Muñoz et al. 2001). Therefore, spawning generally occurs within a specific range of temperatures suitable for embryological development (Brett 1970), and often occurs under thermal conditions that maximize survival, energy conversion (Blaxter 1969), and individual size at specific developmental stages (Atkinson 1994). However, thermal conditions that are optimal for embryological development may result in hatching when thermal conditions are sub-optimal for later life stages (Brett 1970).

In this experiment, examining the effects of temperature on survival to hatch and survival to the larval stage essentially allowed us to examine potential changes in the effects of temperature on survival for pre and post hatch individuals. Overall, there was a significant decrease in survival from the time of hatch ( $155.6 \pm 10.8$  DD) to the time that individuals reached the larval stage ( $294.0 \pm 10.2$  DD) indicating that mortality continued after hatch. However, a change in the trend of individuals surviving over time is apparent when survival is plotted against degree-days (Figure 13). From fertilization to hatch the overall proportion of individuals surviving decreased from 1.00 to 0.85, whereas the proportion of individuals surviving from hatch to the larval stage only decreased from 0.85 to 0.82. Because lampreys

exposed to 10° C, 14° C, and 18° C exhibited high survival rates throughout the duration of the experiment, individuals exposed to 22° C likely had the greatest influence on the observed trend (Figure 12). Nevertheless, interactions were not observed among the factors examined; therefore, changes in survival rates were statistically systematic among temperatures. These data suggest a shift in the effect of temperature on survival based on ontogenetic stage, which may allow survival of post-hatch individuals over a broad range of environmental conditions.

### **River lampreys in the Columbia River Basin**

The main objectives of this study were to examine questions regarding the biology of three species of lampreys found in the CRB. While both Pacific lampreys and western brook lampreys were conveniently found in the mainstem or tributaries to the Columbia River, river lampreys have proven more elusive. River lampreys are an anadromous species historically found in the CRB, and have been collected in the CRB as recently as 1980 (Bond et al. 1983). For this project, we were unable to locate river lampreys in the CRB. Their absence from catch records suggests that they are scarce in this area or may be locally extinct. In 1999, we started to search for river lampreys and have since discovered a need for basic information on the biology of this species.

The river lamprey is an understudied species; much of the information that we have comes from studying other predatory lamprey species. We do know that post-metamorphic river lampreys can be distinguished from other predatory lamprey species using dentition and coloration patterns. The dentition consists of two large teeth on the supraoral lamina, a large middle tooth on the transverse lingual lamina, and 3 points (rarely 2) on each central lateral tooth plate (Eddy and Underhill 1978). Coloration patterns consist of a counter shaded silver body, with a slightly green tint on the upper and mid sections of the body. We also observed a dark

black-pigmented line along the base of both dorsal fins and a patch of pigmentation in the caudal fin. It is believed that river lampreys metamorphose in late July, with downstream migration occurring the following year from late April to July (Beamish 1980b). During the predatory phase, river lampreys attach to their prey, rasp through the outer layers of skin and scales, and feed on the host's body fluids. In laboratory studies they have been observed to feed on small salmonids (Family: Salmonidae), Pacific herring (*Clupea pallasii*), shiner perch (*Cymatogaster aggregata*), and English sole (*Parophrys vetulus*) (Beamish 1980b). The final phase of the life cycle, the spawning phase, begins once feeding ceases; occurring from late fall to May of the following year (Beamish 1980b). During this time, river lampreys will start their upstream migration into the fresh-water system to spawn.

Collection records indicate that river lampreys historically inhabited coastal stream systems from Taku River, AK, south to the San Francisco Bay, CA (Hart 1980). All of the specimens for which records are available are predatory phase individuals that were collected as incidental catch from estuaries and bays along the northwest Pacific coast. Documentation of larval river lampreys has proven extremely difficult and uncommon, which has been attributed to the difficulty in distinguishing among larval lampreys. Analyses of mitochondrial DNA from northern hemisphere lampreys has helped to distinguish between 11 species within the *Lampetra* genus. This method used a 735 base pair sequence from the cytochrome *b* and NADH dehydrogenase subunit 3 (ND3) to aid in differentiating between species. Presently, this form of genetic testing indicates a distinct difference between Pacific lampreys when compared to western brook and river lampreys. However, river and western brook lampreys are considered satellite species and genetically inseparable using this method of testing. This would suggest a divergence time of less than 70,000 years ago (Docker et al. 1999). The lack of general

knowledge and inability to distinguish between the three CRB species in the field has led to misidentification and inaccurate reporting, which complicated our search.

In the fall of 1999, we started looking for river lampreys and originally restricted our search to the CRB. By 2001 we realized that a more extensive search area was required, so we expanded it to include coastal rivers and estuaries from California to Canada (for an overview of agencies and organizations contacted see Appendix 4). Within the CRB, 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 CRB 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 (NOAA Fisheries), 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 specimens of predatory phase river lampreys collected in the CRB, but none collected after 1980. The University of Washington has river lamprey specimens that were collected as recently as 2000 from Lake Washington, WA (outside the CRB). Also contacted were the Point No Point Treaty Council and the Lower Elwha Klallam Tribe from the Puget Sound region of Washington. 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 reported collecting river lampreys in the past, but has not had any recent sightings. Local WDFW and ODFW offices were contacted for Puget Sound, the Klickitat River Basin, the Willamette River Basin, the Umpqua River Basin, and the Smith River Basin. None of these offices recorded sightings or collections of river lampreys. The Hatfield Marine Science Center in Newport, OR, was unable to provide us with new information on search locations. In California, we contacted both the Steinhart Aquarium and the Monterey Bay Aquarium, 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 was collected in Marin County, CA, in 1971. 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 were hesitant to supply us with any river lampreys until more accurate population data are available.

In June 2002, 54 river lampreys were located and captured by NOAA Fisheries in northern Skagit Bay, WA. Specimens were captured as incidental catch during surface trawls using a tow net (6 m wide by 3 m deep) with mesh sizes ranging from 8.9 cm at the front to 0.6 cm at the codend. NOAA Fisheries catch records indicated that river lampreys were present in the surface waters of Skagit Bay, WA, from April until October, peaking from June to August (Table 5). These predatory phase river lampreys were identified using dentition and color patterns.

The river lampreys collected were sexually immature and therefore could not be used for this project. However, due to the difficulty we encountered in locating and collecting live river

lampreys, we explored the feasibility of maintaining river lampreys in a laboratory setting through the time of sexual maturation. River lampreys were transported to the CRRL and held for 32 d in a flow through, 189 L aquarium (see above; Laboratory environment) until a suitable seawater capable facility could be found. During this period, they were provided a diet of Chinook salmon (*Oncorhynchus tshawytscha*) smolts, but they only fed sporadically and some mortality occurred. On July 9, 2002, the remaining 36 river lampreys were transferred to USGS Marrowstone Marine Field Station, Nordland, WA. River lampreys were held for 195 d in a 530 L tank provided with 3.8 L/min to 5.7 L/min of filtered seawater. Water was pumped from the Puget Sound at a depth of 15 m, sand filtered to 40  $\mu\text{m}$ , and sterilized using UV sterilizers. Temperature and salinity fluctuated with that of ambient Puget Sound water.

Measurements were taken periodically over the 318 d that we held the river lampreys in captivity. A mean length of 139 mm (SE = 0.55) (Figure 15) and mean mass of 2.89 g (SE = 0.14) (Figure 16) was recorded in early July. Once the river lampreys were returned to a seawater environment, they resumed feeding on a diet of Pacific herring. The river lampreys were voracious feeders, in many cases tearing sections of flesh from the herring and feeding on recently deceased fish. Lampreys ceased feeding in early December, even though herring were still present in the tank. Herring were offered until January when, due to increased mortality, the river lampreys were transferred back to the CRRL and held for 91 d until the time of spawning. Although sample sizes varied over time due to mortality, mean growth rates were calculated to examine general trends. At the time of transfer, the nine remaining river lampreys had a mean length of 241.33 mm (SE = 1.25) (Figure 15) and mean mass of 22.63 g (SE = 0.63) (Figure 16). This suggests a growth rate of 0.52 mm/d, for the 195 d that the river lampreys were held in seawater. Once feeding ceased the river lampreys went into a period of negative growth. During

this time, the mean length of the river lampreys decreased to 198.0 mm (SE = 1.74) (Figure 15) and mean mass of 15.02 g (SE = 0.63) (Figure 16), which indicates a growth rate of -0.48 mm/d over the remaining 91 d that the river lampreys were held. In April of 2003, the nine remaining river lampreys started to exhibit secondary sexual characteristics such as swelling on the leading edge of the second dorsal fin, development of an anal fin fold behind the cloaca, an oedematous region developed in front of the cloaca, and visible papilla in males (Hardisty and Potter, 1971b). In late April, four river lampreys were artificially spawned and the embryos were incubated in hatching jars (6.86 L McDonald type) until hatch. At that time, they were transferred into an aquarium with suitable substrate and continue to be held at the CRRL (see above; Laboratory environment).

Initially we had projected spawning of the river lampreys to occur in the spring of 2001 and 2002 along with Pacific lampreys and western brook lampreys. Although river lampreys were located in the spring of 2002, they were not sexually mature and therefore could not be included in our experiments. The river lampreys that were held through sexual maturity and artificially spawned produced viable offspring, which indicates that we will be able to successfully spawn river lampreys in the future. While searching for river lampreys, vast gaps in the basic knowledge of this species emerged. Future studies should be conducted on basic biology as virtually nothing is known of the river lamprey, in particular the larval stage of the life cycle. There are also issues of identification such that if species are not distinguishable, much of the published distribution data may be incorrect. This leads to the question of their historic distribution; are they still present in some of the areas that they were found in the past? There are also questions about habitat, migration, feeding, and basic requirements for survival that need

to be addressed. Very little is known, which makes studying river lampreys all the more important in order to preserve their place in the ecosystem.

#### **Future goals**

1. Inclusion of meristic data in morphological examination of early larval stage lampreys.
2. Preparation and submission of manuscripts to peer-reviewed scientific journals.
3. Preparation of final report of research to Bonneville Power Administration.

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Table 1: Sex, sample size, mean total length, and mean wet mass of Pacific and western brook lampreys used to provide gametes for experiments in 2001 and 2002.

Species	Sex	Year	<i>N</i>	Length (mm $\pm$ SD)	Mass (g $\pm$ SD)
Pacific lamprey	Female	2001	5	459 $\pm$ 42	318.4 $\pm$ 65.4
		2002	6	446 $\pm$ 28	292.0 $\pm$ 42.0
	Male	2001	6	508 $\pm$ 41	287.5 $\pm$ 87.9
		2002	6	480 $\pm$ 37	267.6 $\pm$ 69.5
Western brook lamprey	Female	2001	31	122 $\pm$ 5	4.236 $\pm$ 0.656
		2002	29	122 $\pm$ 10	4.545 $\pm$ 1.130
	Male	2001	19	127 $\pm$ 7	3.938 $\pm$ 0.668
		2002	28	124 $\pm$ 9	3.758 $\pm$ 0.995

Table 2: Component loadings for principle components I and II associated with larval length measurements (log transformed standard length and truss elements).

Variable	Eigenvectors	
	Principle component I	Principle component II
LnSL	0.237276	0.169002
LnA	0.290241	-0.313597
LnB	0.255130	-0.342844
LnC	0.244516	-0.207822
LnD	0.244260	-0.060370
LnE	0.277947	-0.194244
LnF	0.250561	-0.096640
LnG	0.264667	-0.146598
LnH	0.189921	0.063315
LnI	0.195816	0.368770
LnJ	0.192611	0.354244
LnK	0.192092	0.367000
LnL	0.195288	0.350751
LnM	0.132638	0.334643
LnN	0.340133	0.028515
LnO	0.380790	-0.064578

Table 3: Parameter estimates for classification functions for Pacific (PCL) and western brook (WBL) lamprey larvae derived from discriminant analyses. Classification takes the form:  $C_j = C_{j0} + C_{j1}X_1 + C_{j2}X_2 + \dots + C_{jp}X_p$ , and membership is assigned to the group with the highest classification score (Tabachnick and Fidell, 1996).

Variable	Full model		Reduced model	
	PCL	WBL	PCL	WBL
Constant	-45472	-45399	-19897	-19831
LnSL	45499	45479	.	.
LnA	-2329	-2330	.	.
LnB	-1447	-1464	-94.66590	-111.31499
LnC	-11476	-11435	-12027	-11987
LnD	-141596	-141341	-123293	-123031
LnE	-139556	-139286	135749	135484
LnF	145082	144824	135749	135484
LnG	152009	151693	137643	137312
LnH	-12984	-12951	-11582	-11546
LnI	-25867	-25945	.	.
LnJ	-22596	-22734	484.47835	428.43440
LnK	6478	6572	.	.
LnL	14057	14201	-1451	-1388
LnM	854.13438	814.84661	296.33265	259.16398
LnN	-2229	-2200	-173.94647	-145.59001
LnO	-4226	-4241	-390.11622	-406.68821

Table 4: Effective temperature ( $E_T$ ), days required to reach 50% hatch ( $D_{H50} \pm SE$ ), and days required to reach 95% hatch ( $D_{H95} \pm SE$ ) for Pacific and western brook lampreys reared at four temperatures.  $D_{H50}$  and  $D_{H95}$  were estimated independently for each replicate using logistic regression.

Species	Temperature (° C)	$E_T$ (° C)	$D_{H50}$ (± SE)	$D_{H95}$ (± SE)
Pacific lamprey	10	5.15	26.22 ± 0.57	29.26 ± 0.50
	14	9.15	16.95 ± 0.20	18.85 ± 0.36
	18	13.15	11.10 ± 0.03	12.22 ± 0.10
	22	17.15	8.38 ± 0.05	9.08 ± 0.08
Western brook lamprey	10	5.03	26.93 ± 0.53	29.34 ± 0.60
	14	9.03	15.82 ± 0.18	17.00 ± 0.19
	18	13.03	10.84 ± 0.10	11.90 ± 0.06
	22	17.03	8.05 ± 0.10	9.03 ± 0.09

Table 5: NOAA Fisheries river lamprey catch records for 2002 in Skagit Bay, WA. Lampreys were captured as incidental catch during surface trawls using a 6 m (wide) by 3 m (deep) tow net.

Date	North Hope Island	South Hope Island	Lone Tree Point	Similk Bay	South Fork Flats	Strawberry Point	Dugwalla Bay	North Fork Flats	Utsalady	PBD Flats	Snee Oosh	Hoypus Point	Crescent Harbor
04/10/2002	1												
06/03/2002	4												
06/04/2002			1	13	4	23							
06/05/2002							4	3	2				
07/10/2002			7				4			3			
07/11/2002		1		2							2	3	
07/12/2002					6	1		7	2				6
07/30/2002					5	1			3				4
07/31/2002	1	1					1						
08/01/2002			4	24							1		
08/28/2002				1			1						
08/29/2002			1										
10/30/2002							1						
Total	6	2	13	40	15	25	11	10	7	3	3	3	10

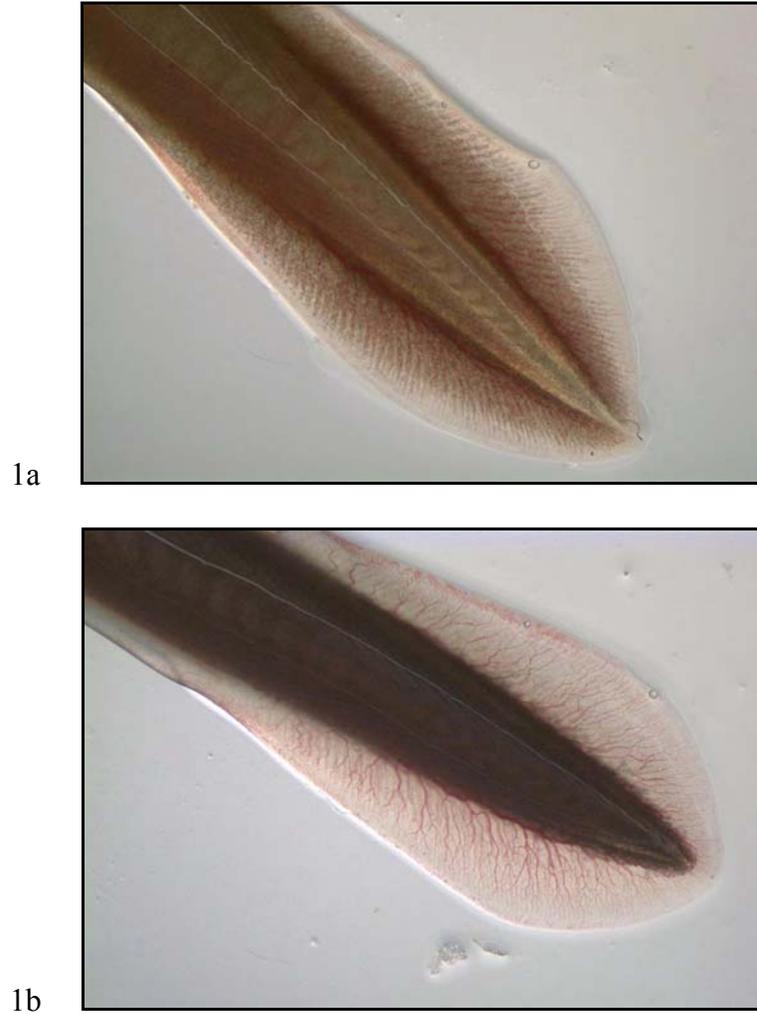
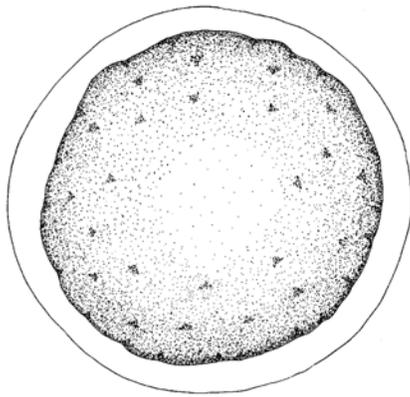
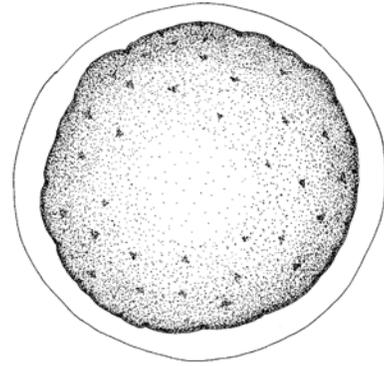


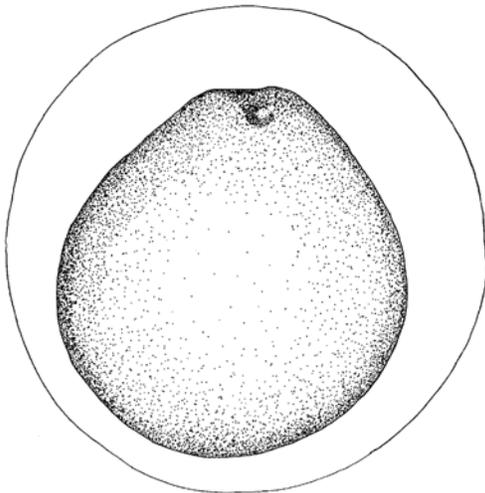
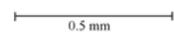
Figure 1: Examples of digital images of caudal region of: 1a) Pacific lamprey; characterized by light pigmentation along the caudal ridge, and 1b) western brook lamprey; characterized by dark, even pigmentation along the caudal ridge (Richards et al. 1982).



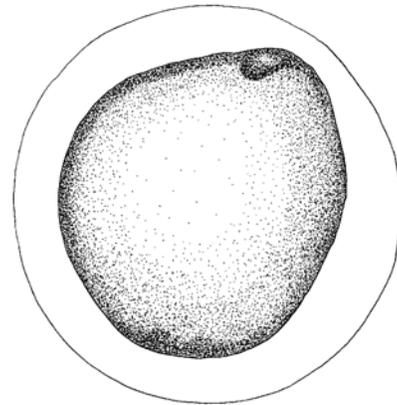
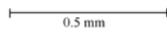
2a



2b



2c



2d

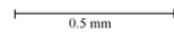


Figure2: Continued on the following page.

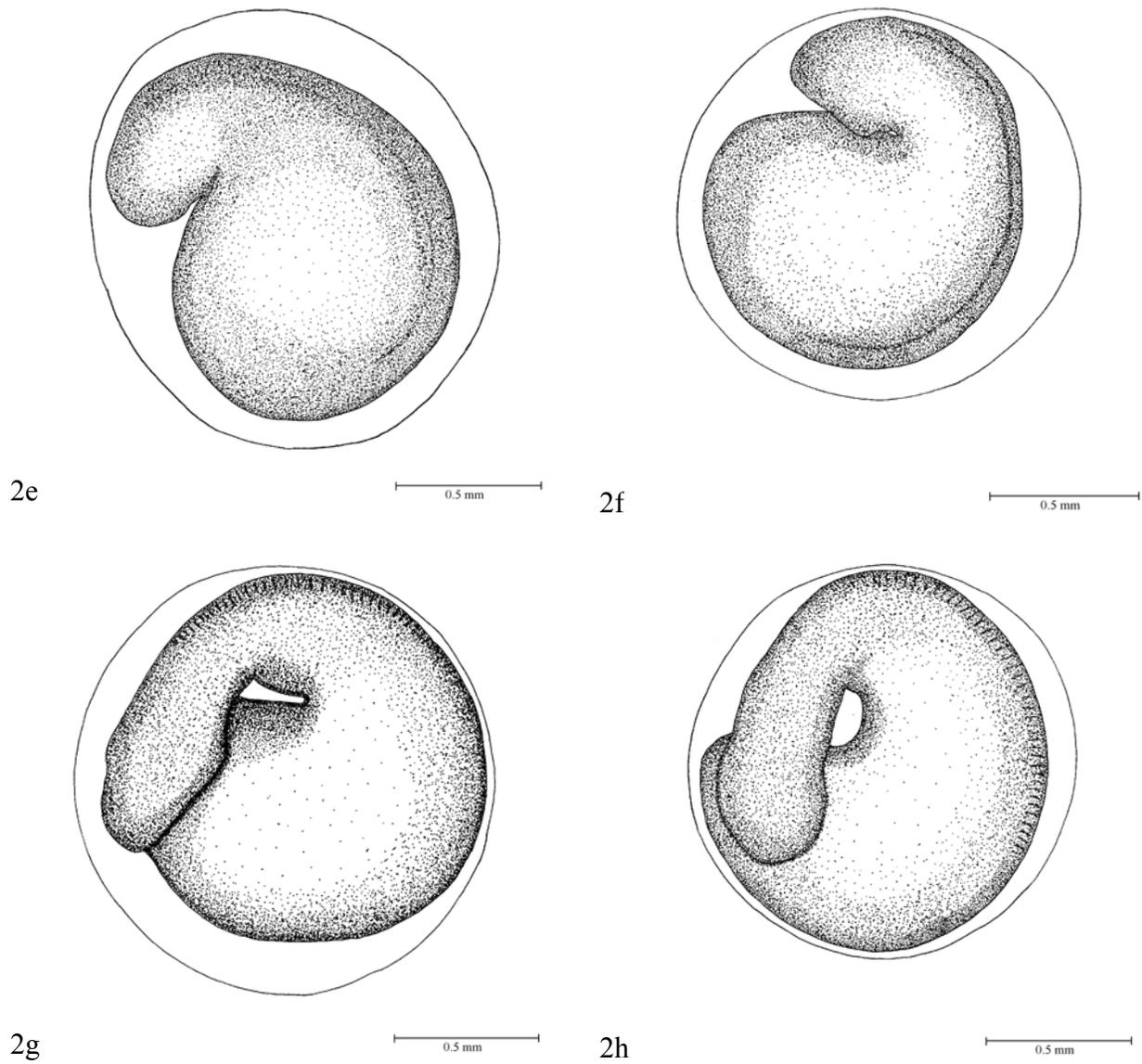


Figure 2: Time series of Pacific and western brook lamprey embryos reared at 14° C; day 1 Pacific (2a) and western brook (2b) lamprey, day 7 Pacific (2c) and western brook (2d) lamprey, day 10 Pacific (2e) and western brook (2f) lamprey, and day 14 Pacific (2g) and western brook (2h) lamprey.

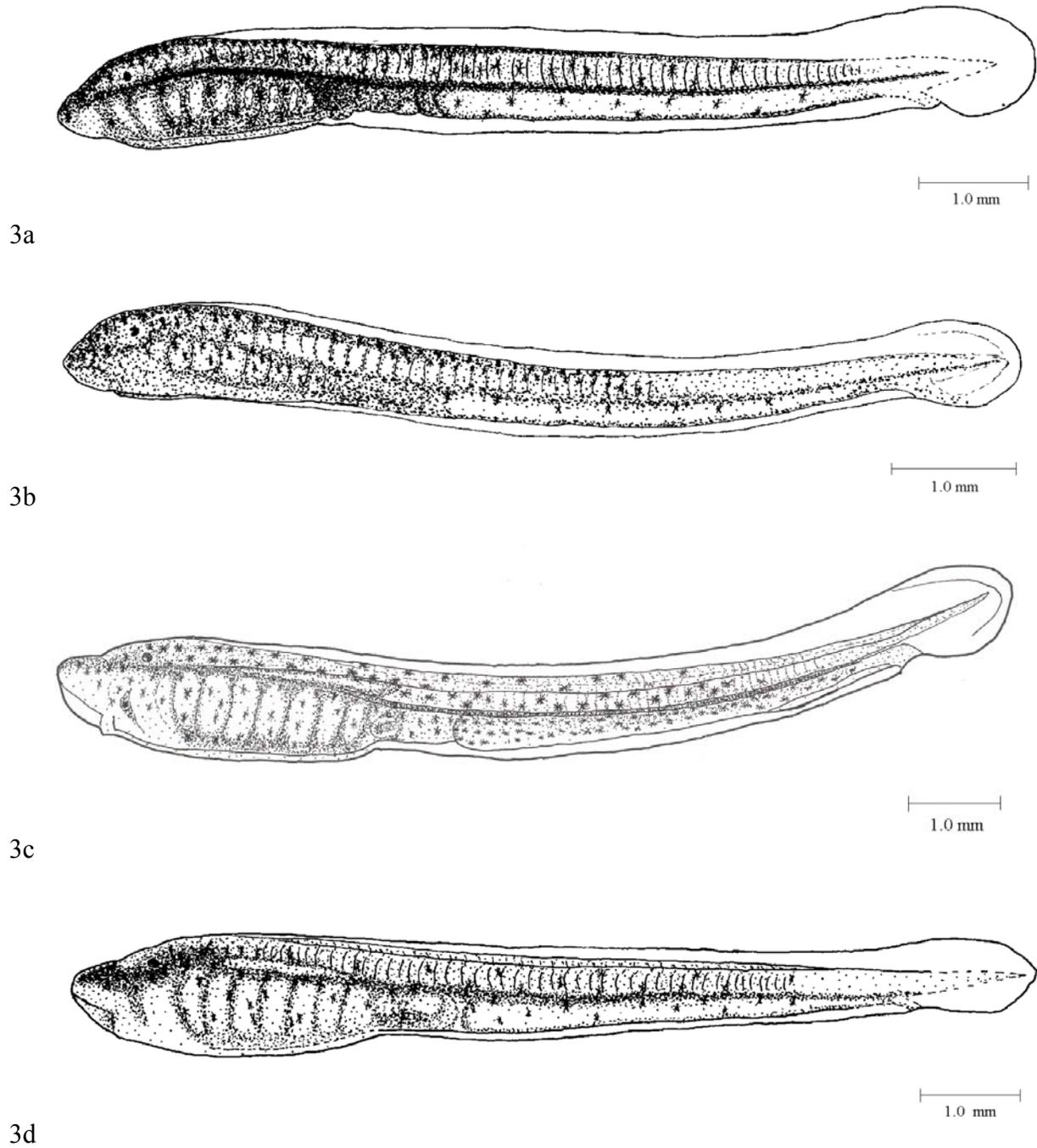


Figure 3: Line drawing of stage 17 Pacific lamprey (3a), stage 17 western brook lamprey (3b), stage 18 Pacific lamprey (3c), and stage 18 western brook lamprey (3d).

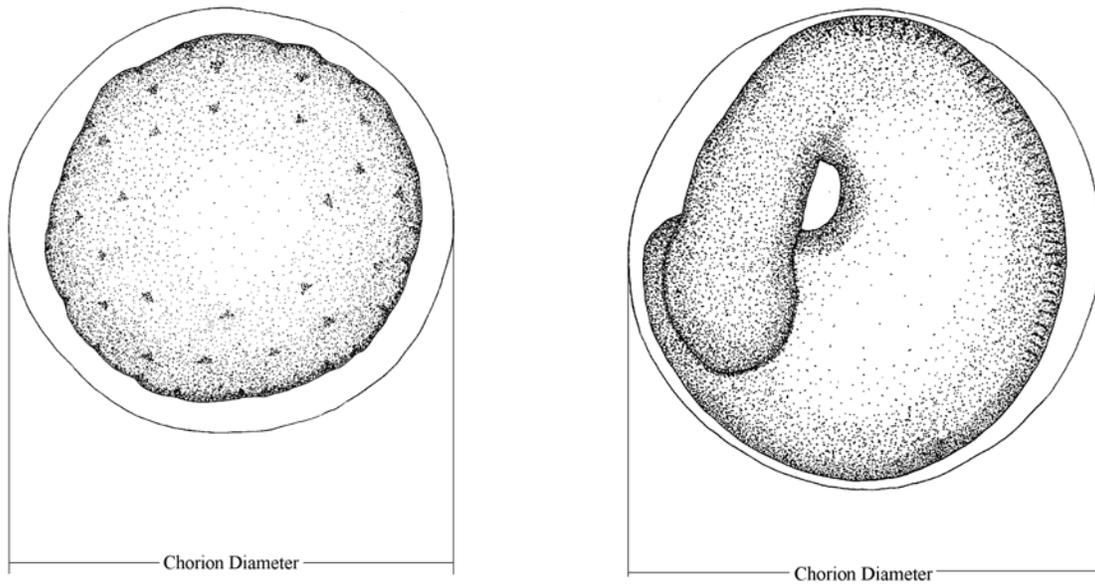
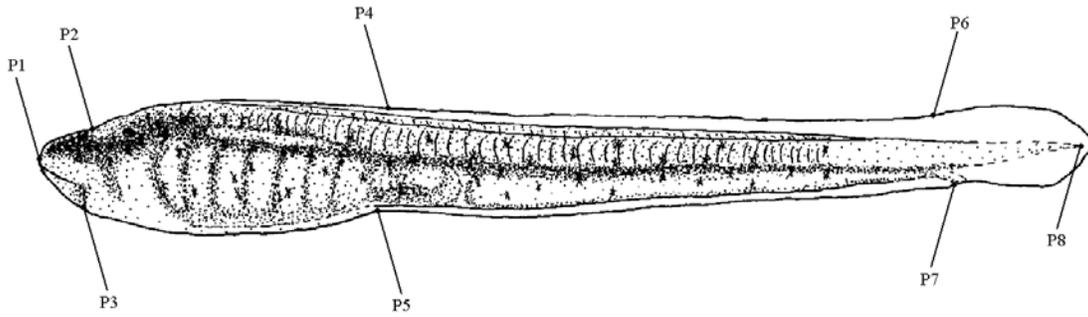
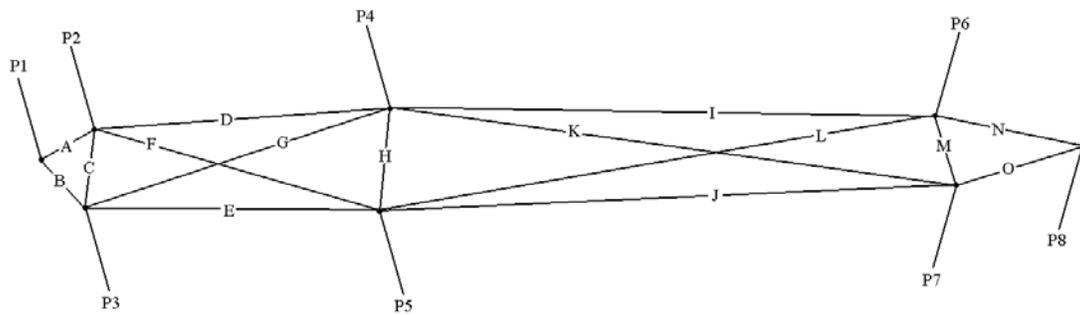


Figure 4: Line drawings of lamprey embryos indicating the chorion diameter. For each individual, 180 measurements were made through the embryo's centroid at 2 degree intervals and the mean chorion diameter was calculated.



5a



5b

Figure 5: Line drawing of a larval lamprey indicating the locations of the eight homologous landmarks (5a), and a representation of the structure of the two-cell truss network with two appended triangles (5b).

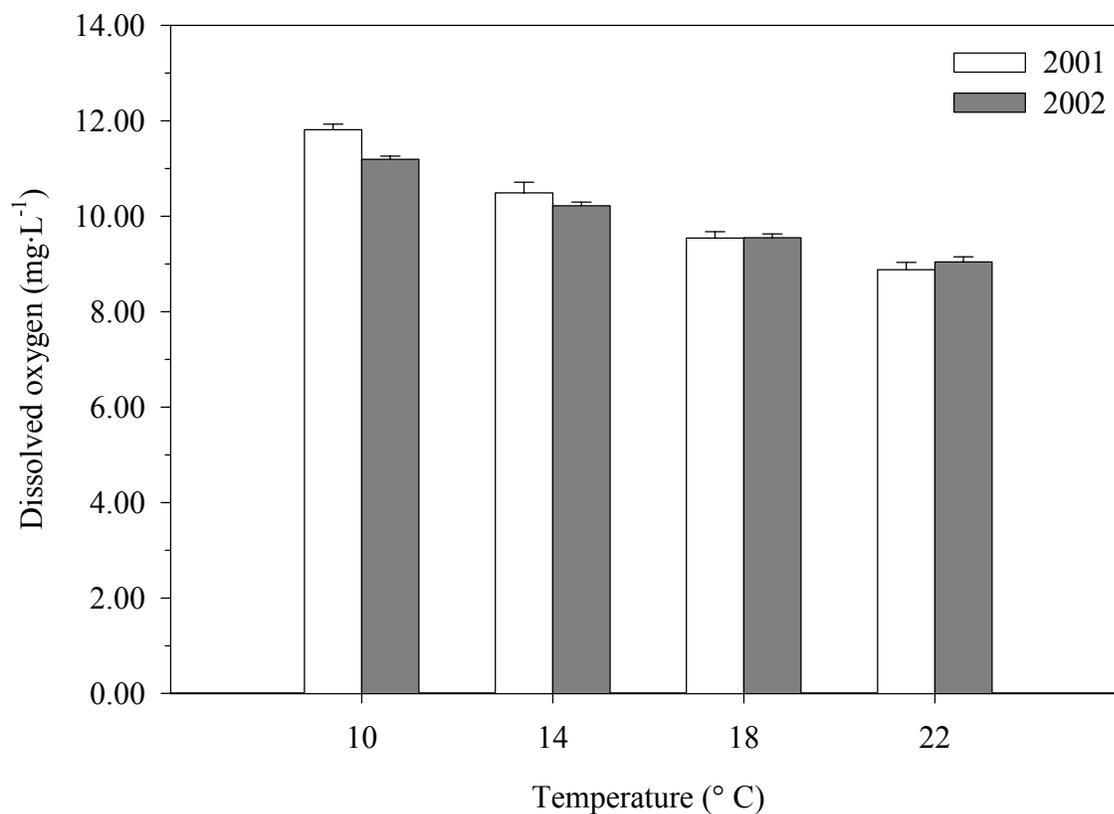


Figure 6: Mean dissolved oxygen ( $\text{mg}\cdot\text{L}^{-1}$ ) (+ SE) of water baths at  $10^{\circ}\text{C}$ ,  $14^{\circ}\text{C}$ ,  $18^{\circ}\text{C}$ , and  $22^{\circ}\text{C}$  for experiments conducted in 2001 and 2002. Measurements were taken daily for the duration of the experiments.

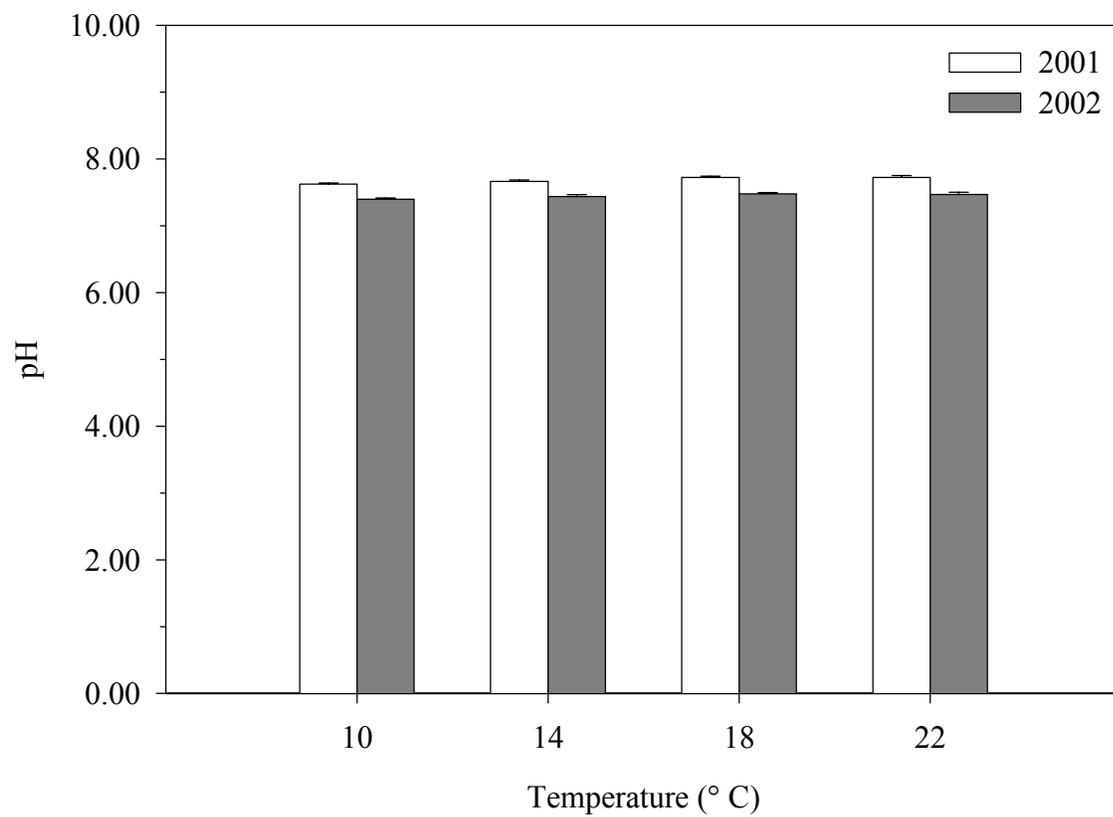


Figure 7: Mean pH (+ SE) of water baths at 10° C, 14° C, 18° C, and 22° C for experiments conducted in 2001 and 2002. Measurements were taken daily for the duration of the experiments.

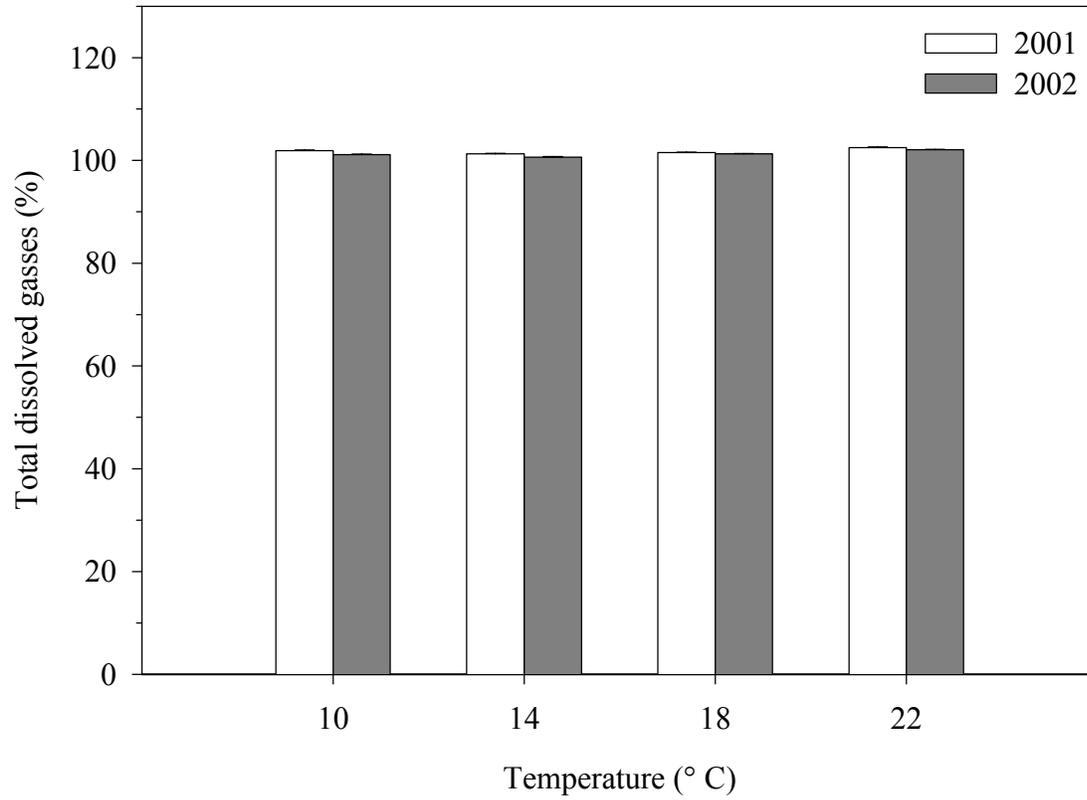


Figure 8: Mean total dissolved gasses (% saturation) (+ SE) of water baths at 10° C, 14° C, 18° C, and 22° C for experiments conducted in 2001 and 2002. Measurements were taken daily for the duration of the experiments.



Figure 9: Time series of normal larval development of Columbia River Basin lampreys; 9a) recently hatched larva exhibiting well-pronounced ventral flexion, 9b) less pronounced ventral flexion, 9c) slight ventral flexion in posterior region, and 9d) fully developed larva.

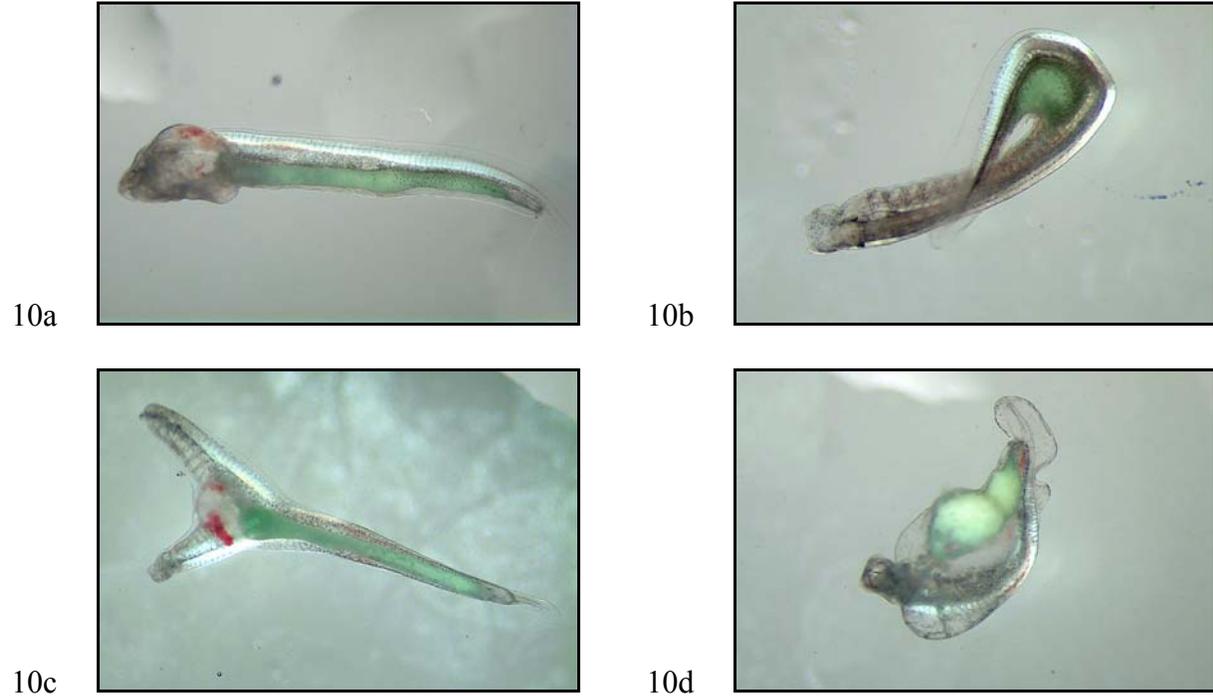


Figure 10: Series of abnormal Columbia River Basin lamprey larvae; 10a) larva with malformed head, branchial, and trunk regions, 10b) larva with malformed trunk region, 10c) larva with superfluous head and branchial region, and 10d) larva with extreme morphological malformations.

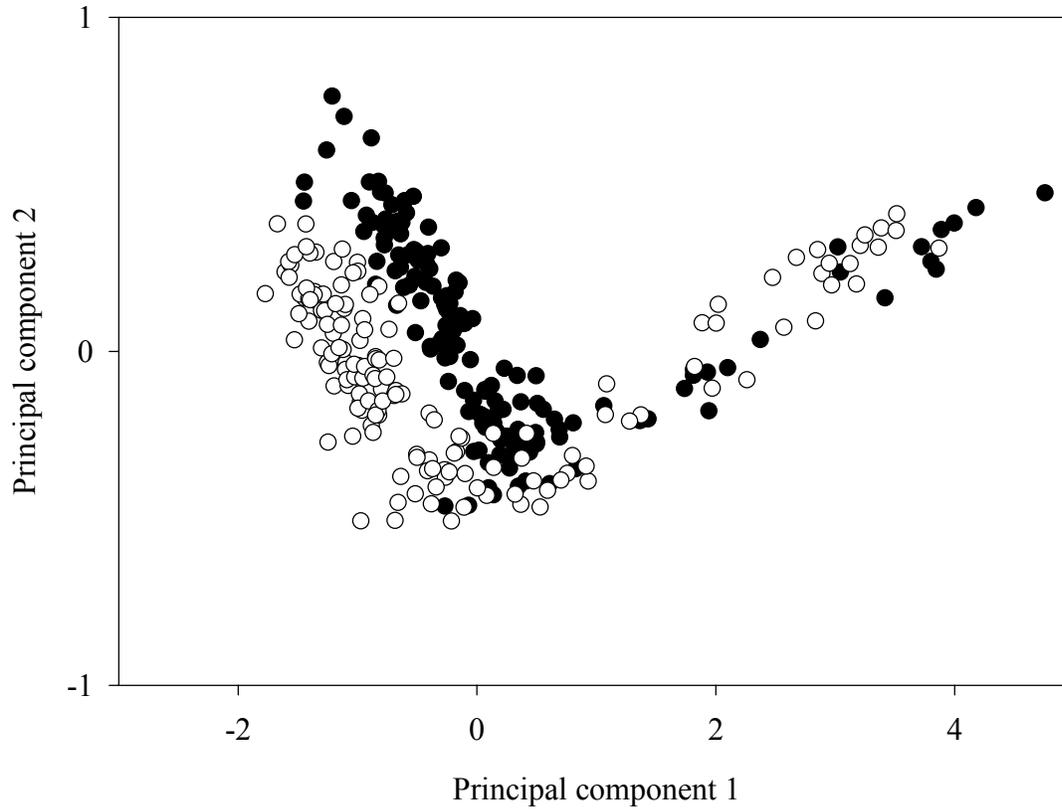


Figure 11: Components plot for principal components 1 and 2 associated with larval length measurements (log transformed standard length and truss elements). Filled circles (●) represent Pacific lampreys and empty circles (○) represent western brook lampreys.

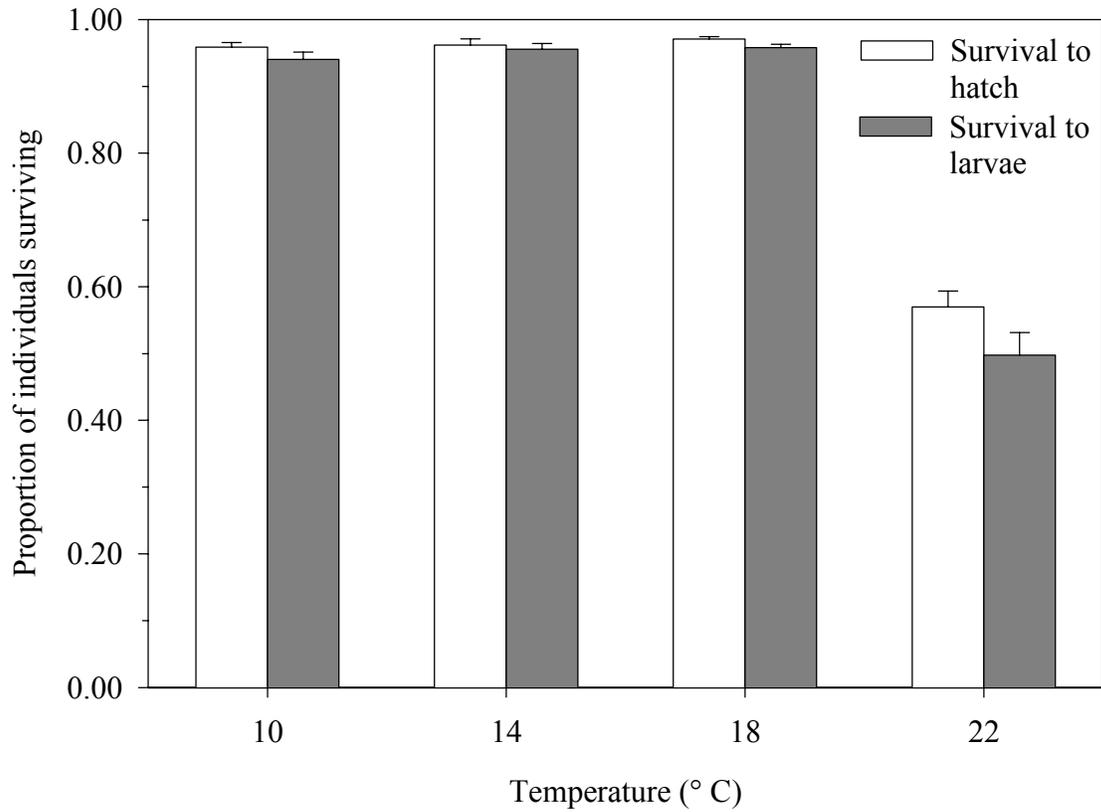


Figure 12: Proportion of individuals surviving (+ 1 SE) at hatch ( $S_H$ ) and at the larval stage ( $S_L$ ).

Overall, survival was significantly reduced at 22° C when compared to other temperatures.

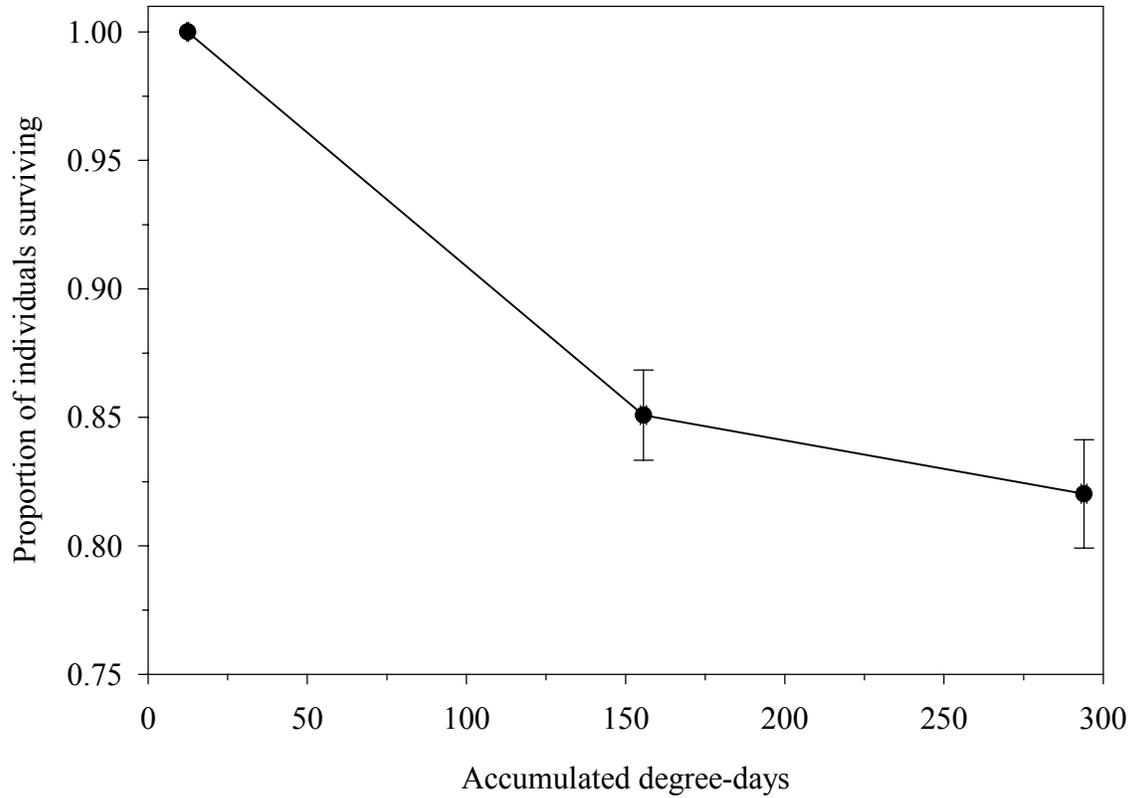


Figure 13: Trend in the proportion of individuals surviving ( $\pm 1$  SE) from the initiation of experiment (12.5 *DD*) to hatch (155.6 *DD*) and from hatch to the larval stage (294.0 *DD*). A slight, but significant, decrease in survival was observed from the time of hatch to the larval stage.

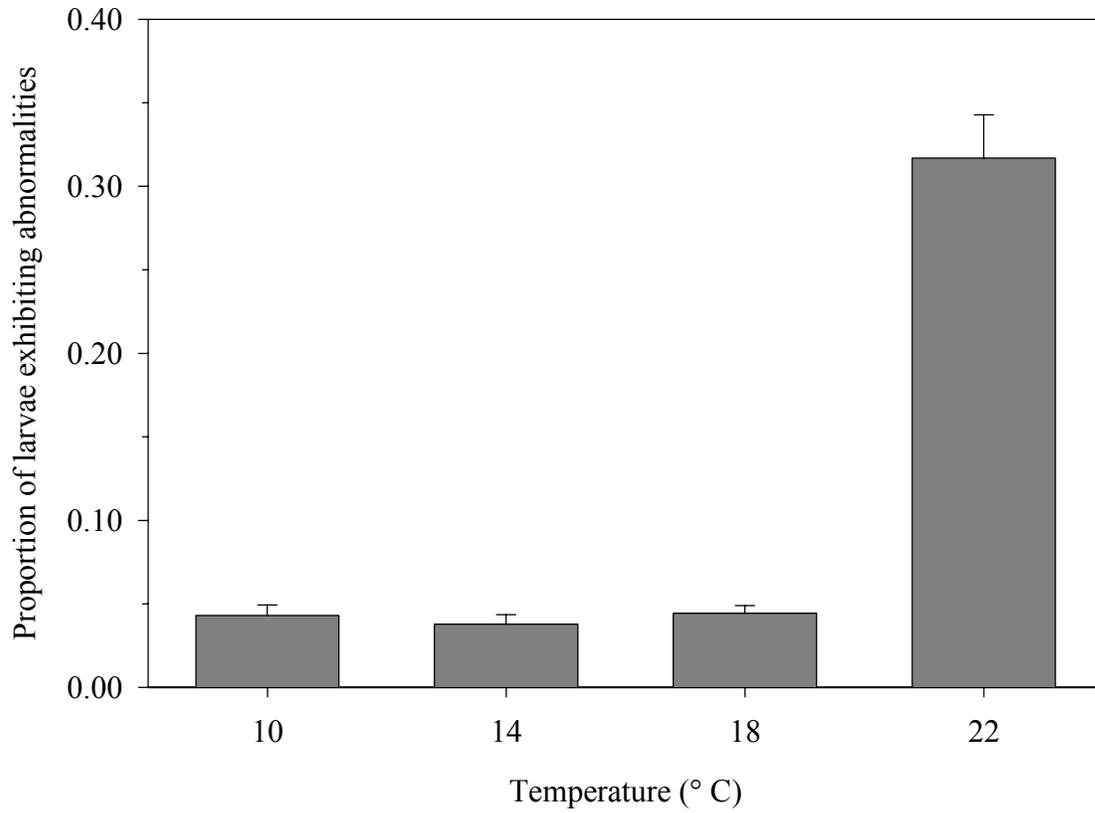


Figure 14: Proportion of larvae exhibiting abnormalities (+ 1 SE). Significantly more larvae exhibited abnormalities at 22° C when compared to other temperatures.

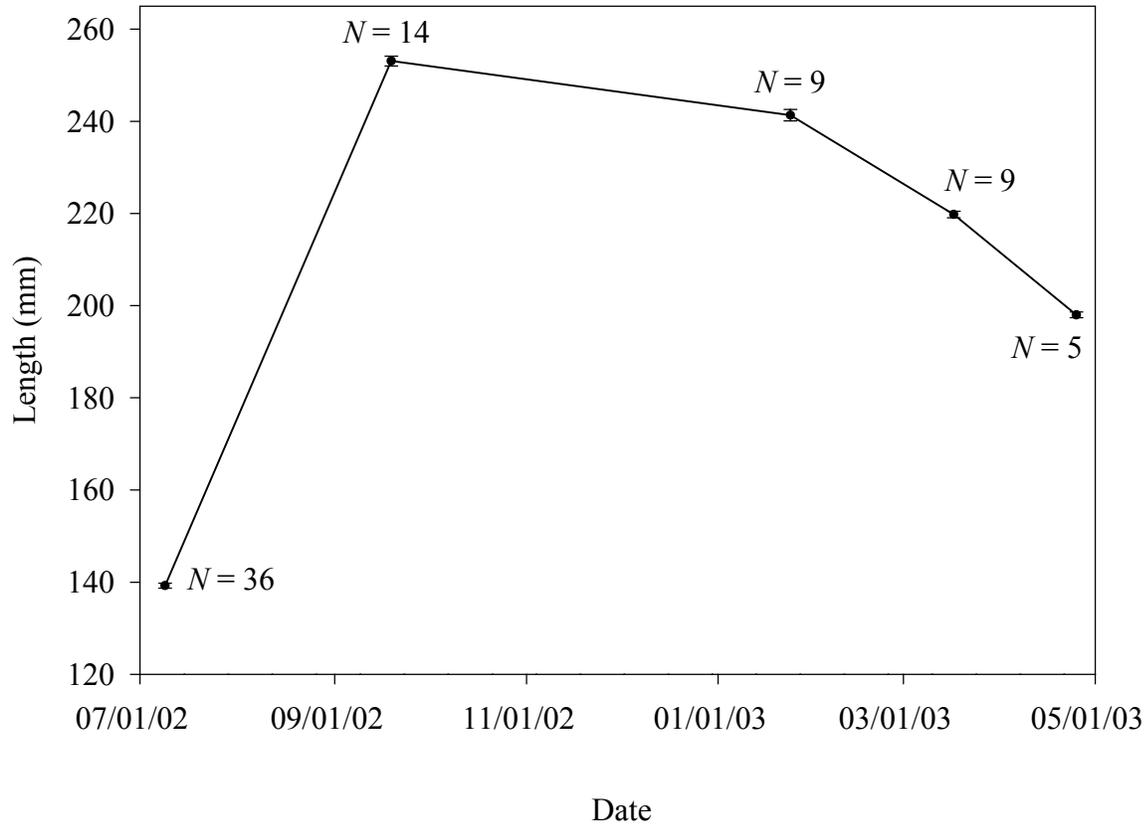


Figure 15: Mean total length (mm  $\pm$  1 SE) and sample size for river lampreys held in captivity and measured at intervals from July 2002 until April 2003.

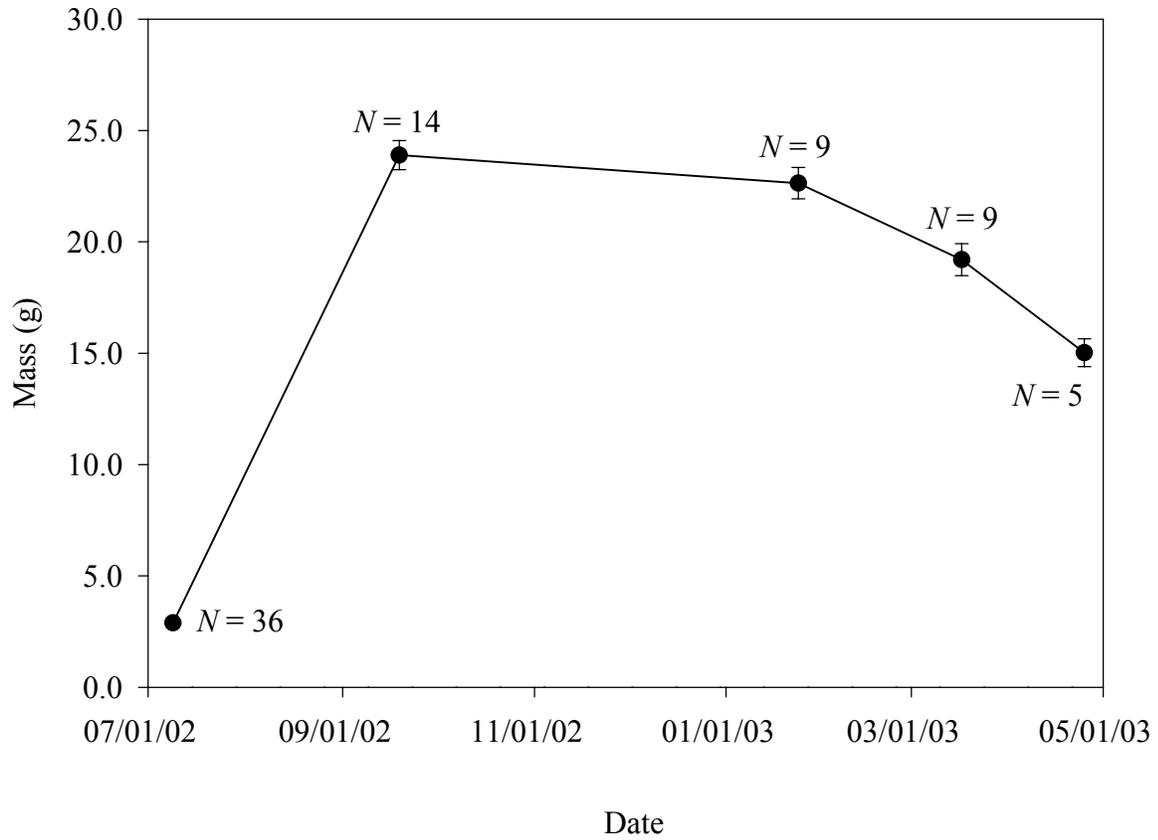


Figure 16: Mean wet mass ( $g \pm 1$  SE) and sample size for river lampreys held in captivity and measured at intervals from July 2002 until April 2003.

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	92	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	30	127	2.859	100	0	
RED	30	134	3.436	100	0	
RED	30	126	2.684	100	0	
RED	6	142	4.500	100	0	
RED	30	139	3.581	100	0	
ENT	30	128	2.977	100	0	
ENT	30	123	2.450	100	0	
ENT	13	108	1.713	100	0	
ENT	18	124	2.919	100	0	PCL
ENT	30	127	2.569	100	0	
ENT	26	132	3.272	100	0	
ENT	30	121	2.660	100	0	
ENT	25	120	2.307	100	0	
JDW	20	125	2.752	100	0	
JDW	27	122	2.275	0	100	
JDW	27	117	2.263	0	100	
JDW	15	114	1.921	0	100	
JDW	27	123	2.605	100	0	
JDW	22	116	2.138	100	0	
JDW	27	120	2.291	100	0	
JDW	20	111	1.841	0	100	

Appendix 3: Locations and descriptions of landmarks and truss elements used for morphometric descriptions of lampreys.

Feature	Label	Description
Landmark	P1	Anterior most portion of the larva (snout)
Landmark	P2	Dorsal margin of the oral hood and the head
Landmark	P3	Anterior most portion of the transverse lip of the oral hood
Landmark	P4	Point at the terminus of a line drawn from P5 to, and perpendicular to, the dorsal surface of the larva
Landmark	P5	Ventral margin of the branchial region and the trunk
Landmark	P6	Point at the terminus of a line drawn from P7 to, and perpendicular to, the dorsal surface of the larva
Landmark	P7	Anterior most portion of the vent
Landmark	P8	Posterior most portion of the notochord
Truss element	A	Line connecting P1 and P2
Truss element	B	Line connecting P1 and P3
Truss element	C	Line connecting P2 and P3
Truss element	D	Line connecting P2 and P4
Truss element	E	Line connecting P3 and P5
Truss element	F	Line connecting P2 and P5
Truss element	G	Line connecting P3 and P4
Truss element	H	Line connecting P4 and P5
Truss element	I	Line connecting P4 and P6
Truss element	J	Line connecting P5 and P7
Truss element	K	Line connecting P4 and P7
Truss element	L	Line connecting P5 and P6
Truss element	M	Line connecting P6 and P7
Truss element	N	Line connecting P6 and P8
Truss element	O	Line connecting P7 and P8

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