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

Amended Final Report
2002 - 2004

DOE/BP-00004695-4

May 2004

This Document should be cited as follows:

Meeuwig, Michael, Jennifer Bayer, 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", 2002-2004 Amended Final Report, Project No. 200002900, 97 electronic pages, (BPA Report DOE/BP-00004695-4)

Bonneville Power Administration
P.O. Box 3621
Portland, OR 97208

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

Amended Final Report of Research 2004

Prepared by:

Michael H. Meeuwig

Jennifer M. Bayer

and

Rebecca A. Reiche

U.S. Geological Survey
Western Fisheries Research Center
Columbia River Research Laboratory
5501A Cook-Underwood Road
Cook, WA 98605

Prepared for:

U.S. Department of Energy
Bonneville Power Administration
Environment, Fish, and Wildlife Department
P.O. Box 3621
Portland, OR 97208-3621

Project Number 2000-029
Contract Number 00AI23249

May 2004

Table of contents

Contents		Page
Title page		1
Table of contents		2
Acknowledgements		3
Chapter 1:	Executive summary	4
	Project overview	4
	References	9
Chapter 2:	Evaluation of color patterns for identification of larval Pacific lampreys (*Lampetra tridentata*) and western brook lampreys (*L. richardsoni*) from the Columbia River Basin	12
	Abstract	12
	Introduction	13
	Methods	14
	Results	15
	Discussion	16
	References	20
	Figures	23
	Appendices	27
Chapter 3:	Morphometric discrimination of early life stage Pacific lampreys (*Lampetra tridentata*) and western brook lampreys (*L. richardsoni*) from the Columbia River Basin	30
	Abstract	30
	Introduction	31
	Methods	34
	Results	38
	Discussion	40
	References	44
	Tables	48
	Figures	53
	Appendices	60
Chapter 4:	Effects of temperature on survival and development of early life stage Pacific lampreys (*Lampetra tridentata*) and western brook lampreys (*L. richardsoni*)	61
	Abstract	61
	Introduction	62
	Methods	64
	Results	67
	Discussion	69
	Tables	74
	Figures	78
	References	80
Chapter 5:	River lampreys (*Lampetra ayresi*) in the Columbia River Basin	84
	Synopsis	84
	References	91
	Figures	92
	Appendices	94

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.

Chapter 1

Executive summary

Project overview

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 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 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; NPPC 1994; Close et al. 1995), has stimulated interest in recovery actions within the Columbia River Basin.

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

The goal of this project was 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 Columbia River Basin. In particular, our objectives were 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 helps to 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 Columbia River Basin.

This document presents results of research conducted by the U.S. Geological Survey, Western Fisheries Research Center, Columbia River Research Laboratory. Unless otherwise noted, animals were held under the following conditions in the laboratory. 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 Columbia River Basin. Temperatures followed ambient Columbia River water temperatures (± 0.5° C) at Bonneville Dam (University of Washington 2004) with the exception that they never exceeded 15° C (± 0.5° 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 $L \cdot min^{-1} \cdot kg^{-1}$), 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.

Chapter 2 presents an examination of current and widely used identification techniques for larval lampreys. Chapter 3 presents an examination of alternative methods for identifying Pacific lampreys and western brook lampreys during early life stages. Specifically, Chapter 3 presents the results of meristic and multivariate morphometric analyses of lamprey size and shape in an effort to discriminate between species. Chapter 4 presents results of research designed to examine the thermal requirements of lampreys during embryogenesis and early larval stage development. Although the projects reported in this document intended to include all lamprey species historically documented to occur within the Columbia River Basin, river lampreys were not included in our analyses. Information regarding the current distribution of river lampreys within the Columbia River Basin is essentially non-existent with specimens collected near Astoria, OR (1980), being the most recent documented occurrence of river lampreys within the Columbia River Basin. Therefore, Chapter 5 provides a synopsis of information with regard to river lampreys within the Columbia River Basin, and data we have obtained from river lampreys collected from outside the basin.

References

Beamish, F. W. H. 1980. Biology of the North American anadromous sea lamprey, *Petromyzon marinus*. Canadian Journal of Fisheries and Aquatic Sciences 37:1924-1943.

Beamish, R. J., and C. D. Levings. 1991. Abundance and freshwater migrations of the anadromous parasitic lamprey, *Lampetra tridentata*, in a tributary of the Fraser River, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 48:1250-1263.

Close, D. A., M. Fitzpatrick, H. Li, B. Parker, D. Hatch, and G. James. 1995. Status report of the Pacific lamprey (*Lampetra tridentata*) in the Columbia Basin. Report (Contract 95BI39067) to Bonneville Power Administration, Portland, Oregon.

Hammond, R. J. 1979. Larval biology of the Pacific lamprey, *Entosphenus tridentatus* (Gairdner), of the Potlach River, Idaho. Master's thesis. University of Idaho, Moscow.

Hardisty, M. W., and I. C. Potter. 1971a. The behaviour, ecology, and growth of larval lampreys. Pages 85-126 *in* M. W. Hardisty and I. C. Potter, editors. The biology of lampreys, volume 1. Academic Press, New York, New York.

Hardisty, M. W., and I. C. Potter. 1971b. The general biology of adult lampreys. Pages 127-206 *in* M. W. Hardisty and I. C. Potter, editors. The biology of lampreys, volume 1. Academic Press, New York, New York.

Holmes, J. A., and P. Lin. 1994. Thermal niche of larval sea lamprey, *Petromyzon marinus*. Canadian Journal of Fisheries and Aquatic Sciences 51:253-262.

Houde, E. D. 1987. Fish early life history dynamics and recruitment variability. American Fisheries Society Symposium 2, pp. 17-29.

Kan, T. T. 1975. Systematics, variation, distribution, and biology of lampreys of the genus *Lampetra* in Oregon. Doctoral thesis. Oregon State University, Corvallis.

McCauley, R. W. 1963. Lethal temperatures of the developmental stages of the Sea Lamprey, *Petromyzon marinus* L. Journal of the Fisheries Research Board of Canada 20:483-490.

Moyle, P. B., and J. J. Cech, Jr. 1996. Fishes: An introduction to ichthyology, 3rd edition. Prentice Hall, Upper Saddle River, New Jersey.

NPPC (Northwest Power Planning Council). 1994. Columbia Basin Fish and Wildlife Program. Portland, Oregon.

Pletcher, F. T. 1963. The life history and distribution of lampreys in the Salmon and certain other rivers in British Columbia, Canada. Master's thesis. University of British Columbia, Vancouver.

Piavis, G. W. 1961. Embryological stages in the sea lamprey and effects of temperature on development. Fishery Bulletin 61:111-143.

Potter, I. C., and F. W. H. Beamish. 1975. Lethal temperatures in ammocoetes of four species of lampreys. Acta Zoologica 56:85-91.

Quinn, T. P., and D. J. Adams. 1996. Environmental changes affecting the migratory timing of American shad and sockeye salmon. Ecology 77:1151-1162.

Richards, J. E. 1980. The freshwater biology of the anadromous Pacific lamprey (*Lampetra tridentata*). Master's thesis. University of Guelph, Guelph, Ontario.

Richards, J. E., R. J. Beamish, and F. W. H. Beamish. 1982. Descriptions and keys for ammocoetes of lampreys from British Columbia, Canada. Canadian Journal of Fisheries and Aquatic Sciences 39:1484-1495.

Rodríguez-Muñoz, R., A. G. Nicieza, and F. Braña. 2001. Effects of temperature on developmental performance, survival and growth of sea lamprey embryos. Journal of Fish Biology 58:475-486.

University of Washington. 2004. Columbia River DART: Data Access in Real Time. School of Aquatic and Fishery Sciences. Available: www.cbr.washington.edu/dart/dart.html. (November 2004).

Young, R. J., J. R. M. Kelso, and J. G. Weise. 1990. Occurrence, relative abundance, and size of landlocked sea lamprey (*Petromyzon marinus*) ammocoetes in relation to stream characteristics in the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences 47:1773-1778.

Youson, J. H., J. A. Holmes, J. A. Guchardi, J. G. Seelye, R. E. Beaver, J. E. Gersmehl, S. A. Sower, and F. W. H. Beamish. 1993. Importance of condition factor and the influence of water temperature and photoperiod on metamorphosis of sea lamprey, *Petromyzon marinus*. Canadian Journal of Fisheries and Aquatic Sciences 50:2448-2456.

Chapter 2

Evaluation of color patterns for identification of larval Pacific lampreys (*Lampetra tridentata*) and western brook lampreys (*L. richardsoni*) from the Columbia River Basin

Abstract

We examined the efficacy of using larval color patters, and temporal variation in color patterns, associated with species identification of Pacific lampreys (*Lampetra tridentata*) and western brook lampreys (*L. richardsoni*). Variation in mitochondrial DNA was not observed among a sample population believed to contain both Pacific and western brook lampreys based on currently used diagnostic characteristics (i.e., caudal region color patterns). Caudal region color patterns of larval lampreys held in the laboratory did not change appreciably through time (sample interval ≈ 6 weeks; sampling events ≤ 31). Only two of 31 lampreys held in the laboratory metamorphosed allowing positive species identification based on dentition patterns. These lampreys were positively identified as Pacific lamprey and had been consistently identified as Pacific lamprey during larval periods. One individual was inconsistently identified based on caudal region color patterns during its observational period (83% Pacific lamprey; 17% western brook lamprey). Twenty-eight individuals were consistently identified throughout the observational period. Mortality and lack of growth and metamorphosis in the laboratory indicate that requirements for development of these species are poorly understood.

Introduction

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; NPPC 1994; Close et al. 1995), has stimulated interest in recovery actions within the Columbia River Basin for this and other lamprey species.

Due to the sedentary nature and protracted duration of a lamprey's larval life stage (Hardisty and Potter 1971), a significant amount of information regarding distribution patterns, abundance, and species composition may be obtained through surveys of larval lampreys. Larval lampreys may be easily censused through electrofishing surveys (Richards et al. 1982); however, information regarding species composition and abundance require accurate species identification.

Species identification of larval lampreys has typically been achieved through examination of color patterns (Hubbs and Potter 1971). Richards et al. (1982) examined larval color patterns of four species of lampreys found in British Columbia Canada. Among the species described, Pacific lampreys (*Lampetra tridentata*) and western brook lampreys (*L. richardsoni*) are currently distributed within the Columbia River Basin. Based on their findings, Richards et al. (1982) concluded that larval Pacific and western brook lampreys could be distinguished based on color patterns of the caudal area. However, color patterns varied from previously published descriptions of Pacific and western brook lampreys (Pletcher 1963 *in* Richards et al. 1982; Vladykov and Follett 1965; Vladykov and Kott 1976 *in* Richards et al. 1982). Also, regional fishery managers have indicated difficulty in using this technique for species identification (Columbia River Basin Lamprey Technical Workgroup 2004). Difficulty associated with using

color patterns as a means of species identification may result from variation in color patterns associated with environmental conditions (Moyle and Cech 1996). Therefore, regional examination of this technique may be required prior to implementation.

The purpose of this study was to examine the efficacy of using color patterns of larval Pacific and western brook lampreys for species identification. Specifically we examined variation in color patterns through time and the accuracy of species identification based on larval color patterns.

Methods

In the fall of 1999, larval lampreys were collected from five locations in the Columbia River Basin. CCollection sites included: Red River (HUC 17060305), Entiat River (HUC 17020010), John Day River (HUC 17070202), Walla Walla River (HUC 17070102), and Cedar Creek (HUC 17080002). 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. 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 color patterns (Richards et al. 1982). Fifty larvae 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 laboratory, and sampled at intervals of approximately six-weeks (Appendix 2; Appendix 3). 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 color patterns (Richards et al. 1982), and 2) if there is a change in color patterns over time, specifically with regard to diagnostic characteristics of these species. This process was repeated until individuals metamorphosed, at which time species identification could be confirmed based on dentition (Vladykov and Follett 1965; Eddy and Underhill 1978), or individuals died, at which time tissue samples were collected for genetic analysis.

Results

Of the larvae sacrificed for genetic analysis, 42 were identified as Pacific lampreys and eight were identified as western brook lampreys based on color patterns of the caudal region (Richards et al. 1982; Appendix 1). Researchers at the University of Idaho did not observe genetic variation indicative of multiple species among the samples that they examined.

The 31 larvae held at the laboratory for repeated examination were sampled up to 31 times (some individuals less due to mortality) (Appendix 2). In the case of mortality, tissue samples were taken for later genetic analysis. 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. Of the two individuals for which species

identification could be confirmed, one lamprey was originally collected from the Red River and metamorphosis and species confirmation was made after 11 sampling events, and the other lamprey was from the Entiat River and metamorphosis and species confirmation was made after 18 sampling events. These individuals were consistently identified as Pacific lampreys (100% of the sampling events). Species identification was also consistent for the 28 lampreys that did not metamorphose. Only one individual was identified inconsistently (Pacific lamprey in 83% of the sampling events; western brook lamprey in 17% of the sampling events). This individual was from Cedar Creek and was sampled 12 times prior to mortality (Appendix 2). After a maximum of 31 sampling events remaining lampreys were sacrificed and tissue samples were archived due to project constraints.

Throughout the duration of observation, mean length and mass of individuals varied greatly (Figure 2; Figure 3); however, for the majority of individuals the net change in length and mass was negative (Appendix 3).

Discussion

The inability to separate Columbia River Basin lampreys based on genetic analysis 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 (*L. ayresi*); however, they were unable to genetically distinguish western brook and river lampreys from each other. Based on their data, Docker et al. (1999) suggests western brook and river lampreys diverged within the past 70,000 years. Although river lampreys have not been documented to occur in the Columbia River Basin since 1980 (Bond et al. 1983), their occurrence within the basin can not be ruled out. Therefore, mitochondrial DNA may not be suitable for separation of lamprey species

found in the Columbia River Basin, and other techniques, such as microsatellite analysis, may merit investigation.

Our results indicate that over time there is not a noticeable change in color patterns associated with species identification. All but one individual were consistently identified based on color patterns of the caudal region suggesting that color patterns did not change through time. However, only two individuals metamorphosed throughout the sampling duration. Although these individuals were accurately identified as Pacific lamprey, confirmation of this technique based on such a small sample size may be premature.

The low occurrence of metamorphosis and high incidence of mortality (individuals sampled less than 31 times; Appendix 2, Appendix 3) suggest that conditions in our laboratory were not adequate for development of Pacific and western brook lampreys. Examination of sea lamprey (*Petromyzon marinus*) metamorphosis has indicated that individual size, temperature, and food availability may all affect the onset of metamorphosis (Holmes and Youson 1994). The minimum size of landlocked sea lampreys prior to metamorphosis was observed to be 120 mm and 3.0 g with a condition factor of 1.5, where condition factor was calculated as:

$$CF = (\text{Mass} / \text{Length}^3) \cdot (10^6)$$

In this experiment size varied greatly through time; however, at least some individuals met the size requirements outlined by Holmes and Youson (1994) for metamorphosis of landlocked sea lampreys through most of the observational period. The mean length and mass of lampreys in this experiment tended to overlap the values of 120 mm and 3.0 g (Figure 2; Figure 3); however, condition factor was generally lower than 1.5 (Figure 4). Based on these observation it may be predicted that a larger portion of our sample should have initiated metamorphosis. A number of hypotheses for why we did not observe a higher proportion of

individuals metamorphosing may be developed based on these findings and the results of other experiments.

First, the size requirements for metamorphosis may differ among species, and minimum size requirements for both Pacific and western brook lamprey metamorphosis may be greater than those of sea lampreys. General trends suggest that brook lampreys attain a greater size prior to metamorphosis, presumably to develop energy reserves sufficient to equip them for their non-trophic adult phase (Hardisty and Potter 1971). However, differences in size at metamorphosis among parasitic species have not been empirically examined.

Second, laboratory thermal regime may have also played a role in the lack of observed metamorphosis. Holmes and Youson (1994) observed significantly more sea lampreys metamorphosing under conditions of seasonally variable temperature (approximately 5° C to 21° C) than at a constant temperature of 21° C over a nine month period. They hypothesize that the decreased metabolic demands at low temperatures during the winter may allow for protein synthesis and lipogenesis. Another possible explanation was that lampreys that did not experience seasonal thermal variation lacked ecological cues essential for the onset of metamorphosis (Holmes and Youson 1994). Although seasonal variation was experienced in the laboratory in this study, the increase in temperature experienced in the spring and summer may not have been of sufficient magnitude to trigger the physiological processes necessary for metamorphosis.

Finally, the diet supplied to the lampreys in this study may not have been adequate for development of sufficient lipid reserves. The hypothesis that metamorphosis is partially dependent on lamprey size, including condition factor, suggests that metamorphosis may be delayed indefinitely in the absence of adequate food sources. For this study we provided food at

a similar rate to other studies (e.g., Murdoch et al. 1991, 1992; *in* Holmes and Youson 1994; Youson et al. 1993; Holmes et al. 1994). The specific dietary requirements of the species examined in this study have not been documented; therefore, it is difficult to speculate on the degree to which food availability affected the low rate of metamorphosis.

In conclusion, for the lampreys examined in this study color patterns of larval Pacific and western brook lampreys do not appear to vary temporally. Although species identification was confirmed for two Pacific lampreys consistently identified as Pacific lamprey based on color patterns of the caudal region, this sample size is too small to provide validation for this technique within the Columbia River Basin. Analysis of mitochondrial DNA suggests that the recent divergence of these and other species may require examination of molecular techniques that have not traditionally been used for species identification, such as analysis of microsatellites. This study also indicates that there is a general lack of knowledge regarding the ecological and dietary requirements of these species. Prior to future long-term laboratory studies it may be necessary to refine methods for culturing these species in captivity.

References

Bond, C. E., T. T. Kan, and K. W. Myers. 1983. Notes on the marine life of the river lamprey, *Lampetra ayresi*, in Yaquina Bay, Oregon, and the Columbia River estuary. Fishery Bulletin 81:165-167.

Close, D. A., M. Fitzpatrick, H. Li, B. Parker, D. Hatch, and G. James. 1995. Status report of the Pacific lamprey (*Lampetra tridentata*) in the Columbia River Basin. Report of Oregon State University, Columbia River Inter-Tribal Fish Commission, and Confederated Tribes of the Umatilla Indian Reservation to U.S. Department of Energy, Portland, Oregon.

Columbia River Basin Lamprey Technical Workgroup. 2004. Columbia River Basin Lamprey Technical Workgroup Minutes, March 9, 2004. Available: http://columbiariver.fws.gov/lampreywg/docs/March%209%20Notes.pdf (April 2004).

Docker, M. F., J. H. Youson, R. J. Beamish, and R. H. Devlin. 1999. Phylogeny of the lamprey genus *Lampetra* inferred from mitochondrial cytochrome *b* and ND3 gene sequences. Canadian Journal of Fisheries and Aquatic Sciences 56:2340-2349.

Eddy, S., and J. C. Underhill. 1978. How to know the freshwater fishes, 3rd edition. Wm. C. Brown Company Publishers, Dubuque, Iowa.

Hardisty, M. W., and I. C. Potter. 1971. The behaviour, ecology, and growth of larval lampreys. Pages 85-126 *in* M. W. Hardisty and I. C. Potter, editors. The biology of lampreys, volume 1. Academic Press, New York, New York.

Holmes, J. A., and J. H. Youson. 1994. Fall condition factor and temperature influence the incidence of metamorphosis in sea lampreys, *Petromyzon marinus*. Canadian Journal of Zoology 72:1134-1140.

Hubbs, C. L., and I. C. Potter. 1971. Distribution, phylogeny and taxonomy, p. 1-66. In: The Biology of Lampreys, Volume 1. M. W. Hardisty and I. C. Potter (eds.). Academic Press, New York, New York.

Kan, T. T. 1975. Systematics, variation, distribution, and biology of lampreys of the genus *Lampetra* in Oregon. Doctoral thesis. Oregon State University, Corvallis.

Moyle, P. B., and J. J. Cech, Jr. 1996. Fishes: An introduction to ichthyology, 3rd edition. Prentice Hall, Upper Saddle River, New Jersey.

Murdoch, S. P., F. W. H. Beamish, and M. F. Docker. 1991. Laboratory study of growth and interspecific competition in larval lampreys. Transactions of the American Fisheries Society 120:653-656.

Murdoch, S. P., M. F. Docker, and F. W. H. Beamish. 1992. Effect of density and individual variation on growth of sea lamprey (*Petromyzon marinus*) larvae in the laboratory. Canadian Journal of Zoology 70:184-188.

NPPC (Northwest Power Planning Council). 1994. Columbia Basin Fish and Wildlife Program. Portland, Oregon.

Pletcher, F. T. 1963. The life history and distribution of lampreys in the Salmon and certain other rivers in British Columbia, Canada. Unpubl. master's thesis.

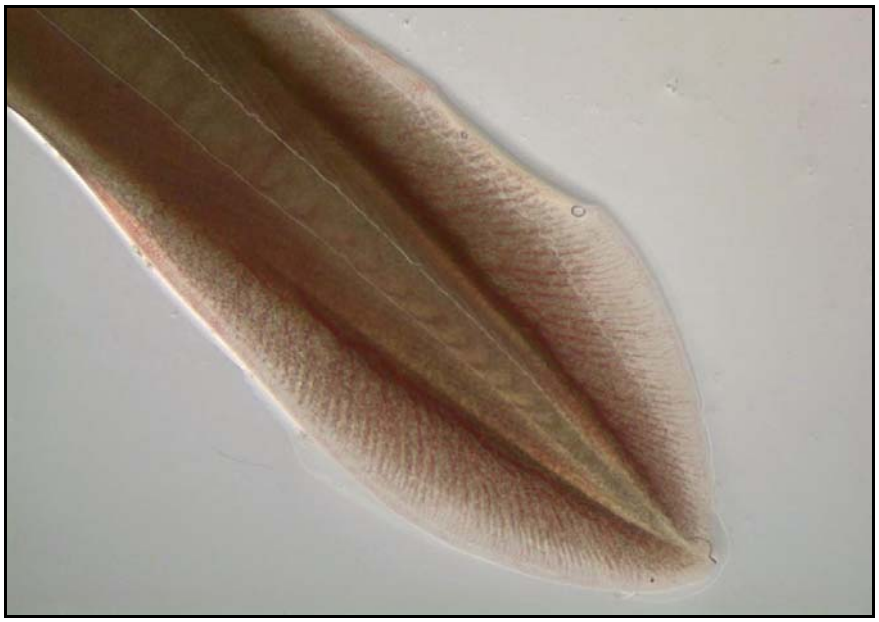
Richards, J. E., R. J. Beamish, and F. W. H. Beamish. 1982. Descriptions and keys for ammocoetes of lampreys from British Columbia, Canada. Canadian Journal of Fisheries and Aquatic Sciences 39:1484-1495.

Vladykov, V. D., and E. Kott. 1976. A second nonparasitic species of *entosphenus* Gill, 1962 (Petromyzonidae) from Klamath River system, California. Canadian Journal of Zoology 54:974-989.

Vladykov, V. D., and W. I. Follett. 1965. *Lampetra richardsoni*, a new nonparasitic species of lamprey (Petromyzonidae) from western North America. Journal of the Fisheries Research Board of Canada 22:139-158.

Youson, J. H., J. A. Holmes, J. A. Gurchardi, J. G. Seelye, R. E. Beaver, J. E. Gersmehl, S. A. Sower, and F. W. H. Beamish. 1993. Importance of condition factor and the influence of water temperature and photoperiod on metamorphosis of sea lampreys, *Petromyzon marinus*. Canadian Journal of Fisheries and Aquatic Sciences 50:2488-2456.

(a)



(b)

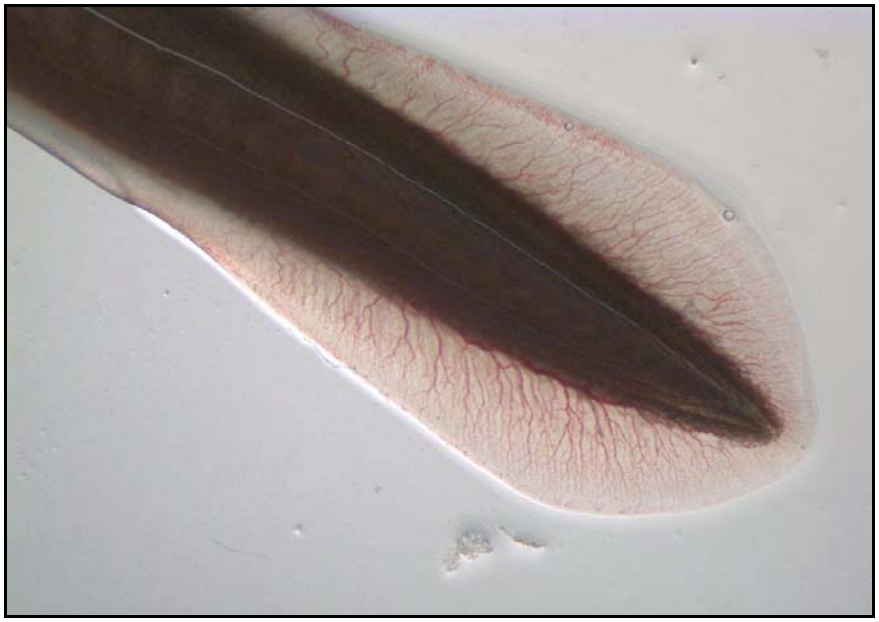


Figure 1: Examples of digital images of caudal region of: (a) Pacific lamprey; characterized by light pigmentation along the caudal ridge, and (b) western brook lamprey; characterized by dark, even pigmentation along the caudal ridge (Richards et al. 1982).

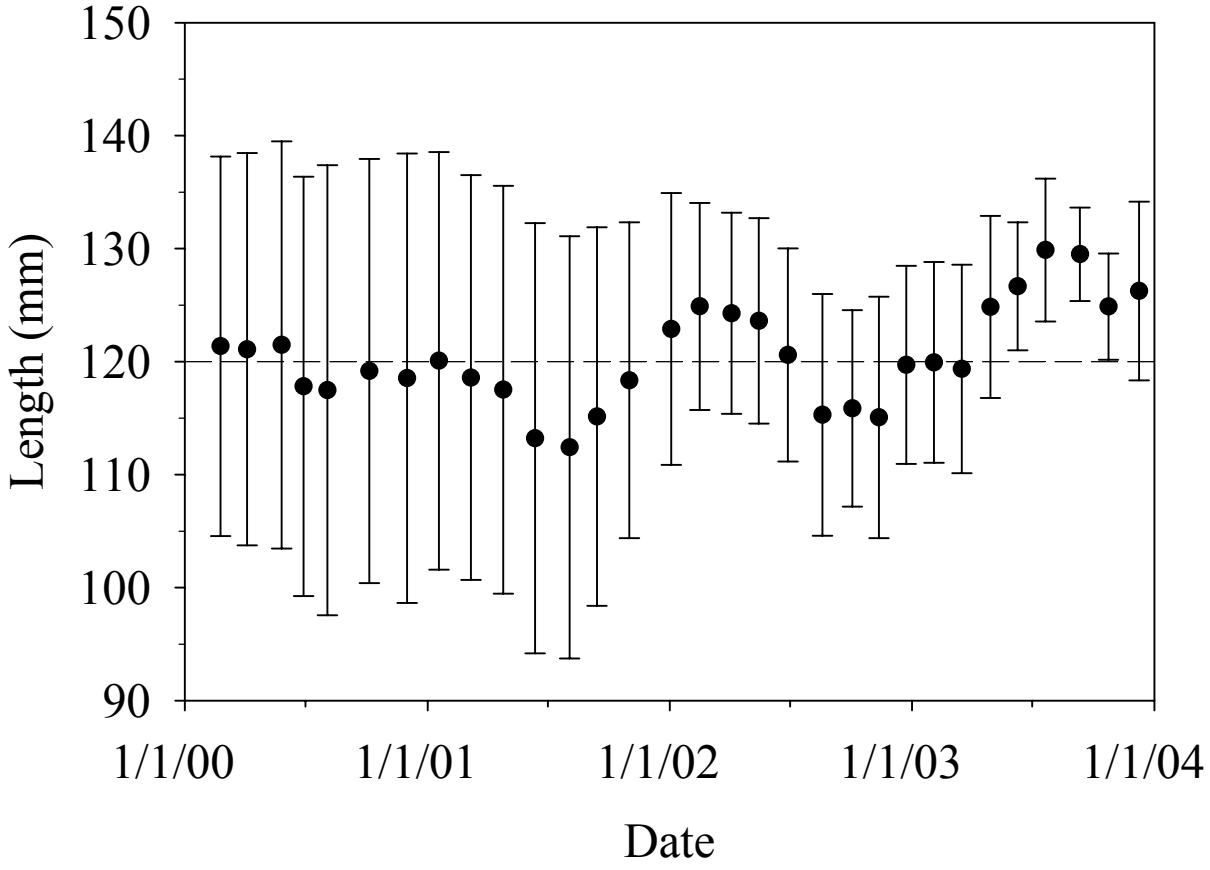


Figure 2: Mean length (mm ± 1 SD) of larval lampreys throughout the duration of observation. A reference line at 120 mm indicates the minimum length for sea lamprey metamorphosis (Holmes and Youson 1994).

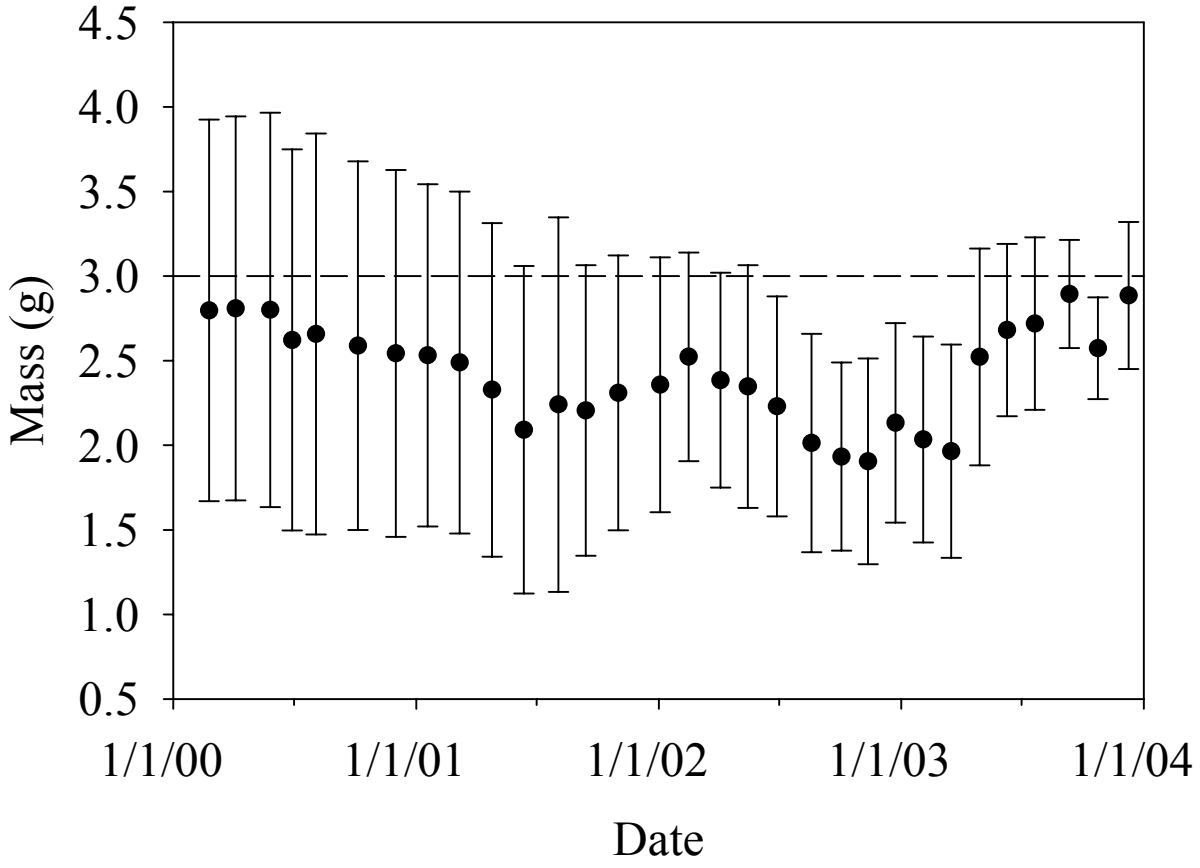


Figure 3: Mean mass (g ± 1 SD) of larval lampreys throughout the duration of observation. A reference line at 3.0 g indicates the minim mass for sea lamprey metamorphosis (Holmes and Youson 1994).

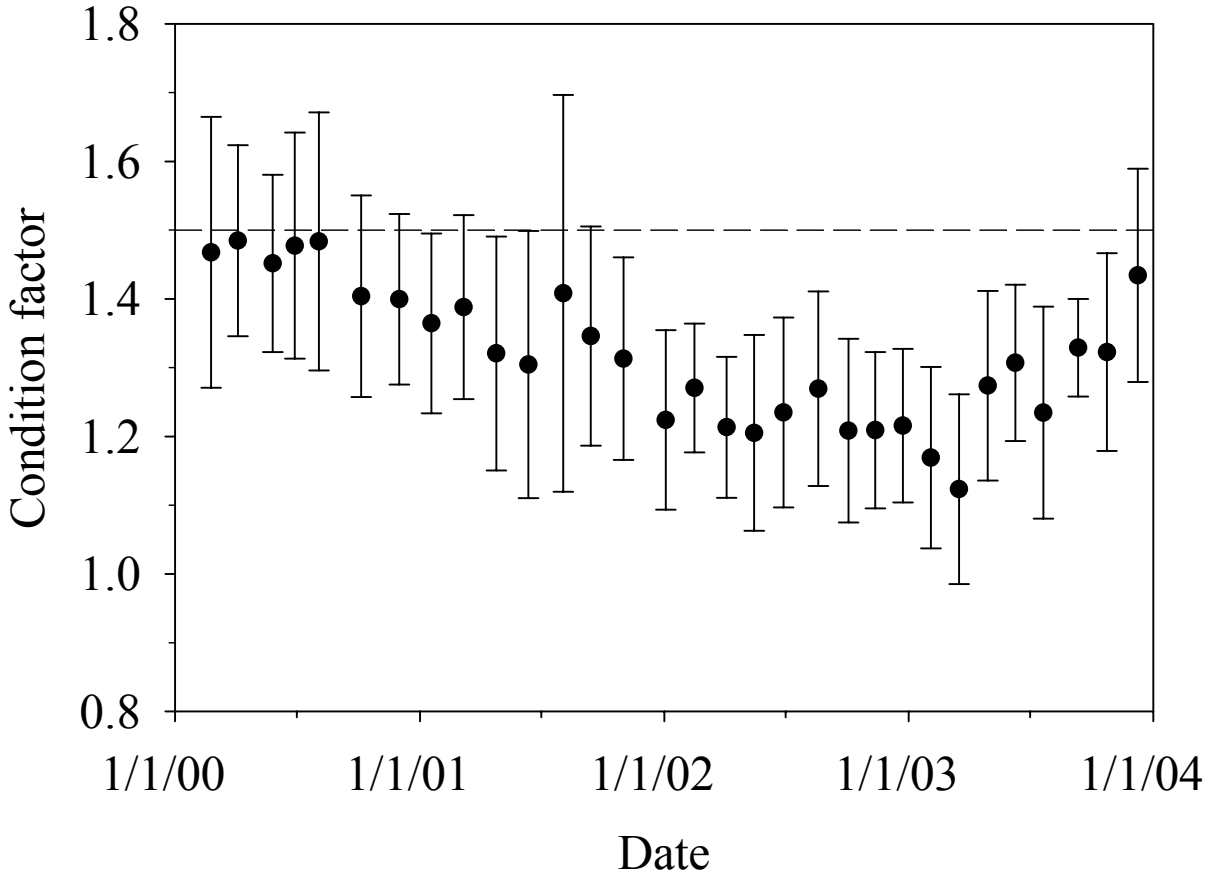


Figure 4: Mean condition factor (± 1 SD) of larval lampreys throughout the duration of observation. A reference line at 1.5 indicates the minim condition factor for sea lamprey metamorphosis (Holmes and Youson 1994).

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

Lamprey ID	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: Lamprey identification number, collection location (ENT=Entiat River, JDW=John Day/Walla Walla Rivers, RED=Red River, and CED=Cedar Creek), the number of times each individual was sampled (*N*), percent of sampling events individual was identified as Pacific lamprey and western brook lamprey, and species confirmation based on morphology of metamorphosed individual.

Lamprey ID	Collection location	*N*	Percent Pacific lamprey	Percent western brook lamprey	Species confirmation
51	CED	7	100	0	.
52	CED	11	100	0	.
53	CED	4	100	0	.
54	CED	11	100	0	.
55	CED	5	100	0	.
56	CED	13	100	0	.
57	CED	12	83	17	.
58	CED	14	100	0	.
59	CED	12	100	0	.
60	RED	11	100	0	Pacific lamprey
61	RED	31	100	0	.
62	RED	31	100	0	.
63	RED	31	100	0	.
64	RED	6	100	0	.
65	RED	31	100	0	.
66	ENT	31	100	0	.
67	ENT	31	100	0	.
68	ENT	13	100	0	.
69	ENT	18	100	0	Pacific lamprey
70	ENT	31	100	0	.
71	ENT	26	100	0	.
72	ENT	31	100	0	.
73	ENT	25	100	0	.
74	JDW	20	100	0	.
75	JDW	27	0	100	.
76	JDW	27	0	100	.
77	JDW	15	0	100	.
78	JDW	27	100	0	.
79	JDW	22	100	0	.
80	JDW	27	100	0	.
81	JDW	20	0	100	.

Appendix 3: Lamprey identification number, date of initial and final sampling event, the number of times each individual was sampled (*N*), initial length and mass, and net change in length and mass over the duration of sampling for larval lampreys held in the laboratory for repeated sampling.

Lamprey ID	Initial sample	Final sample	*N*	Initial length (mm)	Initial mass (g)	Net change in length (mm)	Net change in mass (g)
51	02/24/2000	12/01/2000	7	101	1.537	-27	-1.032
52	02/24/2000	06/12/2001	11	124	2.566	-8	-1.819
53	02/24/2000	06/28/2000	4	96	1.229	-39	-0.377
54	02/24/2000	06/12/2001	11	106	1.817	-12	-1.410
55	02/24/2000	08/03/2000	5	86	0.965	-20	-0.392
56	02/24/2000	09/13/2001	13	92	0.439	-19	-0.033
57	02/24/2000	08/03/2000	12	90	1.319	-27	-0.825
58	02/24/2000	11/01/2001	14	105	1.818	-24	-1.352
59	02/24/2000	08/03/2000	12	102	1.353	-9	-0.931
60	02/24/2000	06/12/2001	11	141	4.425	-10	-1.874
61	02/24/2000	12/09/2003	31	139	4.075	-1	-1.321
62	02/24/2000	12/09/2003	31	134	3.339	-14	+0.186
63	02/24/2000	12/09/2003	31	134	3.525	-6	-1.171
64	02/24/2000	10/05/2000	6	145	4.661	-12	-0.448
65	02/24/2000	12/09/2003	31	147	4.523	+3	-1.307
66	02/24/2000	12/09/2003	31	132	3.630	-8	-0.265
67	02/24/2000	12/09/2003	31	133	3.554	-33	-0.845
68	02/24/2000	09/13/2001	13	120	2.625	-12	-1.907
69	02/24/2000	05/15/2002	18	130	3.411	-18	-1.811
70	02/24/2000	12/09/2003	31	135	3.483	-26	-1.074
71	02/24/2000	04/29/2003	26	143	4.851	-9	-3.282
72	02/24/2000	12/09/2003	31	125	3.101	-31	-0.348
73	02/24/2000	03/17/2003	25	131	3.217	-22	-2.219
74	02/25/2000	08/19/2002	20	125	3.063	+6	-1.999
75	02/25/2000	06/09/2003	27	121	2.500	+7	+0.133
76	02/25/2000	06/09/2003	27	118	2.443	-31	+0.409
77	02/25/2000	01/03/2002	15	123	2.462	+1	-1.583
78	02/25/2000	06/09/2003	27	122	2.817	-38	-0.501
79	02/25/2000	11/12/2002	22	127	3.107	+9	-2.315
80	02/25/2000	06/09/2003	27	116	2.409	-26	+0.021
81	02/25/2000	08/19/2002	20	119	2.460	-27	-1.418

Chapter 3:

Morphometric discrimination of early life stage Pacific lampreys (*Lampetra tridentata*) and western brook lampreys (*L. richardsoni*) from the Columbia River Basin

Abstract

The effectiveness of morphometric and meristic characteristics for taxonomic discrimination of Pacific lampreys (*Lampetra tridentata*) and western brook lampreys (*L. richardsoni*) during embryological, pro-larval, and early larval stages (i.e., age class 1) were examined. Mean chorion diameter increased with time from fertilization to hatch and was significantly greater for Pacific lampreys than for western brook lampreys at 1, 8, and 15 days post-fertilization. Pacific lamprey larvae had significantly more trunk myomeres than western brook lampreys; however, trunk myomere numbers were highly variable within species and deviated from previously published data. Multivariate examinations of pro-larval and larval Pacific lampreys (7.2 mm to 31.8 mm; standard length) and western brook lampreys (6.6 mm to 25.9 mm) were conducted based on standard length and truss element lengths established from eight homologous landmarks. Principal components analysis indicated allometric relationships among the morphometric characteristics examined. Changes in body shape were indicated by groupings of morphometric characteristics associated with body regions (e.g., oral hood, branchial region, trunk region, and tail region). Discriminant function analysis using morphometric characteristics was successful in classifying a large proportion (> 90%) of the lampreys sampled.

Introduction

Documenting the distribution and abundance of individuals can provide information regarding habitat requirements, population dynamics, and community structure, as well as provide the basis for conservation and management actions. Because fishery census data often rely on the ability to accurately identify individuals to the species level, many aspects of a fish's biology have been used for species discrimination. Taxonomic descriptions most often rely on morphological measurements, meristic traits, anatomical characteristics, color patterns, karyotypes, and biochemical characteristics (Moyle and Cech 1996). Of these, morphological characteristics such as morphometrics, meristics, and color patterns continue to be used extensively for taxonomic descriptions (Strauss and Bond 1990).

Lampreys pose an interesting problem for morphological methods of species description and identification. Species discrimination of juvenile and adult lampreys (i.e., post-metamorphic) is most often accomplished based on dentition pattern (Hubbs and Potter 1971) and overall size of individuals, as in the case of closely related or satellite species (Salewski 2003). However, larval lampreys lack many of the features commonly used for morphological descriptions (Strauss and Bond 1990). Univariate morphometrics based on body proportions often overlap (e.g., Richards et al. 1982), and color patterns, which are often used for describing larval lampreys (Hubbs and Potter 1971), may vary in relation to environmental conditions and individual age (Moyle and Cech 1996).

Lampreys have a protracted freshwater larval stage of several years, during which time they live burrowed in soft substrate of lotic (Hardisty and Potter 1971a), or occasionally lentic (Russell et al. 1987), systems. After metamorphosis lampreys adopt a species-specific pattern of either a migratory or a resident life history phase. Migratory species move into oceanic,

estuarine, or lacustrine systems where they prey on a variety of fishes and other animals (Hardisty and Potter 1971b) before returning to riverine systems to reproduce. After metamorphosis, resident species do not feed and normally live for less than one year during which time they become sexually mature, spawn, and die (Hardisty and Potter 1971b). While the occurrence of post-metamorphic individuals of resident species may be recorded along with larvae, the movements and feeding behavior of migratory species make it difficult to include these individuals when censusing populations (Richards et al. 1982). Based on these life history patterns, there are several aspects of the larval biology of lampreys that make them particularly valuable for population censusing. Larval lampreys are easily collected from riverine systems where they burrow in soft substrates and are relatively inactive (Richards et al. 1982). The protracted duration of the larval stage of lamprey, as compared to the duration of the larval life of other fishes, results in a major component of lamprey populations existing as larvae. Consequently, larval abundance may provide information about recruitment and survival rates, as multiple year classes are represented within the larval population.

Two native species of lampreys have been documented to occur within the Columbia River Basin of northwestern USA and southwestern Canada, the Pacific lamprey (*Lampetra tridentata*), a migratory species, and the western brook lamprey (*L. richardsoni*), a resident species. Past collection records (Bond et al. 1983), published distribution data (Moyle 2002), and the presence of the western brook lamprey (Docker et al. 1999; Salewski 2003) indicate that a third species of lamprey, the river lamprey (*L. ayresi*), potentially inhabits the Columbia River Basin; however, their presence in this basin has not been documented since 1980 (Bond et al. 1983; Oregon State University catalogue numbers OS 007319-21, OS 007370, OS 007545, OS 010920, and OS 010922-4). Aside from their general life history patterns little is known about

the ecology of lampreys in the Columbia River Basin. Dam passage data from hydropower projects along the Columbia River and anecdotal information indicate that Pacific lamprey populations within the Columbia River Basin are in decline (Close et al. 1995). These declines have led to interest in recovery efforts for lampreys in the Columbia River Basin.

Management actions require system-wide quantification of the distribution and abundance of lamprey species inhabiting the Columbia River Basin, but current information is limited to geographically localized areas (e.g., Cochnauer and Claire 2001; Close 2002). Surveys of larval lampreys may provide data necessary to evaluate the status of lamprey populations; however, proper identification techniques will be required for population censuses, especially in areas where lamprey species are sympatric. In a revised description of several lamprey species endemic to Pacific coastal regions, Richards et al. (1982) observed that color patterns could distinguish among larvae of closely related lamprey species in British Columbia, Canada. However, color patterns varied from previously published descriptions of Pacific and western brook lampreys (Pletcher 1963 *in* Richards et al. 1982; Vladykov and Follett 1965; Vladykov and Kott 1976 *in* Richards et al. 1982), and color patters indicative of larger larvae were not developed within the first year of life for western brook and river lampreys (Richards et al. 1982).

Due to the variability in color patterns among populations and age classes, alternative methods for describing and distinguishing lamprey species should be examined, especially with respect to early larval stage individuals that may not have developed characteristic color patterns. Under normal conditions (i.e., population stability), age class 1 individuals (age 0 to 1 year; Gotelli 1995) comprise the single largest age class within a population and therefore may provide an early indicator of population growth or decline. The purpose of this study was to examine

size variation among embryos of Pacific and western brook lampreys, describe the gross morphology of pro-larval and early larval stages of these species, and to examine the ability of multivariate morphometric techniques to discriminate between these species.

Methods

Embryological, pro-larval, and larval material for this project was collected from animals artificially spawned in the laboratory. In the spring of 2001 and of 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). Sub-adult lampreys were defined as individuals that exhibited adult morphology, but were not yet sexually mature. Both species were transported to the Columbia River Research Laboratory, Cook, WA, and held until sexually mature. At the laboratory Pacific lampreys were held in 1400 L circular tanks provided with a continuous inflow of water (approximately 0.3 $L \cdot min^{-1} \cdot kg^{-1}$), and western brook lampreys were held in 38 L aquaria provided with burrowing substrate and a continuous inflow of water (approximately 0.3 L/min). Water provided to all lampreys was from the Little White Salmon River (Skamania County, WA). Water was sand-filtered and heated to simulate seasonal thermal trends at Bonneville Dam (University of Washington 2001). All lampreys 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.

The following procedures were performed in 2001 and 2002 on both species of lampreys examined (see Table 1 for sample sizes and morphometrics of sexually mature lampreys). Once lampreys reached sexual maturity, individuals were anesthetized in 250 mg/L of tricaine methane sulfonate (MS-222) buffered with an equal concentration of sodium bicarbonate and rinsed in

fresh water to remove traces of anesthetic. Female lampreys were positioned over a glass bowl filled with about 2 L of fresh water at the approximate temperature of 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 from all females and males were mixed with a gentle flow of water from a large pipette for 5 min and allowed to rest undisturbed for 30 min to allow fertilization to occur. After 30 min, the 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 a flow-through hatching jar (6.86 L McDonald type). Hatching jars were provided with a continuous inflow (approximately 1.5 L/min) of aerated, sand-filtered, and ultraviolet-sterilized river water maintained at a temperature of 14° C. Hatching jar temperature control was lost for a portion of the incubation period in 2001 due to equipment failure (Pacific lamprey, days 18 to 23 post-fertilization; western brook lamprey, days 13 to 18 post-fertilization), during which time temperature decreased to approximately 10° C.

Individuals were held in hatching jars from fertilization until they had hatched and reached stage 17 (Piavis 1961). Stage 17, classified as a pro-larval stage, is marked by the formation of the cloacal slit, formation of the oral hood, and action of the tail and head allowing for burrowing activity (for a comprehensive descriptions of developmental stages referenced in this study see Piavis 1961). At stage 17, lampreys were transferred to 19 L aquaria and provided with burrowing substrate, a continuous inflow of water (approximately 0.3 L/min), aeration, and a simulated photoperiod (see above). Water temperature in aquaria was adjusted to follow seasonal thermal trends at Bonneville Dam (University of Washington 2001), but was not raised above 15° C during summer months. Once stage 18 was reached, the larval stage, lampreys were

fed a suspension of active yeast and commercial fry feed three times per week. Stage 18 is characterized by the onset of exogenous feeding and differentiation of all systems, except genital (Piavis 1961).

Lampreys were sampled periodically to provide morphometric and meristic information (see Table 2 for sample sizes of embryos and post-hatch individuals used in analyses). Embryos were sampled daily from the time of fertilization until just prior to hatching (approximately 15 days; Figure 1). Post-hatch lampreys were sampled from the time that they reached stage 17 and at various times during the first year of their larval stage (stage 18). Post-hatch individuals sampled over the two-year period (2001 and 2002) were represented by lampreys sampled daily from 12 to 19 days post-hatch, and at approximately 1 month, 1.5 months, 2 months, 6 months and 1 year post-hatch. Post-hatch individuals were not sampled before stage 17, as the morphological characteristics examined were not present (Figure 2; Figure 3). At each sampling event, ten individuals were removed from their holding vessel (flow-through hatching jar or aquaria), anesthetized in 250 mg/L of buffered MS-222, digitized, and preserved in 10% formalin. Digital images were taken 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 make measurements on digitized specimens.

For embryos, the mean chorion diameter (Figure 4) was determined by tracing the chorion circumference and making 180 diameter measurements through the embryo's centroid at 2-degree intervals. For post-hatch individuals, a set of eight homologous landmarks was established (Figure 3; Appendix 1) and landmark coordinates were quantified. The locations of the landmarks defined a two-cell truss network with two appended triangles (Bookstein et al.

1985) (Figure 3; Appendix 1) for which the truss element lengths were calculated (Table 3). The truss network divided body regions into the oral hood (section I), the branchial region (section II), the trunk region (section III), and the tail region (section IV) (Figure 3). Myomeres were more easily resolved from preserved material; therefore, preserved larvae were later digitized using a Spot Insight digital camera mounted to a stereomicroscope and trunk myomeres were counted from digital images. Trunk myomeres included those between the last branchial opening and the anterior edge of the cloacal slit (Vladykov and Follett 1965).

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. Due to substantial deviation from normality, differences in chorion diameter between years were examined using a Wilcoxon two-sample test (Sokal and Rohlf 1995). Differences in chorion diameter were not observed between year classes for Pacific lampreys ($Z = 1.36$, $P = 0.18$) or western brook lampreys ($Z = -1.89$, $P = 0.06$) so data for both years were combined. A power transformation was used to stabilize variance in chorion diameter among species (Kuehl 1994; Sokal and Rohlf 1995). The empirical relationship between the standard deviation and the mean of thetreatment groups indicated a transformation to the power of -2 (Kuehl 1994). A factorial analysis of variance (ANOVA) with species (fixed factor) and days post-fertilization (continuous factor) was used to examine the effect of species and days post-fertilization on the transformed chorion diameter (PROC GLM; SAS Institute 1989b). Planned comparisons (Sokal and Rohlf 1995) of the transformed chorion diameter were made between Pacific and western brook lampreys at 1, 8, and 15 days post-fertilization.

Length measurements, standard length and truss element lengths, were $\log_e$-transformed to stabilize variance caused by variability in size (Jolicoeur 1963; Humphries et al. 1981), then

used for morphometric descriptions of larval lampreys. Principal components analysis was used to examine the relationship among the $\log_e$-transformed length measurements of all larvae (PROC PRINCOMP; SAS Institute 1989a, 1989b; Tabachnick and Fidell 2001). Discriminant analysis was performed on the $\log_e$-transformed length measurements (PROC DISCRIM; SAS Institute 1989a; Tabachnick and Fidell 2001). Both a full model and a reduced model discriminant analysis were examined for their ability to accurately predict group membership (i.e., species). 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 92% of the Pacific lampreys and 88% of the western brook lampreys was used to produce classification functions (Tabachnick and Fidell 2001). A test dataset composed of the remaining animals was used to test the classification functions.

The number of trunk myomeres differed significantly between year classes for Pacific lampreys; therefore, the effects of species and year were included in analyses. A reciprocal transformation (myomer number to the power of –1) was used to stabilize variance among treatments (Kuehl 1994; Sokal and Rohlf 1995). A factorial ANOVA with species (fixed factor), year (fixed factor) and standard length (continuous factor) was used to examine the effect of species, year, and individual size on the transformed number of trunk myomeres (PROC GLM; SAS Institute 1989b). The interaction between species and year was also included in the statistical model. Planned comparisons (Sokal and Rohlf 1995) of the transformed data were made between years for a given species and between species for both years combined.

Results

Over the entire incubation period, mean chorion diameter was greater and more variable for Pacific lampreys (mean ± standard deviation, 1.47 ± 0.11 mm, $N = 320$) than for western

brook lampreys (1.24 ± 0.06 mm, N = 290). The overall ANOVA showed that the factors examined had a significant effect on chorion diameter (Table 4). Species ($F_{1,607}$ = 1697.60, P < 0.0001) and days post-fertilization ($F_{1,607}$ = 314.76, P < 0.0001) had a significant effect on mean chorion diameter. Chorion diameter was significantly greater for Pacific lampreys than for western book lampreys at 1, 8, and 15 days post fertilization (P < 0.0001; Figure 5).

The first two principal components accounted for 97% of the variability in the length measurements, with 93% of the variability accounted for by principal component 1 (PC1). Positive loadings on PC1 were observed for all variables (Table 5), indicating positive correlations between all measured dimensions and PC1, and suggesting PC1 may be a good descriptor of the general size of the animals examined (Bookstein et al. 1985; Bookstein 1991). Although relatively similar in magnitude, slight differences in loadings indicate allometric growth (Bookstein et al. 1985). For principal component 2 (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 oral hood and branchial regions, and large positive loadings were observed for truss elements I, J, K, L, and M, associated with the trunk region (Table 5). Examination of the component scores indicated that multivariate analyses might be successful in classifying species (Figure 6).

The full model discriminant analysis correctly classified 91.7% of the Pacific lampreys and 92.7% of the western brook lampreys in the classification data set (see Table 6 for parameter estimates for the classification functions). When applied to the test data set, the classification functions correctly classified 90.9% of the Pacific lampreys and 85.0% 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 91.0% of the Pacific lampreys and 93.4% of the western brook lampreys in the classification data set. When applied to the test data set, the classification functions correctly classified 90.9% of the Pacific lampreys and 85.0% of the western brook lampreys. For both the full and reduced models, correlations between the discriminant function and the predictors were relatively low with the exception of the predictor ln(M) (Table 7).

A significant interaction was observed between species and year class on the number of trunk myomeres ($F_{1,329} = 7.67$, $P = 0.01$), but the number of trunk myomeres was not affected by the size (i.e., standard length) of the lamprey ($F_{1,329} = 0.69$, $P = 0.41$). For both species, a greater number of trunk myomeres were observed in the 2002 year class than in the 2001 year class; however, the number of trunk myomeres differed significantly between year classes for Pacific lampreys ($t = 6.01$, $P < 0.0001$) but not for western brook lampreys ($t = 1.87$, $P = 0.06$) resulting in the observed interaction. Combining year classes, Pacific lamprey trunk myomeres ranged from 61 to 77 with a mean of 68.1 and western brook lamprey trunk myomeres ranged from 54 to 67 with a mean of 59.9. Although overlap was observed, Pacific lampreys had significantly more trunk myomeres than western brook lampreys ($t = -26.11$, $P < 0.0001$; Figure 7).

Discussion

Difficulties associated with morphological descriptions for taxonomic classification to the species level have been well documented for lampreys (Hubbs and Potter 1971; Richards et al. 1982). Although dentition patterns and overall animal size have been used to discriminate among juvenile and adult lampreys, larval lampreys do not possess characteristic dentition patterns and the protracted duration of the larval stage results in substantial or complete body

size overlap among species. Color patterns have most widely been used for discrimination of larval lampreys found within the Columbia River Basin, but incomplete development of pigmentation and potential geographic variability in color patterns make this technique less useful for very young larvae (i.e., less than one year; Richards et al. 1982). In this study, we found significant differences in morphometric and meristic traits between Pacific and western brook lampreys during embryological and pro-larval and early larval stages, and we were able to predict species grouping based on morphometric traits with a high degree of accuracy.

The chorion diameter of Pacific lampreys was significantly greater than that of western brook lampreys throughout embryological development (Figure 4). A positive relationship was also observed between chorion diameter and time (i.e., days post-fertilization). This relationship could potentially result in size overlap of early stage Pacific lampreys and late stage western brook lampreys embryos; however, under conditions of similar chorion diameter these species would differ greatly with regard to development (Figure 1). Therefore, information on both size and development could be used to distinguish between these species.

The set of morphometrics used in this study was extremely successful in discriminating between pro-larval and larval Pacific and western brook lampreys. Principal components analysis revealed relationships among measurements that are frequently used to describe lamprey body regions, such as the tail region, trunk region, branchial region, and oral hood (e.g., Vladykov and Follett 1965; Hardisty and Potter 1971a). Both the full and reduced model discriminant function had similar predictive abilities and indicated the derived distance from the cloacal slit to the dorsal surface of the lamprey (truss element M) largely separated these species. Compared to the other measurements, the variability in truss element M was low within species over the size range of lampreys examined (Table 3). This coupled with the difference of this

measurement between species likely resulted in the high correlation between ln(M) and the discriminant function (Table 7).

Meristic characteristics derived from external morphology are limited in larval lampreys. Myomere number is the most commonly used meristic characteristic used to describe lampreys (Hubbs and Potter 1971). Pacific lampreys had significantly more trunk myomeres than western brook lampreys; however, overlap between species and differences between year-classes within species indicated variability in this characteristic (Figure 7). Past descriptions of these species indicate variability in the number and range of trunk myomeres across geographic regions. Kan (1975) reported from 61 to 71 trunk myomeres (mean = 66.5) for larval Pacific lampreys from Oregon and neighboring areas, and Richards et al. (1982) observed from 63 to 69 trunk myomeres (mean = 66.1) for larval Pacific lampreys from four rivers in British Columbia, Canada. Vladykov and Follett (1965) described larval western brook lampreys from British Columbia, Canada, and Washington, USA, as having from 57 to 65 trunk myomeres (mean = 60.7), whereas Richards et al. (1982) observed from 58 to 67 trunk myomeres (mean = 62.9) for larval western brook lampreys from British Columbia, Canada. The mean number of trunk myomeres observed in this study was higher for Pacific lampreys (68.1) and lower for western brook lampreys (59.9) than observed in other studies (Vladykov and Follett 1965; Kan 1975; Richards et al. 1982). The magnitude of variation in trunk myomeres observed in this study was greater for both Pacific and western brook lampreys; however, this may be due to the presence of a limited number of extreme observations, as the range of myomeres bracketed by the 10^{th} and 90^{th} percentiles is similar to that observed in other studies (Figure 7).

The ability to collect lampreys from streams during larval stages provides an efficient method of estimating abundance and recruitment. However, in systems where lamprey species

have sympatric distributions, accurate population estimates require effective techniques for identifying species. Color patterns of larval lampreys have been used to separate closely related species, but are less reliable during the very early larval stages (e.g., the first year of life; Richards et al. 1982). The techniques examined in this study were effective in separating Pacific and western brook lampreys during early life stages. These techniques require measurement accuracy not commonly employed during field sampling; therefore, sub-sampling individuals from age class 1 for subsequent laboratory examination could be conducted when species identification is required. Although individuals used in this study were digitized while live, examination and morphometric identification of preserved specimens may be possible. The effects of preservation on morphometrics have been shown to vary according to species, individual size, and preservation technique, and may not be isometric among morphometric characteristics within an individual (Morkert and Bergstedt 1990; Sagnes 1997; Bayer and Counihan 2001); therefore, documentation of multivariate shape change due to preservation would be required prior to using these techniques for identification of preserved material. Further work is needed to determine if the discriminatory morphometric characteristics used in this study are consistent among populations throughout these species distribution (i.e., outside of the Columbia River Basin), if this technique may be used for older larvae (e.g., greater than one year), and if this technique may be used to discriminate among other closely related species (e.g., the river lamprey).

References

Bayer, J. M., and T. D. Counihan. 2001. Length changes in white sturgeon larvae preserved in ethanol or formaldehyde. Collection Forum 15:57-64.

Bond, C. E., T. T. Kan, and K. W. Myers. 1983. Notes on the marine life of the river lamprey, *Lampetra ayresi*, in Yaquina Bay, Oregon, and the Columbia River estuary. Fishery Bulletin 81:165-167.

Bookstein, F. L. 1991. Morphometric tools for landmark data: geometry and biology. Cambridge University Press, New York, New York.

Bookstein, F., B. Chernoff, R. Elder, J. Humphries, G. Smith, and R. Strauss. 1985. Morphometrics in evolutionary biology. The Academy of Natural Sciences of Philadelphia, Special Publication 15, Philadelphia, Pennsylvania.

Cochnauer, T., and C. Claire. 2001. Evaluate status of Pacific lamprey in the Clearwater River drainage, Idaho. Report of Idaho Department of Fish and Game to U.S. Department of Energy, Portland, Oregon.

Close, D. A. 2002. Pacific lamprey research and restoration project. Report of Confederated Tribes of the Umatilla Indian Reservation to U.S. Department of Energy, Portland, Oregon

Close, D. A., M. Fitzpatrick, H. Li, B. Parker, D. Hatch, and G. James. 1995. Status report of the Pacific lamprey (*Lampetra tridentata*) in the Columbia River Basin. Report of Oregon State University, Columbia River Inter-Tribal Fish Commission, and Confederated Tribes of the Umatilla Indian Reservation to U.S. Department of Energy, Portland, Oregon.

Docker, M. F., J. H. Youson, R. J. Beamish, and R. H. Devlin. 1999. Phylogeny of the lamprey genus *Lampetra* inferred from mitochondrial cytochrome *b* and ND3 gene sequences. Canadian Journal of Fisheries and Aquatic Sciences 56:2340-2349.

Gotelli, N. J. 1995. A primer of ecology. Sinauer Associates, Inc., Sunderland, Massachusetts.

Hardisty, M. W., and I. C. Potter. 1971a. The behaviour, ecology and growth of larval lampreys, p. 85-125. In: The Biology of Lampreys, Volume 1. M. W. Hardisty and I. C. Potter (eds.). Academic Press, New York, New York.

Hardisty, M. W., and I. C. Potter. 1971b. The general biology of adult lampreys, p. 127-206. In: The Biology of Lampreys, Volume 1. M. W. Hardisty and I. C. Potter (eds.). Academic Press, New York, New York.

Hubbs, C. L., and I. C. Potter. 1971. Distribution, phylogeny and taxonomy, p. 1-66. In: The Biology of Lampreys, Volume 1. M. W. Hardisty and I. C. Potter (eds.). Academic Press, New York, New York.

Humphries, J. M., F. L. Bookstein, B. Chernoff, G. R. Smith, R. L. Elder, and S. G. Poss. 1981. Multivariate discrimination by shape in relation to size. Systematic Zoology 30:291-308.

Jolicoeur, P. 1963. The multivariate generalization of the allometry equation. Biometrics 19:497-499.

Kan, T. T. 1975. Systematics, variation, distribution, and biology of lampreys of the genus *Lampetra* in Oregon. Unpubl. Ph.D. thesis, Oregon State University, Corvallis.

Kuehl, R. O. 1994. Statistical principles of research design and analysis. Duxbury Press, Belmont, California.

Morkert, S. B., and R. A. Bergstedt. 1990. Shrinkage of sea lamprey larvae preserved in formalin. North American Journal of Fisheries Management 10:484-486.

Moyle, P. B. 2002. Inland fishes of California. University of California Press, Berkeley, California.

Moyle, P. B., and J. J. Cech, Jr. 1996. Fishes: An introduction to ichthyology, 3rd edition. Prentice Hall, Upper Saddle River, New Jersey.

Piavis, G. W. 1961. Embryological stages in the sea lamprey and effects of temperature on development. Fishery Bulletin 61:111-143.

Pletcher, F. T. 1963. The life history and distribution of lampreys in the Salmon and certain other rivers in British Columbia, Canada. Unpubl. master's thesis. University of British Columbia, Vancouver.

Richards, J. E., R. J. Beamish, and F. W. H. Beamish. 1982. Descriptions and keys for ammocoetes of lampreys from British Columbia, Canada. Canadian Journal of Fisheries and Aquatic Sciences 39:1484-1495.

Russell, J. E., F. W. H. Beamish, and R. J. Beamish. 1987. Lentic spawning by the Pacific lamprey, Lampetra tridentata. Canadian Journal of Fisheries and Aquatic Sciences 44:476-478.

Sagnes, P. 1997. Potential artifacts in morphometric analyses of fish: effects of formalin preservation on 0+ grayling. Journal of Fish Biology 50:910-914.

Salewski, V. 2003. Satellite species in lampreys: a worldwide trend for ecological speciation in sympatry? Journal of Fish Biology 63:267-279.

SAS Institute. 1989a. SAS/STAT user's guide, version 6, volume 1, 4th edition. SAS Institute, Cary, North Carolina.

SAS Institute. 1989b. SAS/STAT user's guide, version 6, volume 2, 4th edition. SAS Institute, Cary, North Carolina.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry, 3rd edition. W. H. Freeman and Company, New York, New York.

Strauss, R. E., and C. E. Bond. 1990. Taxonomic methods: Morphology, p. 109-140. In: Methods for fish biology. C. B. Schreck, and P. B. Moyle (eds.). American Fisheries Society, Bethesda, Maryland.

Tabachnick, B. G., and L. S. Fidell. 2001. Using multivariate statistics, 4th edition. Allyn and Bacon, Boston, Massachusetts.

University of Washington. 2001. Columbia River DART: Data Access in Real Time. School of Aquatic and Fishery Sciences. Available: www.cbr.washington.edu/dart/dart.html. (2001 - 2002).

Vladykov, V. D., and E. Kott. 1976. A second nonparasitic species of *entosphenus* Gill, 1962 (Petromyzonidae) from Klamath River system, California. Canadian Journal of Zoology 54:974-989.

Vladykov, V. D., and W. I. Follett. 1965. *Lampetra richardsoni*, a new nonparasitic species of lamprey (Petromyzonidae) from western North America. Journal of the Fisheries Research Board of Canada 22:139-158.

Table 1: Mean total length (mm ± SD) and mean mass (g ± SD) of adult Pacific and western brook lampreys artificially spawned to provide material for morphometric and meristic descriptions of embryological, pro-larval, and larval lampreys.

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

Table 2: Sample sizes for Pacific and western brook lampreys used for examining chorion diameter, principal components analysis, discriminant analysis, and examining trunk myomeres.

	Species	
Analysis	Pacific lamprey (*N*)	Western brook lamprey (*N*)
Chorion diameter	320	290
Principal components analysis	156	156
Discriminant analysis (classification data)	143	138
Discriminant analysis (test data)	13	18
Myomere count	176	159

Table 3: Mean, standard deviation (SD), minimum (Min), and maximum (Max) values of measurement lengths (mm) [standard length (SL) and truss elements] used to describe pro-larval and larval Pacific lampreys (*N*=156) and western brook lampreys (*N*=156).

	Pacific lamprey				Western brook lamprey			
Measurement	Mean	SD	Min	Max	Mean	SD	Min	Max
SL	10.407	4.140	7.197	31.785	9.675	4.389	6.645	25.899
A	0.498	0.200	0.195	1.297	0.453	0.234	0.199	1.185
B	0.673	0.266	0.312	1.857	0.610	0.251	0.312	1.508
C	0.706	0.249	0.408	1.866	0.653	0.274	0.371	1.499
D	2.797	1.085	1.639	7.956	2.621	1.123	1.655	6.464
E	2.623	1.096	1.423	7.506	2.453	1.211	1.396	6.494
F	2.791	1.088	1.625	7.850	2.613	1.151	1.629	6.512
G	2.865	1.147	1.654	8.128	2.673	1.248	1.578	6.815
H	0.947	0.258	0.650	2.209	0.868	0.290	0.572	1.788
I	5.924	1.957	4.496	16.153	5.490	2.233	3.784	13.871
J	6.132	1.947	4.414	16.163	5.613	2.213	3.674	14.014
K	6.092	1.946	4.482	16.270	5.603	2.224	3.798	14.048
L	6.087	1.971	4.526	16.260	5.603	2.241	3.749	13.957
M	0.812	0.141	0.590	1.574	0.664	0.177	0.377	1.271
N	1.365	0.975	0.529	6.823	1.308	0.846	0.480	4.470
O	1.275	0.993	0.494	6.724	1.196	0.878	0.445	4.534

Table 4: Results of ANOVA examining the effects of species and days post-fertilization on the mean chorion diameter of Pacific and western brook lampreys.

Source of variation	df	Sum of squares	Mean square	*F*	*P* > *F*
Model	2	6.30	3.15	1024.97	< 0.0001
Error	607	1.86	< 0.01		
Corrected total	609	8.16			

Table 5: Component loadings for principal component 1 and principal component 2.

	Eigenvectors	
Variable	Principal component 1	Principal component 2
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 6: Full and reduced model classification function parameter estimates for Pacific and western brook lamprey pro-larvae and larvae derived from discriminant analysis. Where classification takes the form: $C_j = C_{j0} + C_{j1}X_1 + C_{j2}X_2 + \cdots + C_{jp}X_p$, and group membership is assigned to the group with the highest classification score (Tabachnick and Fidell, 1996).

	Full model		Reduced model	
Variable	Pacific lamprey	Western brook lamprey	Pacific lamprey	Western brook lamprey
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 7: Component loadings for the full model and the reduced model discriminant function.

Variable	Full model discriminant function	Reduced model discriminant function
lnSL	0.112754	.
lnA	0.114105	.
lnB	0.098712	0.099478
lnC	0.103183	0.103984
lnD	0.089568	0.090263
lnE	0.087051	0.087727
lnF	0.089725	0.090421
lnG	0.092344	0.093061
lnH	0.145492	0.146621
lnI	0.137939	.
lnJ	0.155684	0.156892
lnK	0.149964	.
lnL	0.147172	0.148314
lnM	0.421119	0.424387
lnN	0.037812	0.038105
lnO	0.059969	0.060435

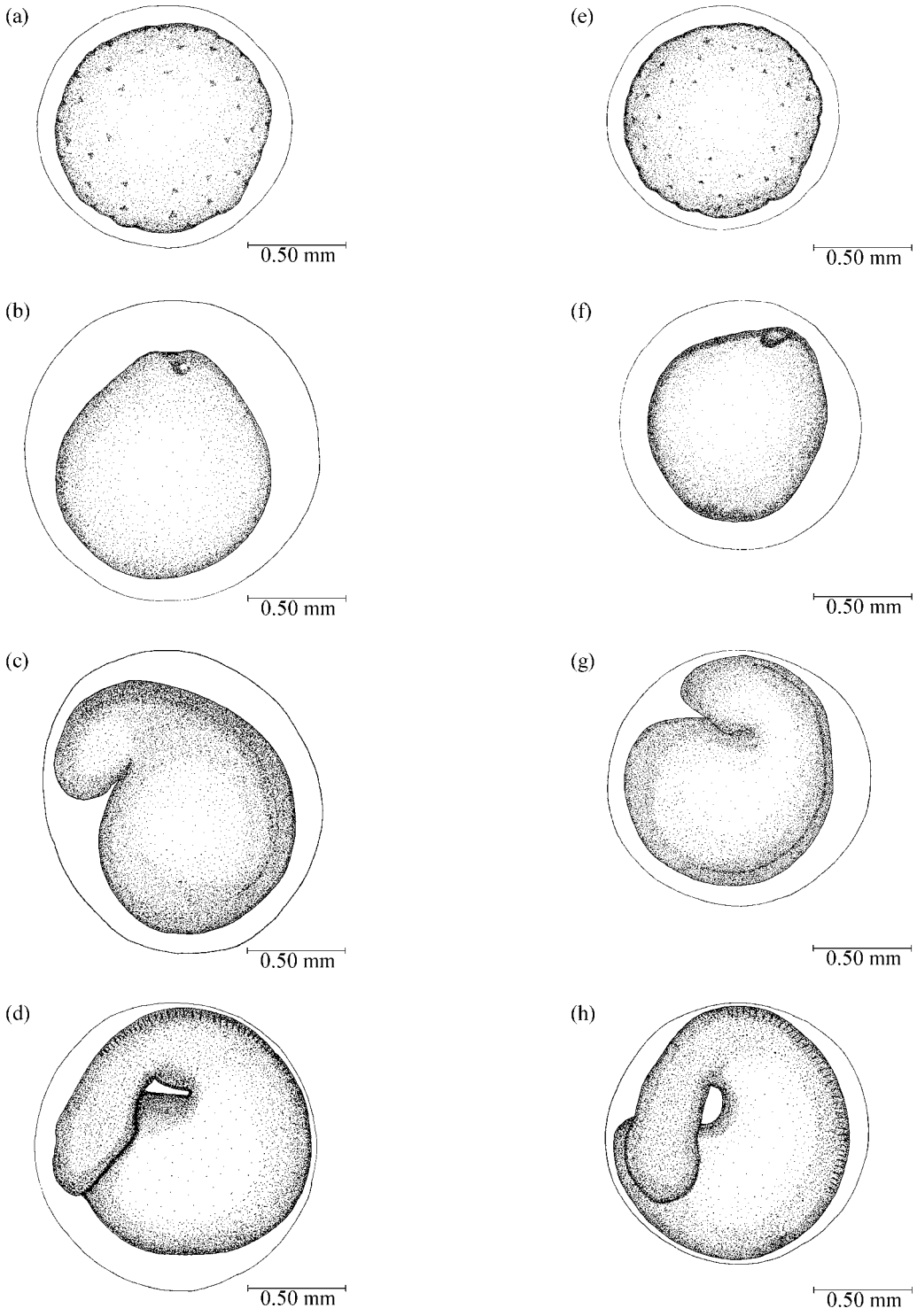


Figure 1: Developmental series of the Pacific lamprey (a-d) and the western brook lamprey (e-h) embryos reared at 14° C. Pacific lampreys at 1 day (a), 7 days (b), 10 days (c), and 14 days (d) post-fertilization. Western brook lampreys at 1 day (e), 7 days (f), 10 days (g), and 14 days (h) post-fertilization.

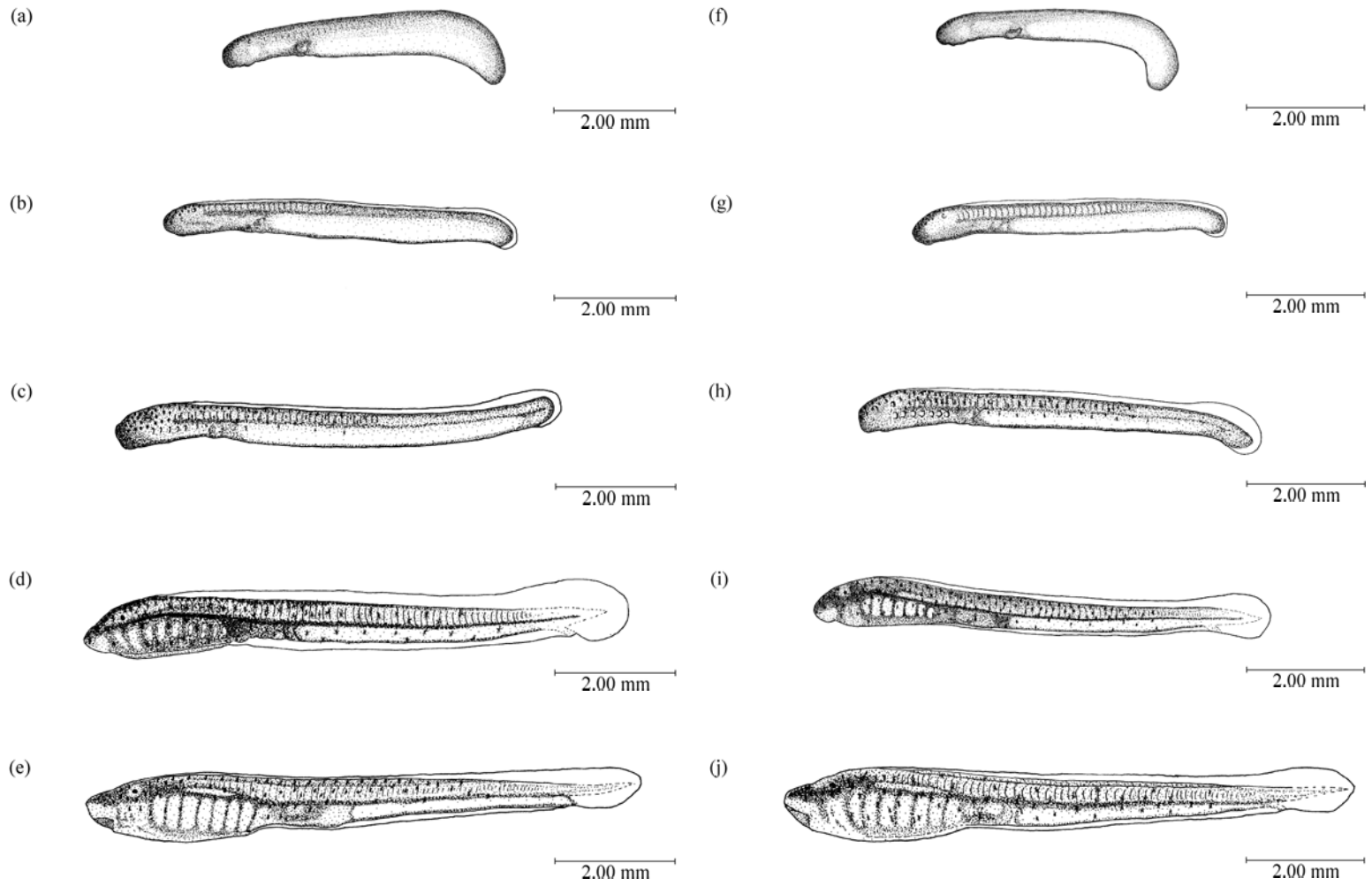


Figure 2: Developmental series of the Pacific lamprey (a-e) and the western brook lamprey (f-j) pro-larvae and larvae. The Pacific lamprey at pro-larval stages 14 (a), 15 (b), 16 (c), and 17 (d), and larval stage 18 (e). The western brook lamprey at pro-larval stages 14 (f), 15 (g), 16 (h), and 17 (i), and larval stage 18 (j).

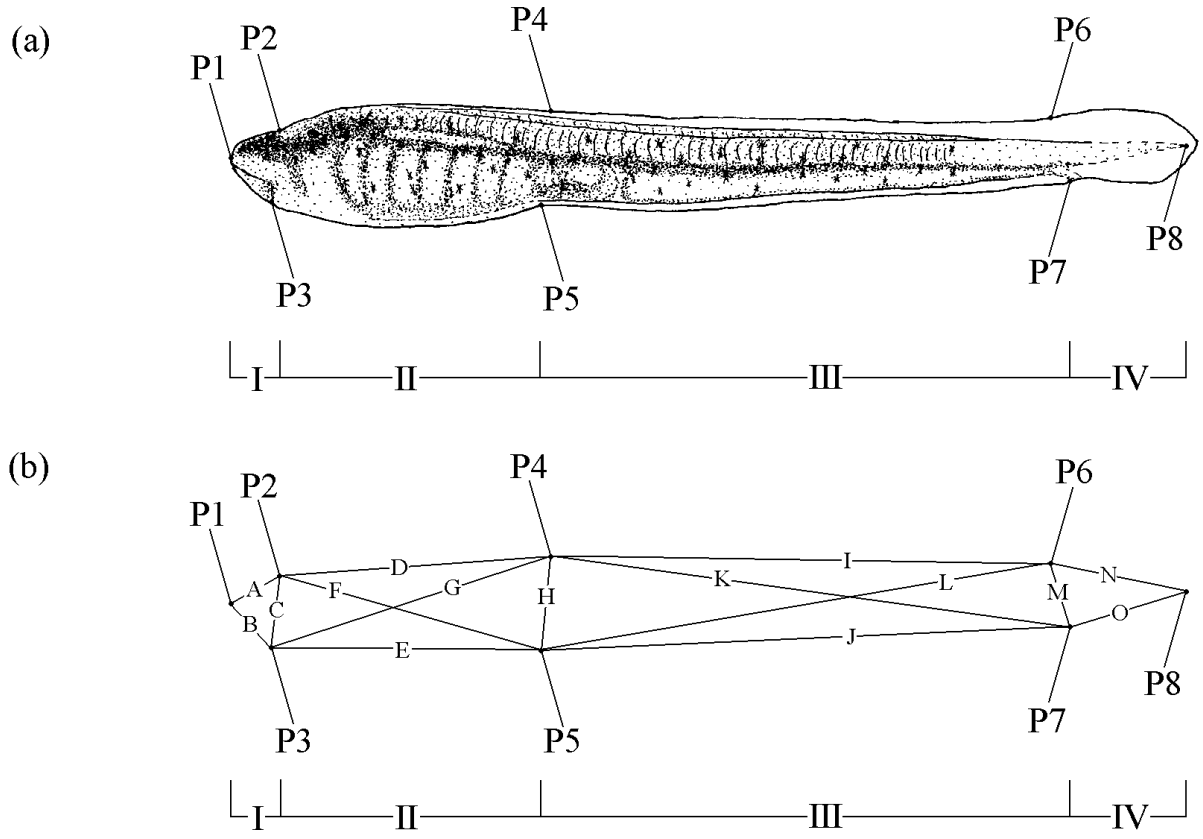


Figure 3: Location of landmarks (P1-P8) (a) and derived truss elements (A-O) (b) used for morphometric analyses of pro-larval and larval lampreys. Truss elements divided lampreys into the oral hood (region I), the branchial region (region II), the trunk region (region III), and the tail region (region IV).

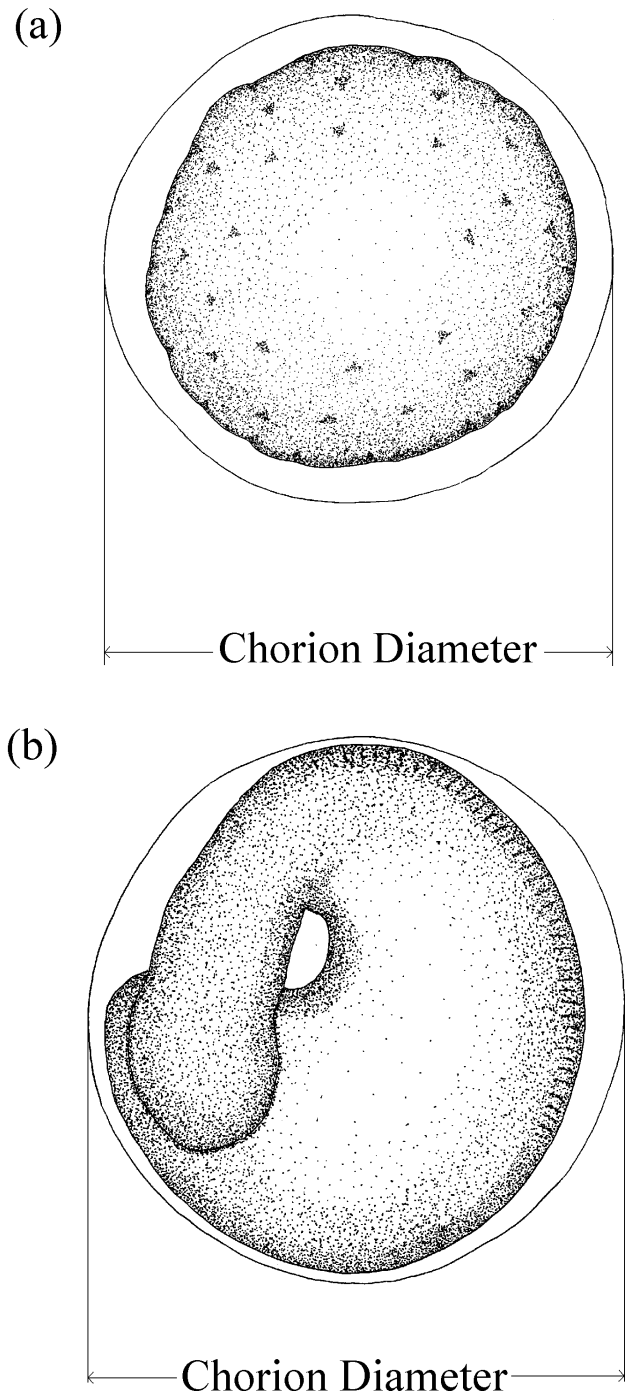


Figure 4: Example of chorion diameter for a day 1 post-fertilization Pacific lamprey (a) and a day 14 post-fertilization western brook lamprey (b) reared at 14° C.

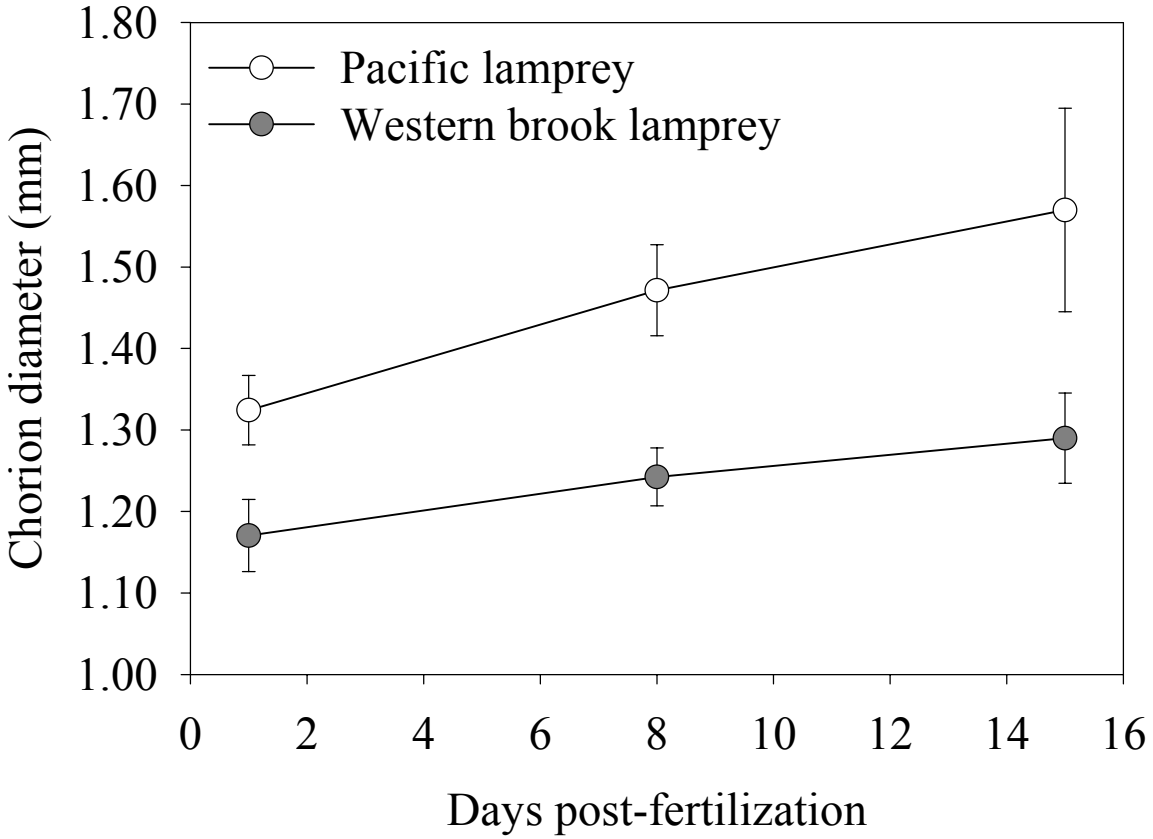


Figure 5: Mean chorion diameter ± 1 standard deviation at 1, 8, and 15 days post-fertilization for Pacific and western brook lampreys reared at 14° C.

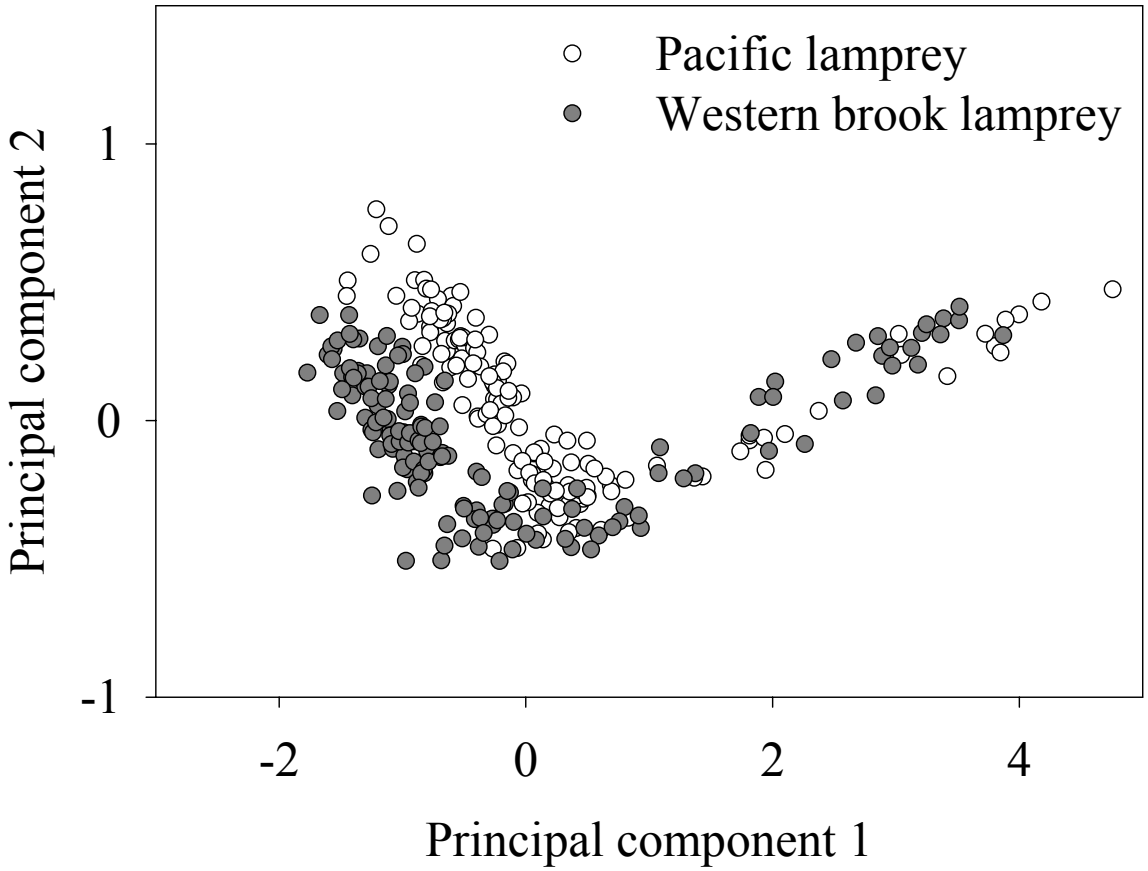


Figure 6: Component scores on principal component 1 and principal component 2 for standard length and truss element lengths measured from pro-larval and larval Pacific and western brook lampreys.

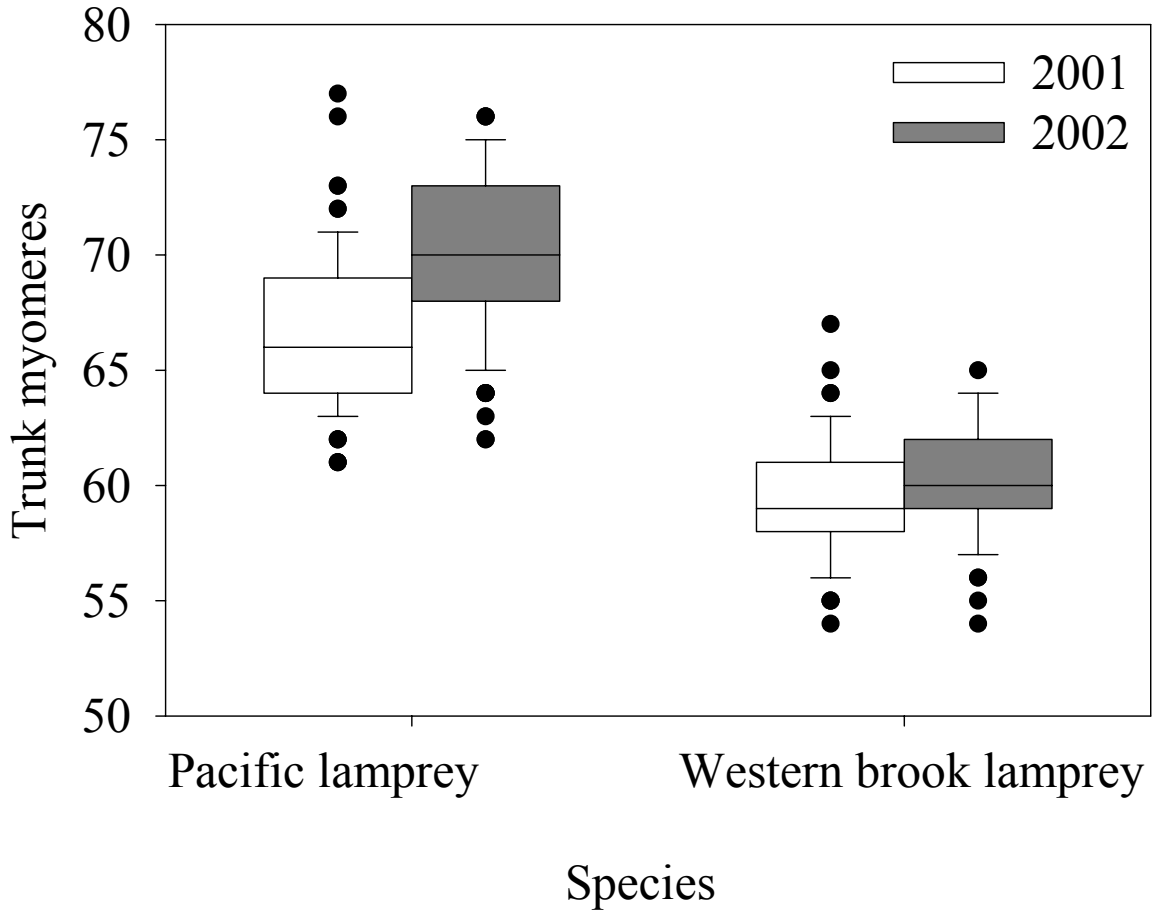


Figure 7: Box plot of the median number of trunk myomeres, 25th and 75th percentiles, and 10th and 90th percentiles for Pacific and western brook lampreys reared in 2001 and 2002. Extreme observations are represented by filled circles (●) outside of the 10th and 90th percentiles.

Appendix 1: Homologous landmarks (P1-P8) and truss elements derived from landmarks used for morphometric examination of pro-larval and larval Pacific lampreys and western brook 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 cloacal slit
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

Chapter 4:

Effects of temperature on survival and development of early life stage Pacific lampreys (*Lampetra tridentata*) and western brook lampreys (*L. richardsoni*)

Abstract

We examined the effects of temperature (10° C, 14° C, 18° C, and 22° C) on survival and development of Pacific lampreys (*Lampetra tridentata*) and western brook lampreys (*L. richardsoni*) during embryological and early larval stages. The temperature for zero development was estimated for each species and the response to temperature was measured as the proportion of individuals surviving to hatch, surviving to the larval stage, and exhibiting abnormalities at the larval stage (i.e., malformations of the body). The estimated temperature for zero development was 4.85° C for Pacific lampreys 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 lampreys than for Pacific lampreys; however, the difference in proportion of individuals surviving was only 0.02. Similarly, survival to the time of hatch was significantly greater than survival to the time of the larval stage, although this represented a difference in proportion of individuals surviving 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. These data provide baseline information on the thermal requirements of early life stage Pacific and western brook lampreys, and will aid in assessment and prediction of suitable spawning and rearing habitats for these species.

Introduction

Due to its pervasive nature, understanding how temperature affects individuals is crucial to understanding the basic ecology of a species. Because of this, the role of temperature in influencing the biology of fishes has been widely demonstrated. For example, water temperature can greatly influence community structure and interactions (Beschta et al. 1987), habitat selection and partitioning (Magnuson et al. 1979; Hofmann and Fischer 2002), physiological rates (Holmes 1990), reproductive timing (Brett 1970), and survival and fitness of individuals (Elliott 1981). While fish taxa can be categorized based on general patterns of thermal tolerance and preference (Hokanson 1977; Magnuson et al. 1979; Elliott 1981), regional adaptations to thermal regimes are not uncommon (Hall et al. 1978; Beschta et al. 1987), and temperature may have differential effects among ontogenetic stages (Magnuson et al. 1979; Elliott 1981; Sanders 1993). Because of this, generalizations about the thermal requirements of a species or groups of species must be broad. More specific information is often needed to elucidate the basic biology of a species or to provide guidance for activities such as determining the suitability of available habitat or developing water quality criteria for species of interest. Addressing these issues often requires species-specific information, and may require examination of multiple life history stages, or those stages that are potentially most sensitive.

Aside from the fundamental influence of temperature on the biology of individuals, populations, and species, current interest in the effects of temperature has been driven by broad scale and regionally localized perturbations to thermal conditions within aquatic systems. Because thermal conditions can greatly influence the quantity and quality of habitat available to aquatic organisms, recent alterations to the thermal regime of the Columbia River (northwestern United States and southwestern Canada), specifically increases in spring and summer

temperatures (Quinn and Adams, 1996), have prompted interest in the habitat requirements and thermal ecology of aquatic species within the Columbia River Basin. Among these are two native species of lampreys, the Pacific lamprey (*Lampetra tridentata*) and the western brook lamprey (*L. richardsoni*). Both Pacific and western brook lampreys have broad geographic distributions within North America, with Pacific lampreys inhabiting Pacific coastal streams from Baja California northward to Alaska and western brook lampreys inhabiting Pacific coastal stream systems from California northward to southeastern Alaska (Moyle 2002).

Like most other fishes, lampreys are obligate poikilotherms and therefore are directly affected by the temperature of their environment (Brett 1970; Elliott 1981). In general, little is known about the thermal requirements of lampreys, with exception of the well-studied sea lamprey (*Petromyzon marinus*) (Piavis 1961; McCauley 1963; Potter and Beamish 1975; Beamish 1980; Manion and Hanson 1980; Swink 1995; Rodríguez-Muñoz et al. 2001). Due to the varied life history patterns and distributions among and within species, lampreys may experience a broad range of thermal conditions. However, the influence of temperature on survival during early life stages is of particular interest. The thermal requirements of early life stages are generally believed to be among the most narrow (Elliott 1981; Rombough 1988), and thermal exposure during early life development may have a potential effect on later life stages (Atkinson 1996); therefore, this period may be a critical determinant of recruitment for many fish populations (Houde 1987).

We examined the effects of four temperatures (10° C, 14° C, 18° C, and 22° C) on survival and development of early life stage Pacific and western brook lampreys. Lampreys were reared in the laboratory under controlled conditions from fertilization until individuals reached the larval stage (Piavis 1961). Lampreys were examined daily and species-specific

development rates, temperatures for zero development, and effective temperatures were calculated. Survival of lampreys was compared between two distinct time periods (95% hatch and fully developed larvae). This allowed us to examine the effects of temperature on survival, changes in survival from the time of hatching to the larval stage, and differences among species. We also examined the effects of temperature on the occurrence of larval abnormalities.

Methods

The following procedures were replicated in 2001 and 2002. In the spring, sub-adult Pacific and western brook lampreys were collected from the wild, transported to the U.S. Geological Survey, Columbia River Research Laboratory, and held until sexually mature (May/June) to provide gametes for experiments. Pacific lampreys were collected from the Columbia River at the Bonneville Dam north shore fish ladder (Skamania County, WA), and western brook lampreys were collected from Gibbons Creek (Clark County, WA) and Yellowhawk Creek (Walla Walla County, WA). Lengths and masses of lampreys used to provide gametes for experiments in 2001 and 2002 are summarized in Table 1. Pacific lampreys were held in 1400 L circular tanks and provided with a continuous inflow of water (approximately 0.3 $L \cdot min^{-1} \cdot kg^{-1}$). Western brook lampreys were held in 38 L aquaria provided with burrowing substrate and a continuous inflow of water (approximately 0.3 L/min). Water provided to all lampreys was from the Little White Salmon River (Skamania County, WA). Water was treated with sand filters and heated to simulate seasonal thermal trends at Bonneville Dam (University of Washington 2001). All lampreys were exposed 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.

Prior to spawning, mature lampreys were anesthetized in 250 mg/L of tricaine methane sulfonate (MS-222) buffered with an equal concentration of sodium bicarbonate and rinsed in fresh water to remove traces of anesthetic. Female lampreys were positioned over a glass bowl filled with approximately 2 L of fresh water at the same temperature as the animal. Eggs were forced out the vent by squeezing the abdomen in a downward motion. This was repeated until blood appeared with the gametes. Sperm was removed from males in a similar fashion. Gametes were mixed with a gentle flow of water from a large pipette for 5 min and allowed to rest undisturbed for 30 min to allow fertilization to occur. After 30 min the fertilized eggs were divided into four glass bowls and the water temperature of each bowl was gradually adjusted through the addition of cool or warm water until the target temperatures of 10° C, 14° C, 18° C, and 22° C were reached (approximately 30 min). Once target temperatures were reached, fertilized eggs were transferred to flow-through hatching jars (6.86 L McDonald type) of the appropriate temperature (one hatching jar per temperature).

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, with the

addition of water treatment by ultraviolet sterilizers. Water supplied to rearing vessels was monitored daily for dissolved oxygen content, pH, and total dissolved gasses (Table 2).

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). 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. 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).

All statistical analyses were performed at $\alpha = 0.05$ using SAS software (SAS version 8.01, SAS Institute Inc., Cary, NC). Less than ten replicates 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 ($DD = E_T \cdot$

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 = proportion of individuals surviving to D_{H95}) and the proportion of individuals surviving to the larval stage (S_L = 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 = 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).

Results

Mean D_{H50} and D_{H95} (Table 3) 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 and 4.97° C for Pacific and western brook lampreys, respectively, and were used to calculate effective temperatures (Table 3).

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 1; Figure 2), 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.

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 3). 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 ($P > 0.05$).

Discussion

Many factors have been used to explain variation in early life stage survival and development of fishes, most notably photoperiod and temperature (Brett 1970). While photoperiod often stimulates the onset of events related to reproduction, such as migratory behavior, temperature often dictates the conditions necessary for embryogenesis. Overall, Pacific and western brook lampreys responded similarly to temperature. Estimated days to 50% and 95% hatch were very consistent among species (Table 3), as were the predicted temperatures for zero development. And 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 this difference is questionable. The scale at which we examined temperature in this experiment did not allow us to isolate the temperature at which survival was maximized during early life stages of these species; however, a reasonable approximation can be made. The slight increase in survival from 10° C to 18° C followed by a sharp decline in survival at 22° C suggests that the survival of early life stage Pacific and western brook lampreys is optimal in the temperature 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 significant increase in abnormalities at 22° C. Similarly, Piavis (1961) and Rodríguez-Muñoz et al. (2001) reported optimal temperatures of 18.3° C and 19° C, respectively, for survival of early life stage sea lampreys.

Our data indicate that Pacific and western brook lampreys have a broader zone of thermal tolerance, with respect to early life stages, than sea lampreys. Although survival of Pacific and

western brook lampreys was significantly reduced at 22° C, the proportion of individuals surviving to the larval stage was greater than 0.50 over the entire range of temperatures examined (range of 12° C; Figure 2). Comparatively, Piavis (1961) observed no survival to the burrowing stage (stage 17) for sea lampreys at temperatures below 15.6° C or above 21.1° C. This corresponds to a range of temperatures in which early life stage sea lampreys can survive of less than 4.5° C. Alternatively, McCauley (1963) suggested that sea lamprey eggs could hatch over a temperature range of 10° C (15° C to 25° C); however, low percent hatch (less than 30%) at temperatures greater than or equal to 20° C and less than or equal to 15° C suggests that much of the reported hatching range included sub-optimal temperatures. More recently, Rodríguez-Muñoz et al. (2001) reported high survival rates (greater than 58%) from fertilization to the burrowing stage for sea lampreys reared at 16° C, 19° C, and 23° C, with no survival to the burrowing stage for individuals reared at 11° C or lower. Rodríguez-Muñoz et al. (2001) also reported an estimated temperature for zero development of 6.93° C, higher than that estimated for lamprey species examined in this experiment, which may further restrict the range of potential temperatures available for embryogenesis of sea lampreys as compared Pacific and western brook lampreys.

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 habitat (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), and distribution data for western brook lampreys within the Columbia River Basin 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 affect 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 ± 10.8 *DD*) to the time that individuals reached the larval stage (294.0 ± 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 4). 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 1). 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.

Temperature can significantly influence the biology of fishes. Regarding early life stages, temperature can influence the timing of reproduction, development rates, survival, and the quantity and quality of habitat available for spawning and rearing. Categories that groups species based on general trends in thermal preference and tolerance (e.g., thermal guilds; Magnuson et al. 1979) provide information useful for determining broad-scale distributions; however, inclusion of a species in these types of categories may be based on the thermal requirements of closely related taxa and often do not take into account fine-scale differences among species and ontogenetic stages or the effects of local adaptation (Hall et al. 1978; Magnuson et al. 1979; Elliott 1981; Beschta et al. 1987; Sanders 1993). Therefore, empirical evidence should be used to provide more specific information when necessary, such as for comparative purposes or to provide a basis for management strategies. As compared to sea lampreys, Pacific and western brook lampreys were less stenothermic over the period of

development examined, with a lower temperature for zero development; however, thermal requirements of other life stages should be defined before generalizations can be made. The differences between the response to temperature of the species examined in this study and sea lampreys indicate the need for species-specific biological examinations prior to initiation of management or conservation efforts. Further work is needed to examine the response of these species to a more dynamic thermal environment and to other habitat components; however, these data provide baseline information that will be useful for predicting reproductive timing, developmental rates, and the suitability and distribution of spawning and rearing habitat available to Pacific and western brook lampreys.

References

Atkinson, D. 1994. Temperature and organism size-a biological law for ectotherms? Advances in Ecological Research 25:1-58.

Atkinson, D. 1996. Ectotherm life-history responses to developmental temperature. Pages 183-204 *in* I. A. Johnston and A. F. Bennett, editors. Animals and temperature: phenotypic and evolutionary adaptations. Society for Experimental Biology, Seminar Series 59, Cambridge University Press, Cambridge, Great Britain.

Beamish, F. W. H. 1980. Biology of the North American anadromous sea lamprey, *Petromyzon marinus*. Canadian Journal of Fisheries and Aquatic Sciences 37:1924-1943.

Beschta, R. L., R. E. Bilby, G. W. Brown, L. B. Holtby, and T. D. Hofstra. 1987. Pages 132-191 *in* E. O. Salo, and T. W. Cundy, editors. Stream temperature and aquatic habitat: fisheries and forestry interactions. Contribution no. 57, Institute of Forest Resources, University of Washington, Seattle.

Blaxter, J. H. S. 1969. Development: eggs and larvae. Pages 177-252 *in* W. S. Hoar and D. J. Randall, editors. Fish physiology, volume III. Academic Press, New York.

Brett, J. R. 1970. Temperature. Pages 515-560 *in* O. Kinne, editor. Marine ecology. Wiley-Interscience, New York.

Close, D. A. 2002. Pacific lamprey research and restoration project. Report of Confederated Tribes of the Umatilla Indian Reservation to U.S. Department of Energy, Portland, Oregon.

Cochnauer, T., and C. Claire. 2001. Evaluate status of Pacific lamprey in the Clearwater River drainage, Idaho. Report of Idaho Department of Fish and Game to U.S. Department of Energy, Portland, Oregon.

Elliott, J. M. 1981. Some aspects of thermal stress on freshwater teleosts. Pages 209-245 *in* A. D. Pickering, editor. Stress and fish. Academic Press, New York.

Hall, L. W. Jr., C. H. Hocutt, and J. R. Stauffer Jr. 1978. Implication of geographic location on temperature preference of white perch, *Morone americana*. Journal of the Fisheries Research Board of Canada 35:1464-1468.

Hofmann, N., and P. Fischer. Temperature preferences and critical thermal limits of burbot: implications for habitat selection and ontogenetic habitat shifts. Transactions of the American Fisheries Society 131:1164-1172.

Hokanson, K. E. F. 1977. Temperature requirements of some percids and adaptations to the seasonal temperature cycle. Journal of the Fisheries Research Board of Canada 34:1524-1550.

Holmes, J. A. 1990. Sea lamprey as an early responder to climate change in the Great Lakes Basin. Transactions of the American fisheries Society 119:292-300.

Houde, E. D. 1987. Fish early life history dynamics and recruitment variability. American Fisheries Society Symposium 2, pp. 17-29.

Kuehl, R. O. 1994. Statistical principles of research design and analysis. Duxbury Press, Belmont, CA.

Lind, O. T. 1985. Handbook of common methods in limnology, 2nd edition. Kendall/Hunt Publishing Company, Dubuque, IA.

Magnuson, J. J., L. B. Crowder, and P. A. Medvick. 1979. Temperature as an ecological resource. American Zoologist 19:331-343.

Manion, P. J., and L. H. Hanson. 1980. Spawning behavior and fecundity of lampreys from the upper three Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences 37:1635-1640.

McCauley, R. W. 1963. Lethal temperatures of the developmental stages of the Sea Lamprey, *Petromyzon marinus* L. Journal of the Fisheries Research Board of Canada 20:483-490.

Moyle, P. B. 2002. Inland fishes of California. University of California Press, Berkeley, CA.

Piavis, G. W. 1961. Embryological stages in the sea lamprey and effects of temperature on development. Fishery Bulletin 61:111-143.

Potter, I. C., and F. W. H. Beamish. 1975. Lethal temperatures in ammocoetes of four species of lampreys. Acta Zoologica 56:85-91.

Quinn, T. P., and D. J. Adams. 1996. Environmental changes affecting the migratory timing of American shad and sockeye salmon. Ecology 77:1151-1162.

Rodríguez-Muñoz, R., A. G. Nicieza, and F. Braña. 2001. Effects of temperature on developmental performance, survival and growth of sea lamprey embryos. Journal of Fish Biology 58:475-486.

Rombough, P. J. 1988. Respiratory gas exchange, aerobic metabolism, and effects of hypoxia during early life. Pages 59-161 *in* W. S. Hoar and D. J. Randall, editors. Fish physiology, volume XI. Academic Press, New York.

Sanders, B. M. 1993. Stress proteins in aquatic organisms: an environmental perspective. Critical Reviews in Toxicology 23:49-75.

Satterthwaite, F. E. 1946. An approximate distribution of estimates of variance components. Biometrics 2:110-114.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry, 3rd edition. Freeman and Company, New York.

Swink, W. D. 1995. Growth and survival of newly parasitic sea lampreys at representative winter temperatures. Transactions of the American Fisheries Society 124:380-386.

University of Washington. 2001. Columbia River DART: Data Access in Real Time. School of Aquatic and Fishery Sciences. Available: www.cbr.washington.edu/dart/dart.html. (2001 - 2002).

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

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

Table 2: Mean (± standard deviation) dissolved oxygen (DO), pH, and total dissolved gasses (TDG). Parameters were measured once daily in each water bath for the duration of the experiment. Dissolved oxygen converted to percent from mg/L as in Lind (1985).

Year	Temperature (° C)	DO (% ± SD)	pH ± SD	TDG (% ± SD)
2001	10	108.15 ± 8.11	7.62 ± 0.13	101.89 ± 1.02
	14	104.85 ± 9.40	7.66 ± 0.10	101.26 ± 0.45
	18	103.70 ± 7.64	7.72 ± 0.11	101.52 ± 0.45
	22	103.73 ± 8.47	7.72 ± 0.14	102.50 ± 0.48
2002	10	103.22 ± 5.24	7.39 ± 0.18	101.14 ± 0.76
	14	103.13 ± 5.19	7.44 ± 0.18	100.66 ± 0.54
	18	104.61 ± 5.00	7.48 ± 0.12	101.27 ± 0.41
	22	106.76 ± 6.48	7.47 ± 0.18	102.08 ± 0.37

Table 3: Effective temperature (E_T), days required to reach 50% hatch (D_{H50} ± SE), and days required to reach 95% hatch (D_{H95} ± 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 (º C)	E_T (º 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

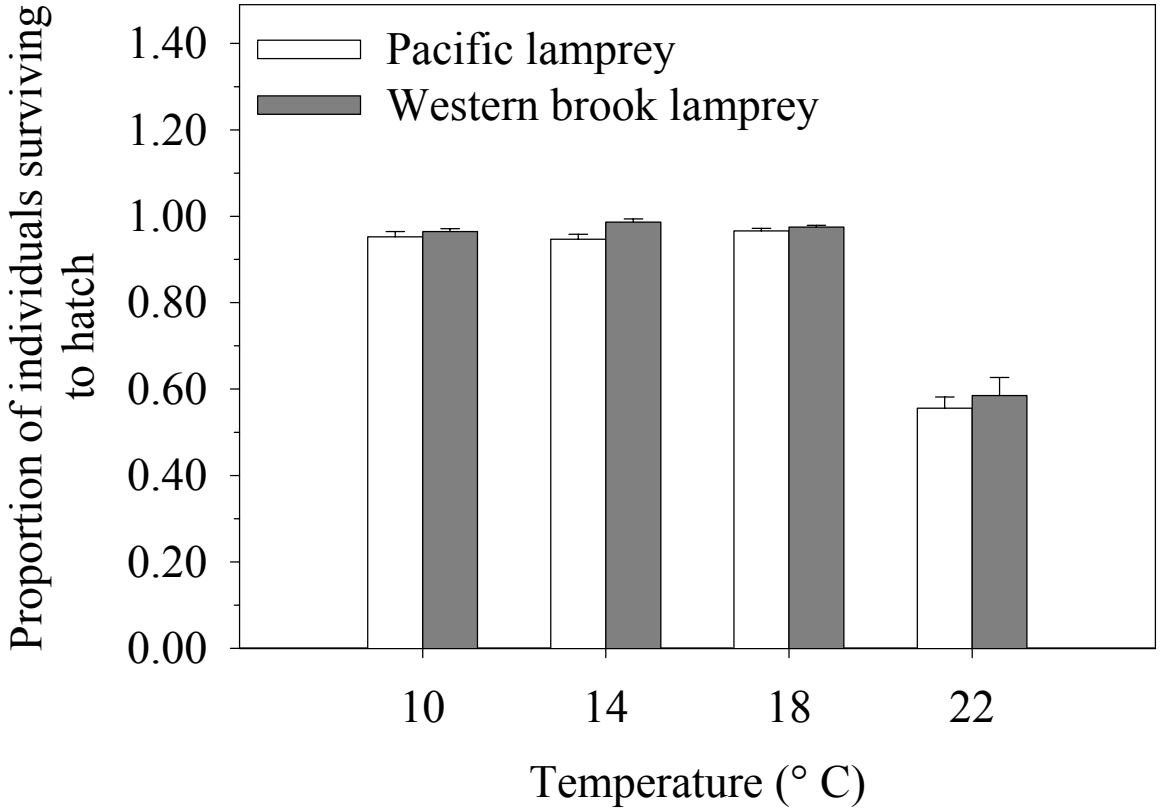


Figure 1: Proportion of Pacific and western brook lampreys surviving (+ 1 SE) from fertilization to hatch (S_H). Overall, survival was significantly reduced at 22° C when compared to other temperatures.

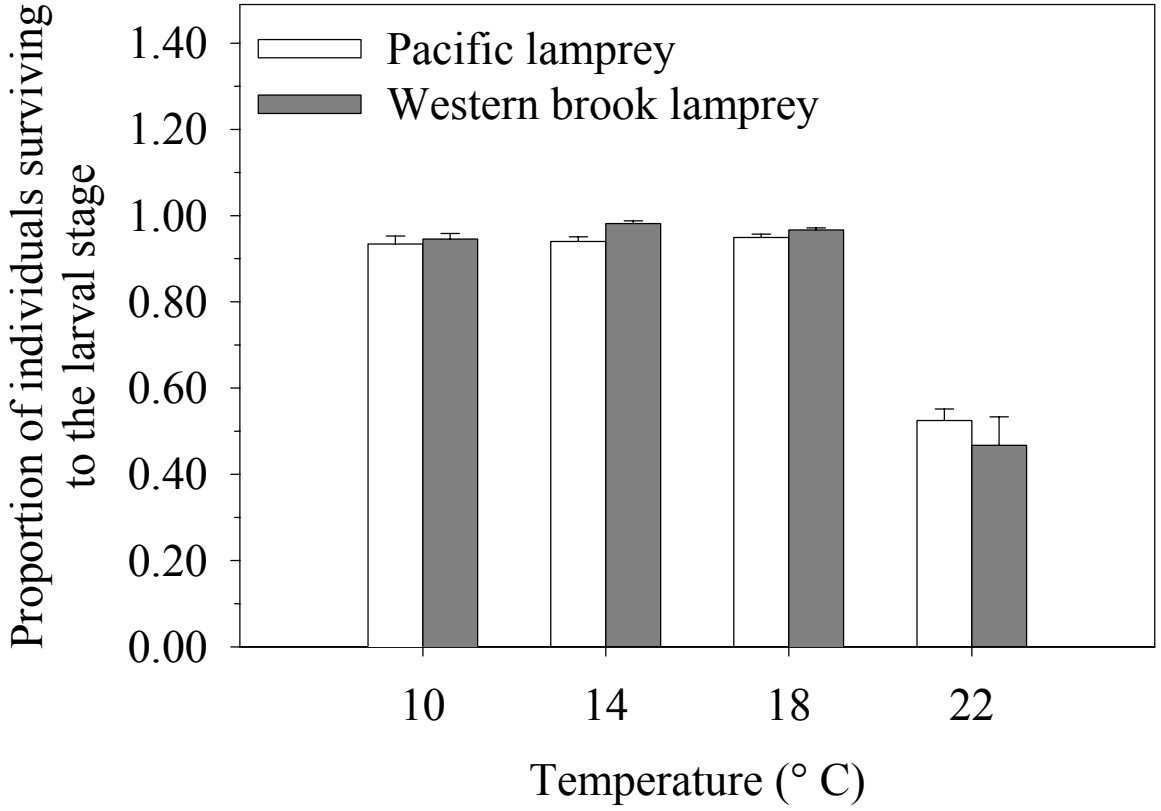


Figure 2: Proportion of Pacific and western brook lampreys surviving (+ 1 SE) from fertilization to the larval stage (S_L). Overall, survival was significantly reduced at 22° C when compared to other temperatures.

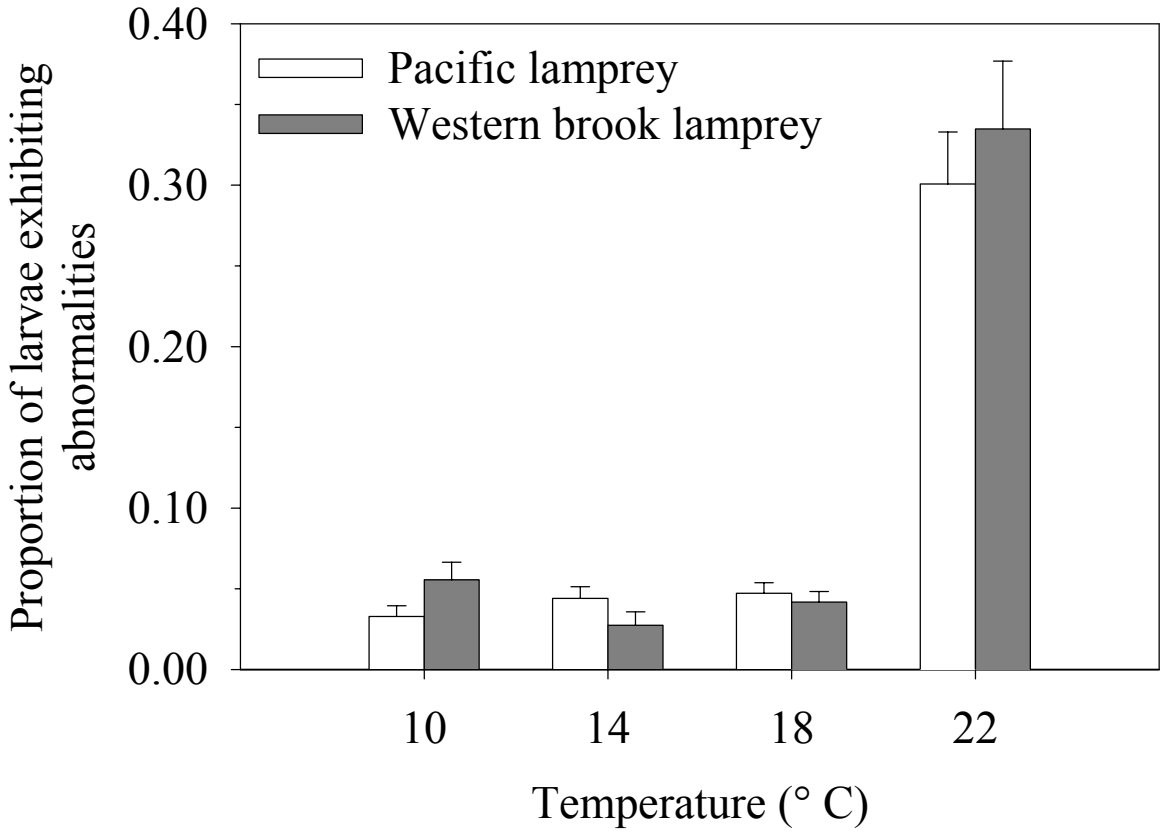


Figure 3: Proportion of Pacific and western brook lamprey larvae exhibiting abnormalities (+ 1 SE). Significantly more larvae exhibited abnormalities at 22° C when compared to other temperatures.

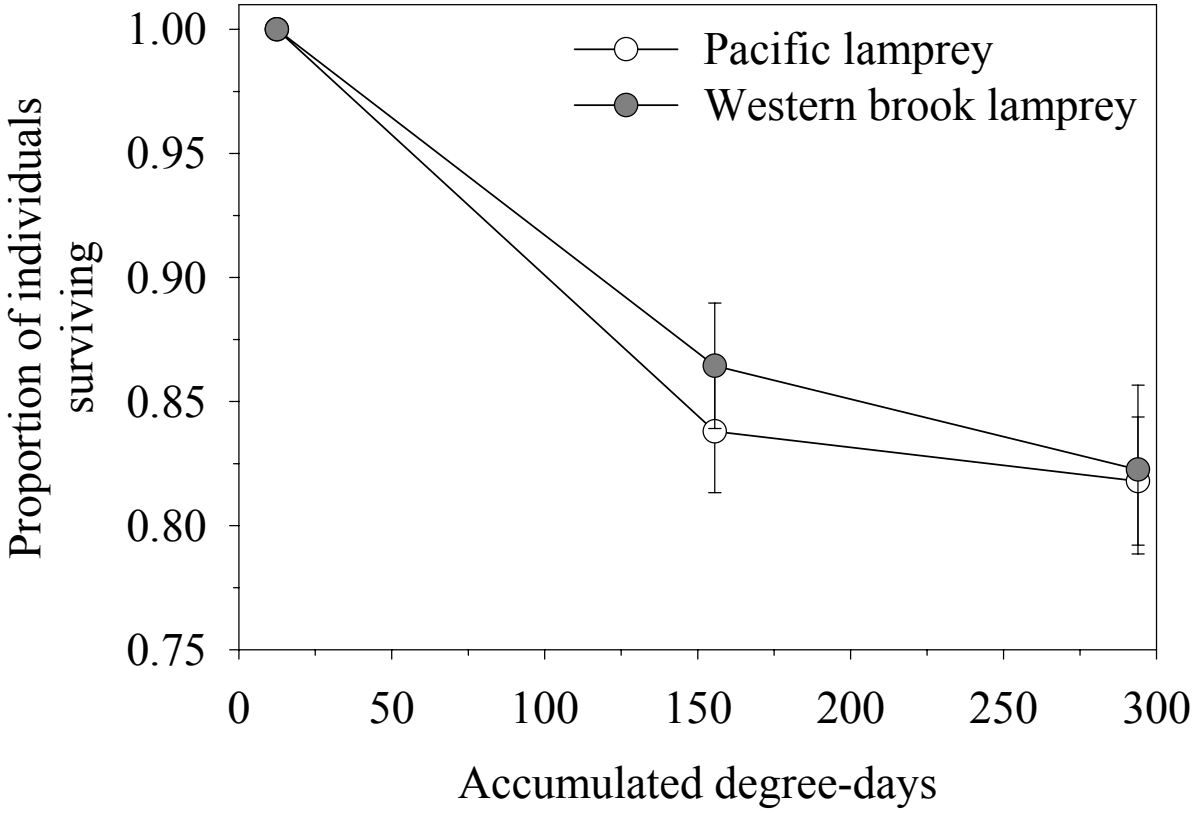


Figure 4: Trend in the proportion of Pacific and western brook lampreys surviving (± 1 SE) from the initiation of experiment (12.5 *DD*) to hatch (155.6 *DD*) and from hatch to the larval stage (294.0 *DD*). A slight, but significant, decrease in survival was observed from the time of hatch to the larval stage.

Chapter 5:

River lampreys (*Lampetra ayresi*) in the Columbia River Basin

Synopsis

The main objectives of this study were to examine questions regarding the biology of three species of lampreys found in the Columbia River Basin. 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 Columbia River Basin; however, documented collections of this species from the Columbia River have not been made since 1980 (Bond et al. 1983). For this project, we were unable to locate river lampreys in the Columbia River Basin. 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 slight green tint on the upper and mid sections of the body. We have 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 1980). 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 1980). 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 1980). 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 we obtained records were of 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 have 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 the western brook lamprey/river lamprey complex. 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 Columbia River Basin 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 Columbia River Basin. 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 1). Within the Columbia River Basin, we spoke with personnel from universities and state, federal, tribal, and private agencies in an attempt to collect river lampreys. Initially, the Oregon Department of Fish and Wildlife (ODFW) and Washington Department of Fish and Wildlife (WDFW) were contacted to establish a list of possible collection locations. Individuals contacted within these agencies stated that there have been no sightings of adult river lampreys and that they have no way of distinguishing between the three Columbia River Basin 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 Columbia River Basin, 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 Columbia River Basin). 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 (Appendix 2). 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 Columbia River Research Laboratory and held for 32 d in a flow through, 189 L aquarium [see Executive Summary (this document) for laboratory conditions] 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 μm, 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 1) and mean mass of 2.89 g (SE = 0.14) (Figure 2) 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 Columbia River Research Laboratory and held for 91 d until the time of spawning. Although sample sizes varied over time due to mortality, mean growth rates where 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 1) and mean mass of 22.63 g (SE = 0.63) (Figure 2). 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 1) and mean mass of 15.02 g (SE = 0.63) (Figure 2), 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 1971). 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 Columbia River Research Laboratory [see Executive Summary (this document) for laboratory conditions].

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 know 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 about river lampreys, which makes studying their biology all the more important in order to preserve their place in the ecosystem.

References

Beamish, R. J. 1980. Adult biology of the river lamprey (*Lampetra ayresi*) and the Pacific lamprey (*Lampetra tridentata*) from the Pacific coast of Canada. Canadian Journal of Fisheries and Aquatic Sciences 37:1906-1923.

Bond, C., T. T. Kan, and K. W. Myers. 1983. Notes on the marine life of the river lamprey, *Lampetra ayresi*, in Yaquina Bay, Oregon, and the Columbia River estuary. Fishery Bulletin 81:165-167.

Docker, M. F., J. H. Youson, R. J. Beamish, and R. H. Devlin. 1999. Phylogeny of the lamprey genus *Lampetra* inferred from mitocondrial cytochrome *b* and ND3 gene sequences. Canadian Journal of Fisheries and Aquatic Sciences 56:2340-2349.

Eddy, S., and J. C. Underhill. 1978. How to know the freshwater fishes, 3rd edition. Wm. C. Brown Company Publishers, Dubuque, Iowa.

Hardisty, M. W., and I. C. Potter. 1971. The general biology of adult lampreys. Pages 127-206 *in* M. W. Hardisty and I. C. Potter, editors. The biology of lampreys, volume 1. Academic Press, New York, New York.

Hart, J. L. 1973. Pacific Fishes of Canada. Fisheries Research Board of Canada, Ottawa, Canada.

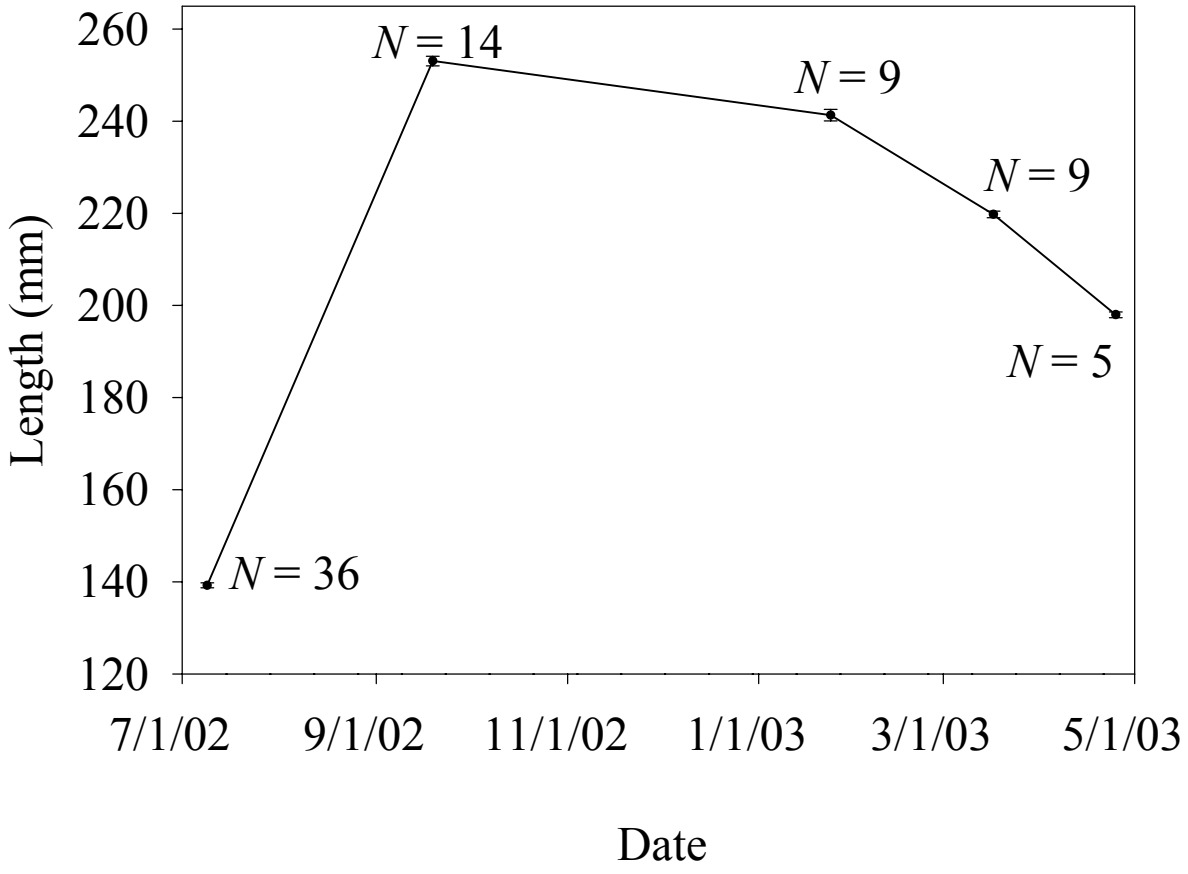


Figure 1: Mean total length (mm ± 1 SE) and sample size for river lampreys held in captivity and measured at intervals from July 2002 until April 2003.

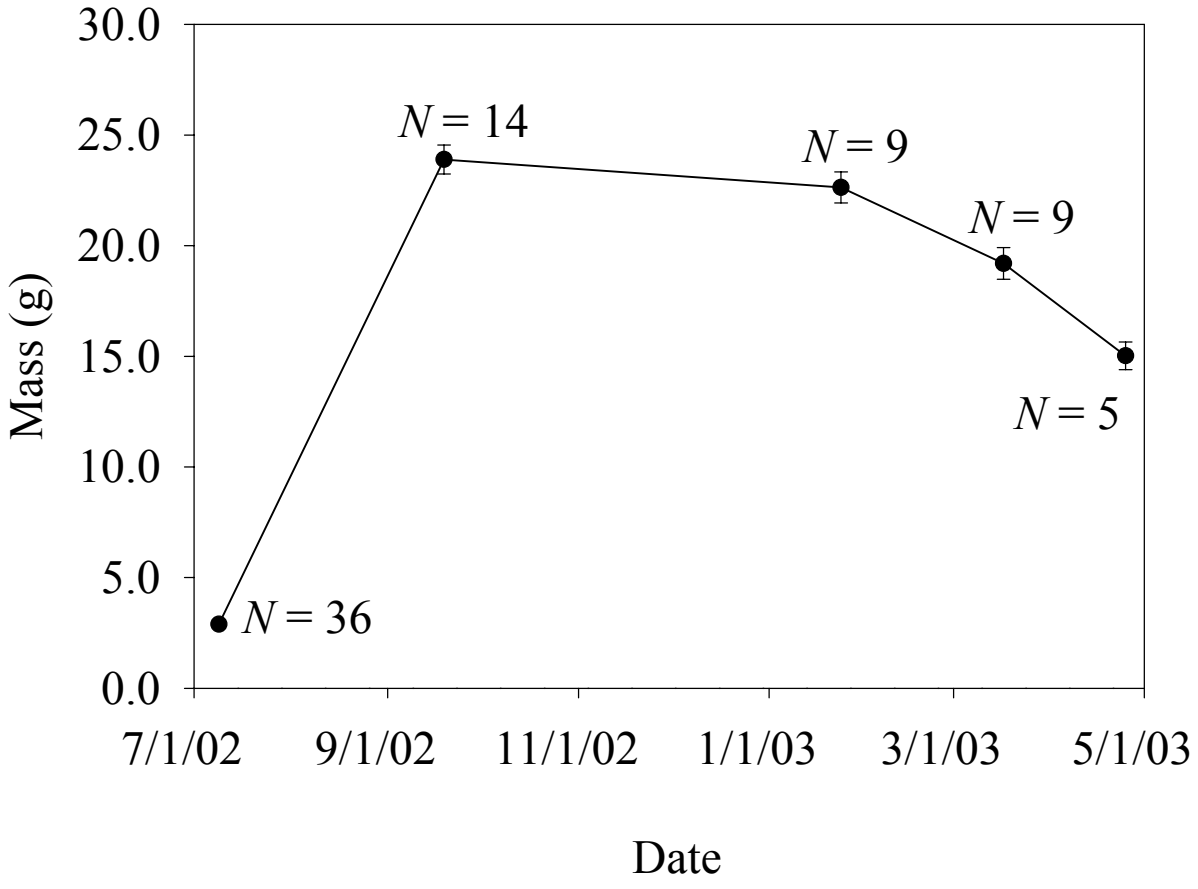


Figure 2: Mean wet mass (g ± 1 SE) and sample size for river lampreys held in captivity and measured at intervals from July 2002 until April 2003.

Appendix 1: 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

Appendix 2: 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. (Courtesy of NOAA Fisheries Service).

Date	North Hope Island	South Hope Island	Lone Tree Point	Similk Bay	South Fork Flats	Strawberry Point	Dugualla 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