

Pacific Lamprey Research and Restoration Project

**Annual Report
2000**



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PACIFIC LAMPREY RESEARCH AND RESTORATION PROJECT

ANNUAL REPORT 2000

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EXECUTIVE SUMMARY

This report summarizes results of research activities conducted in 1999-2000. The findings in these chapters represent the efforts of the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) and collaborative efforts among other researchers working on Pacific lampreys (*Lampetra tridentata*) under this project. The findings in these chapters will help management and recovery of Pacific lampreys in the Columbia River Basin.

Chapter I

We have initiated a reintroduction of Pacific lamprey into the Umatilla River. To initiate the restoration effort, the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) began developing a restoration plan in 1998. In 2000, the CTUIR implemented a pilot project in the Umatilla River. Experiments necessary to maximize the potential for the restoration of lampreys in the Umatilla River began in 2000 after review from ODFW and other agencies. The goal of the lamprey research and restoration project is to restore natural production of Pacific lampreys in the Umatilla River to self-sustaining and harvestable levels.

Outplantings of adult lampreys in the Umatilla River were started in 2000 and the monitoring of several metrics of Pacific lamprey was initiated in 1998 and has been continued yearly. In 2000, we outplanted 600 adult lampreys into the Umatilla River. We observed 81 nests within areas surveyed in the mainstem Umatilla and Meacham Creek.

We monitored existing index plots for larval abundance with electrofishing gear. Larvae were found in 4 of the 30 sampling stations. The mean density of the sites sampled was 0.08 individuals/m². The mean length was 127 mm and ranged from 100 to 155 mm.

Outmigrant metamorphosed and larval lampreys were monitored with a Rotary Screw Trap near the mouth of the Umatilla River. Metamorphosed lampreys were captured from

November to March, with peak migration in December. Larval lampreys were caught from October to March, with peak migration in February. Outmigration of both life stages were significantly correlated with river flow. Abundance of outmigrant metamorphosed lampreys was estimated to be $17,157 \pm 14,902$ (95% CI).

Portable assessment traps were fished to monitor the abundance of adult lampreys entering the Umatilla River. We captured three adults during the trapping period. No adults were observed on video at the Three Mile Falls Dam fishway.

We will continue adult outplantings and monitoring of population metrics in the Umatilla River. Furthermore, we will investigate spawning habitat selection and egg survival of outplanted lampreys. This report describes the work completed in 2000 with regards to outplanting adult lampreys.

Chapter II

Habitat heterogeneity at fine and coarse scales influences the measurement and detection of patterns in the abundance and habitat relationships of larval Pacific lamprey, *Lampetra tridentata*. In a 55-km section of the Middle Fork John Day River, a fourth- to fifth-order stream in northeastern Oregon, we used a nested sampling design and multiple logistic regression to evaluate heterogeneity in larval abundance and habitat within and among sites. Stream habitat variables predicted patterns in larval abundance but played different roles at different spatial scales. The spatial distribution of larvae at large scales (5–10 km) was positively associated with water depth and an open riparian canopy (likelihood ratio χ^2 test, $P < 0.001$). Patchiness in larval occurrence at small scales (< 50 m) corresponded positively with low water velocity, pool habitats, and the availability of suitable burrowing habitat ($P < 0.001$). We determined that habitat variables explain a significant proportion of variation in larval abundance at large and

small scales, but locational factors, such as longitudinal position in the stream section and sample location within the channel unit, explain additional variation that might otherwise be discounted as noise.

Chapter III

Lampreys as a group are primitive creatures, with a more than 300 million year history. The anadromous lamprey species are parasitic and some evidence (based on sea lamprey *Petromyzon marinus* research in the Great Lakes) suggests that homing to the natal streams is not part of their life history. Therefore, rehabilitation takes on a system-wide scope. To properly design a rehabilitation program, managers must know what factors must be modified or controlled to allow lamprey populations to survive and increase in numbers. One avenue to develop this level of understanding is basic physiological measurements with these animals. It is believed that adult sea lampreys use pheromones as migratory and behavioral cues. Before they are sexually mature (during migration), adult sea lampreys are sensitive to pheromones released by conspecific larval lampreys. This compound, the bile salt petromyzonol sulfate, appears to be released only by larval lampreys. Thus, petromyzonol sulfate seems to function as a migratory cue, indicating to upstream migrating adult lampreys locations where lampreys have successfully reproduced before. As upstream migrating sea lampreys become sexually mature, their olfactory sensitivities change. Response to migratory cues is replaced by sensitivity to pheromones that influence adult lamprey interactions during reproduction. These pheromones are produced only by sexually mature adult sea lampreys and seem to function to bring adults together to spawn. The goal of the research presented in this report is to provide data on the relative sensitivity of upstream migrating Pacific lampreys to petromyzonol sulfate as they migrate up the Columbia River, using a similar approach to that of sea lamprey researchers in the Great Lakes.

Information on Pacific lamprey olfactory response to pheromones is especially important to the Confederated Tribes on the Umatilla Indian Reservation because they are conducting a feasibility study designed to rehabilitate the Pacific lamprey population in the Umatilla River. Knowing if upstream migrating Pacific lampreys in the Columbia River mainstem are sensitive to the presence of either larval or other adult lampreys could strongly influence the success of their effort. This report describes the electro-olfactogram apparatus that we built during the first year of this contract.

ACKNOWLEDEMENTS

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CHAPTER ONE

Reintroduction of Pacific Lamprey in the Umatilla River, Oregon: A Case Study

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INTRODUCTION

The Pacific lamprey (*Lampetra tridentata*) is an anadromous fish, which is distributed in rivers from California along the Pacific Rim to Hokkaido, Japan (Ruiz-Campos and Gonzalez-Guzman 1996; Simpson and Wallace 1982). Pacific lamprey is native to the Columbia River Basin, and their spawning migration extends into many inland rivers draining Oregon, Washington, and Idaho (Kan 1975; Hammond 1979; Simpson and Wallace 1982). Pacific lamprey spawn in riffle areas. Eggs hatch in the gravel and become larvae or ammocoetes, which then drift into silty substrate to burrow and begin filter feeding (Beamish 1980). During this stage, larvae spend 4 to 6 years growing in the sediment (Hammond 1979; Pletcher 1963; Beamish 1980). Larvae then go through metamorphosis, changing morphologically and physiologically to begin their parasitic stage as adults. The duration of the parasitic stage in the ocean has been speculated to last approximately 3.5 years (Beamish 1980). Adult Pacific lamprey typically enters the Columbia River in April, over winter, and then spawns the following spring (Kan 1975).

During the spawning migration, lampreys are harvested for subsistence by Native peoples in the Pacific Northwest. Lampreys are culturally important to Native peoples along the West coast of the United States and Canada (Pletcher 1963, Keim 2000, Close et al. 2002).

Lampreys have declined in numbers throughout the world due to habitat destruction from hydroelectric dams, flow regulation, channelization and poor water quality (Kirchhofer 1995, Ojutkangas et al. 1995, Myllynen et al. 1997, Renaud 1997). In the Columbia River Basin, declines in adult Pacific lamprey can be partially attributed to hydroelectric dams (Moser et al. 2002). Dams have impeded passage of adult Pacific lamprey in the Columbia and Snake rivers, thus effecting larval recruitment in the basin. In the early 1900's, low head diversion dams were

built throughout the Columbia River Basin for irrigation. These dams have had negative effects on adult lamprey passage and have reduced larval habitat by dewatering large sections of the rivers. Adult Pacific lamprey has declined in numbers in the Umatilla River, a tributary of mainstem Columbia River (Close and Jackson 2001; Close and Bronson 2001), thus affecting tribal treaty fishing rights and tribal culture (Close et al. 2002)

The tribes initially raised awareness regarding Pacific lamprey declines along the Oregon Coast and interior Columbia River Basin (Downey et al. 1993; Close et. al. 1995). The Northwest Power Planning Council approved the Status Report in 1995 that initiated CTUIR's lamprey research and restoration project in 1996 within the Columbia River basin. The goal of the lamprey research and restoration project is to restore natural production of Pacific lamprey in the Umatilla River to self-sustaining and harvestable levels. Our study objective was to determine if adult Pacific lamprey are limiting in the Umatilla River.

This report summarizes the studies and restoration efforts conducted during 2000 in the Umatilla River.

STUDY AREA

The Umatilla River originates in the Blue Mountains and enters the Columbia River 465-km from the Pacific Ocean (Figure 1.). Elevation ranges from 1768 m in the headwaters to 79 m above sea level at the mouth of the river. The basin drains approximately 5,931 km² in northeastern Oregon. The average annual discharge of the river is 13 m³/s. Precipitation ranges from 22 cm/yr near the mouth to approximately 140 cm/yr near the headwaters.

In the Umatilla basin, human impacts have altered river habitats as in most drainages of the American West. In the Umatilla basin, habitat alterations were as follows: 1) loss of beaver

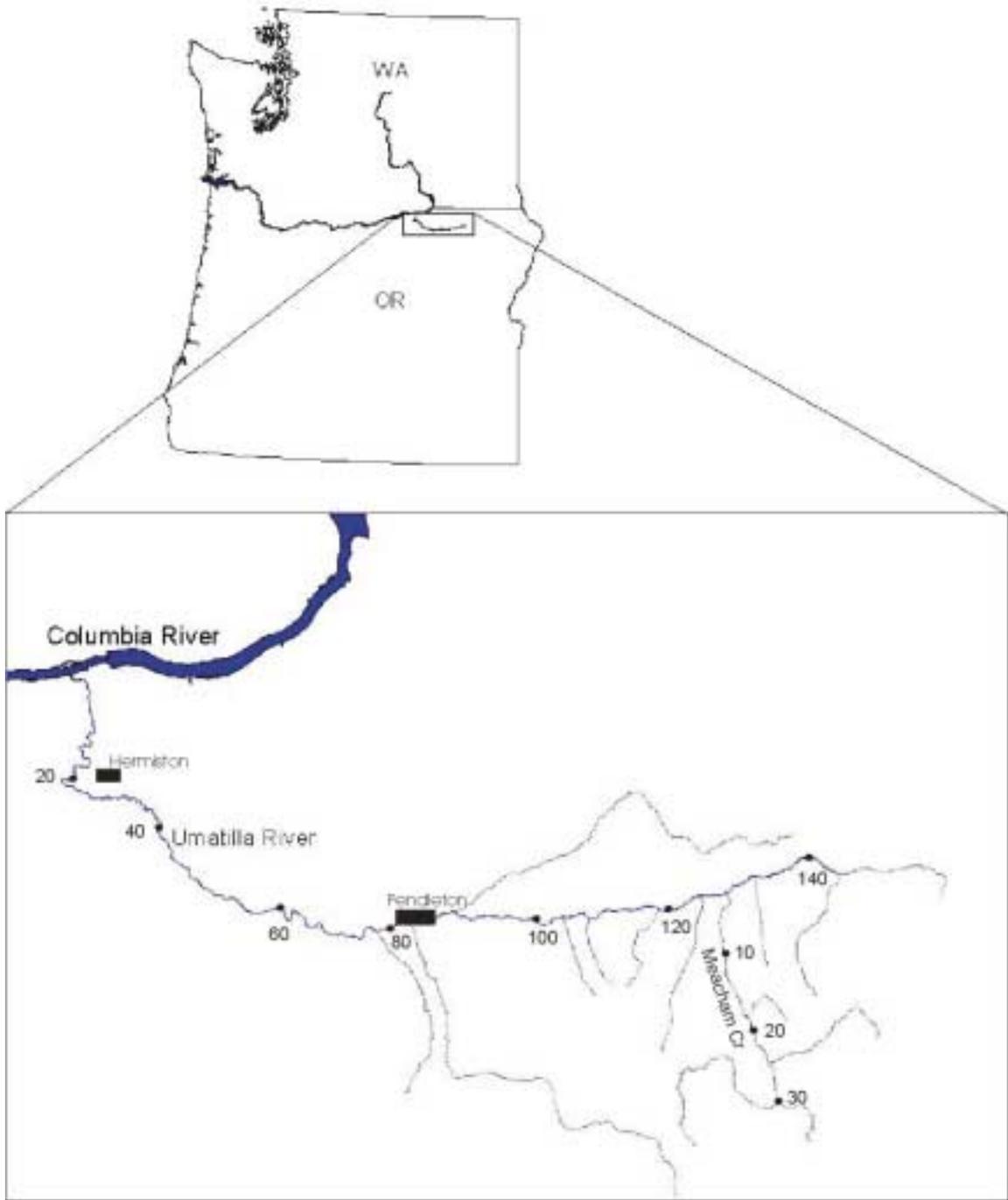


Figure 1. Map of Oregon showing location of the Umatilla River. Numbers represent river kilometers.

(*Castor canadensis*) populations during the beaver trade, 2) livestock overgrazing of native grasses and loss of riparian areas, 3) the conversion of native plants to introduced crops, 4) logging the upper watershed led to changes in forest communities, and 5) irrigation practices to support agriculture that channelized and impounded streams, dewatered rivers, obstructed fish passage, and diverted fish into ditches and onto agricultural fields. Another alteration that obstructed passage was hydroelectric dams built on the mainstem Columbia and Snake Rivers.

METHODS

Collection and holding.- In July and August 1999, we collected 100 adult Pacific lamprey at Tumwater Falls in the John Day River, Oregon. In December 1999 and January 2000, we collected 511 adults in the Fishways of the John Day Dam located on the mainstem Columbia River. Fish were transported and held at the Columbia River Research Laboratory (CRRL) at Cook Washington from July 1999 through March 2000. Upon arrival, lampreys were anaesthetized by immersion in 80 mg/L tricaine methanesulfonate (MS-222) buffered with sodium bicarbonate for handling. All fish were weighed (g) and lengths (mm) measured then recorded. Fish were then injected with oxytetracycline at the dose of 10 mg/kg for bacterial infections. Fish were maintained at CRRL in flow-through 0.9 m tanks supplied by river water. Water temperatures were maintained at river temperatures from 6-8°C through March 16, 2000.

Fish were transported from the CRRL to the Three-Mile Falls holding facility on March 16, 2000 and held there until release. Fish were maintained in a single raceway (27.45 m x 3.05 m x 1.22 m) supplied by Umatilla River water. Temperatures ranged from 4-15°C during holding period. Weekly observations were conducted to determine stage of maturation. Once females exhibited distended body walls (ovaries enlarging) fish were deemed ready to be

released. Before fish were removed from the facility to be released into upper Umatilla River, fish pathologist (Sam Onjukka) from the Oregon Department of Fish and Wildlife's La Grande laboratory, screened fish for disease prior to release into the Umatilla River.

On May 8, 2000, we released 600 adult Pacific lampreys (233-594 g; 358-750 mm) at three locations in the headwaters of the Umatilla River. 150 and 300 fish were released at river kilometers 118.0 and 140, respectively. The remaining 150 fish were released at river kilometer 17.5 in Meacham Creek, a major tributary in the Umatilla River.

Spawning surveys.- Spawning surveys were conducted to assess the distribution of Pacific lamprey nests in the Umatilla River. Surveys were conducted in the following areas: 1) the mainstem Umatilla River from rkm 90 to rkm 144, 2) the North Fork Umatilla from rkm 0 to rkm 4, 3) the South Fork Umatilla from rkm 0 to rkm 1.6, and 4) Meacham Creek from rkm 0 to rkm 24. Surveys were conducted beginning on May 8, 2000 through August 1, 2000. Prior to conducting the survey, field training was necessary to standardize survey methods and to illustrate the typical construction sites for lamprey redds. After surveyors located a high density of spawning individuals and nests, the stream sections of low density was either eliminated from the survey or conducted on a bi-weekly basis. However, survey lengths were shortened due to the time intensive efforts required to locate and identify lamprey nests. Four to five river kilometers were surveyed daily by a two person crew.

Surveyors walked downstream along the margins and traversed from bank to bank at the tail out of each pool and above each riffle. We maximized our ability to view spawning activity by using polarized sunglasses and only walking the stream if visibility was clear enough to view depths of pools and riffles. Once a test nest, a nest, or construction of a nest was located, pink fluorescent flagging was placed in the vicinity of the nest construction. Surveyors recorded the

number of adults on or near nest, date, location, and crew on data sheet. Special care was taken not to disturb active spawning. Approximate location was logged with a hand held Global Positioning System (GPS) unit (Garmin GPS III plus) and mapped with Arcview (GIS version 3.2).

Larval abundance.- We electrofished selected plots along the longitudinal profile of the Umatilla River to estimate lamprey abundance before and after outplanting adults in the Umatilla River. The number of sampling stations located in the river was 30. Each index plot consisted of a 7.5 m² area of silty substrate where larvae are typically most abundant (Potter et al. 1986, Young et al. 1990). In 2000, we sampled during the month of September. We used the Abp-2 electrofishing unit (Engineering Technical Services, University of Wisconsin, Madison, Wisconsin) to sample each plot. The electrofishing unit delivered 3 pulses per second (125 volts DC at 25% duty cycle) to remove larvae from the substrate and 30 pulses per second (125 volts DC at 25% duty cycle) to stun and capture larvae once in the water column. Pulse rates, voltage level, and effort (90 s/m²) used during our study, were based on electrofishing of larval sea lampreys (Hintz 1993, Weisser 1994). We used two passes with the electrofishing unit to remove larvae from each plot. After removing the larvae from the plot, they were anaesthetized in 50 mg/L MS-222 for handling. Larvae were identified to species by tail pigmentation (Richards et al. 1982) and total length (\pm 1 mm) measured. After identification and measurements were taken, larvae were placed in a recovery bucket (~5 minutes) then returned to the stream. GPS hand held units were used to record each index plot location in the Umatilla River.

Outmigrant abundance.- A 1.5 m diameter rotary screw trap was fished September 1st 1999 to March 9th 2000 to estimate the abundance of recently metamorphosed Pacific lampreys

outmigrating from the Umatilla River. The trap was operated 24 hr/day. The trap had a revolving stainless steel 3.5 mm mesh cone (one half submerged) mounted on pontoons. The trap was located approximately 1.9 river kilometers from the mouth of the Umatilla River. Lampreys passing through the cone were collected in a live-box located on the back of the trap. During high flow events, the screw trap was routinely cleaned to prevent debris build up from obstructing the opening of the cone. The trap was checked once in the morning. Captured Pacific lampreys were removed from the live-holding box, anaesthetized with 50 mg/L MS-222, counted and measured to the nearest millimeter. Lampreys were classified as recently metamorphosed when they were at stage 5 based on Youson and Potter's (1979) classification of metamorphosis in sea lampreys (*Petromyzon marinus*).

A mark-recapture method was used to measure trap efficiency. A caudal fin clip was used to mark lampreys for recapture. After marking the fish, they were held 24 to 48 hours and released during the morning approximately 4.0 river kilometers upstream from the rotary screw trap. Oregon Department of Fish and Wildlife owns and operates the screw trap with some assistance from the Confederated Tribes of the Umatilla Indian Reservation's Fisheries Program.

In addition to the rotary screw trap, outmigrant lampreys were captured from March 6, 2000 to October 3, 2000 at the fish collection facility at the West Extension Canal on the west side of Three Mile Falls Dam (rkm 5.9) on the Umatilla River. Oregon Department of Fish and Wildlife operates the fish collection facility. The facility has fish trapping and bypassing capability and generally operates from March through mid-October. During operation the canal flow is 5.1 m³/sec with a bypass flow ranging from 0.14 to 3.5 m³/sec. A detailed description of the juvenile fish collection facility is described in Knapp et al. (1996). Daily catch of outmigrant lampreys were counted, however, trapping efficiency estimates were not conducted.

Adult trapping.- Portable assessment traps were used to estimate the numbers of adult lampreys entering the Three Mile Dam fishway (rkm 5.9). Two portable assessment traps were fished 118 trap nights from September 16th, 1999 to April 29th, 2000 and 43 trap nights from June 19th through July 31st in 2000. Traps were placed on both sides of the entrance of the fish ladder. Adult traps were checked daily and lampreys captured in the trap were measured and marked then released.

Video monitoring.- We used a video recording system in front of the viewing window in the east-bank fish ladder at Three Mile Falls Dam to count upstream migrating adult lampreys passing through the ladder during 2000. Tapes were reviewed on a desktop editor video recorder.

Data Analysis

Spawning surveys.- We visually assessed the spawning distribution of adult Pacific lamprey by mapping their nests. Lamprey nests were mapped using a geographic information system (GIS).

Larval abundance.- For estimating the larval population in each plot, we used the Serber and LeCren (1967) estimator to analyze the data. The population, N , and variance, $\text{var}(N)$, are described as

$$N = \frac{(C_1)^2}{(C_1 - C_2)}$$

$$\text{var}(N) = \frac{(C_1)^2(C_2)^2(C_1 + C_2)}{(C_1 - C_2)^4}$$

where C_1 is the catch at first electrofishing pass and C_2 is the catch at the second pass. For the Serber and LeCren model we assumed 1) larvae could not be lost from the sample plot, 2) all stunned fish were captured, and 3) equal effort was used on each pass. We calculated a population estimate for each 7.5 m² plot. Estimates for each plot were calculated using the Capture software (White et al. 1982). Population estimates and variances for each plot were summed to calculate the total population estimate and 95% confidence interval for the total area sampled. In addition, the population estimates for plots were calculated to density (no./m²).

Outmigrant abundance.- Metamorphosed lamprey were marked and released above rotary screw trap to calculate trap efficiencies. Estimates and variances were summed for the total trapping period. The Bootstrap method (Efron and Tibshirani 1986; Thedinga et al. 1994) with 1,000 iterations was used to determine the variance of all abundance estimates. Confidence intervals (95%) for the abundance estimate were calculated using the square root of the Bootstrap variance estimate (CI = 1.96 x square root of the variance). We enumerated the catch of lampreys collected at the juvenile fish collection facility. The correlation between discharge and the number of caught larvae and metamorphosed lampreys and were tested using Spearman rank correlation test. A nonparametric test was used because the assumption of bivariate normal distribution was not fulfilled. The variable reflecting the flow during the catching night was the average of the mean flow of the day before and the day after each catching night. The time period for the analysis was between the days when the first and last lamprey was caught (12/22/00-5/1/01).

RESULTS

spawning surveys.- Adult lamprey spawning activity began in May and continued through July in the Umatilla River. We observed 81 lamprey nests in the areas surveyed within

the Umatilla River and Meacham Creek (Figure 2). The majority of the lamprey nests were found in the mainstem Umatilla River. We found 51 nests from river kilometer 96 to 142. In Meacham Creek, we found 29 nests from river kilometer 17 to 27 and one located near the mouth of North Fork Meacham Creek.

Larval abundance.- Larval abundance in the mainstem sampling plots did not increase after releasing adults to spawn in 2000. Larvae were found in 4 of the 30 sampling stations (Table 1). Larval lampreys in the mainstem Umatilla River were detected from river kilometer 3.0 to 36.8. The mean density of sites sampled was 0.08 ind. /m². The mean length of larvae was 127 mm and lengths ranged from 100 to 155 mm (Table 1).

Table 1. Site densities of larvae, mean lengths, and ranges collected in 2000.

site (no)	river kilometer	density (ind.m ⁻²)	length (mm)	
			mean	range
4	4.0	0.26	119	118-119
6	9.3	0.40	148	138-155
7	11.9	0.13	144	-
8	36.8	1.66	122	100-152
1-30	0-128.4	0.08	127	100-152

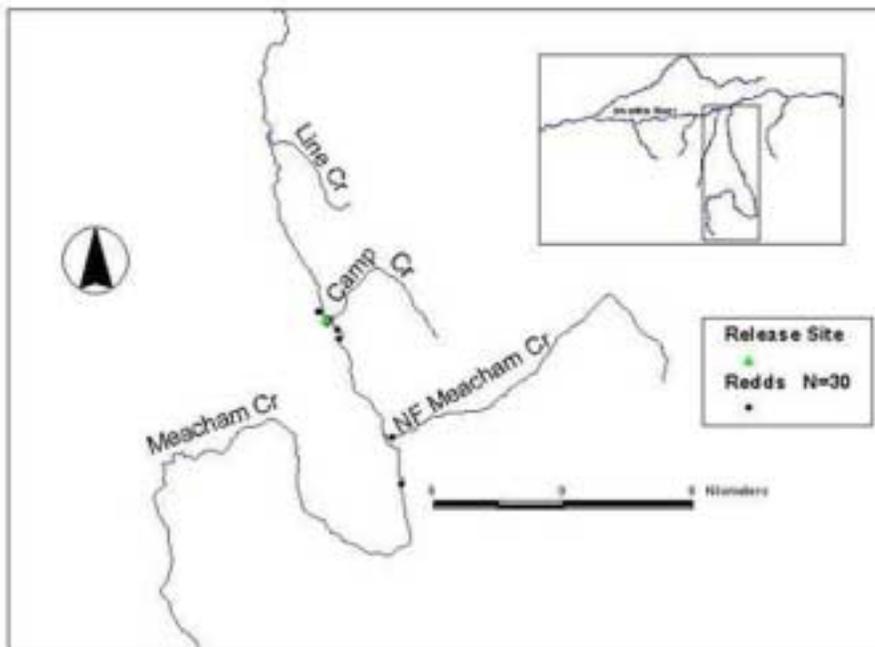
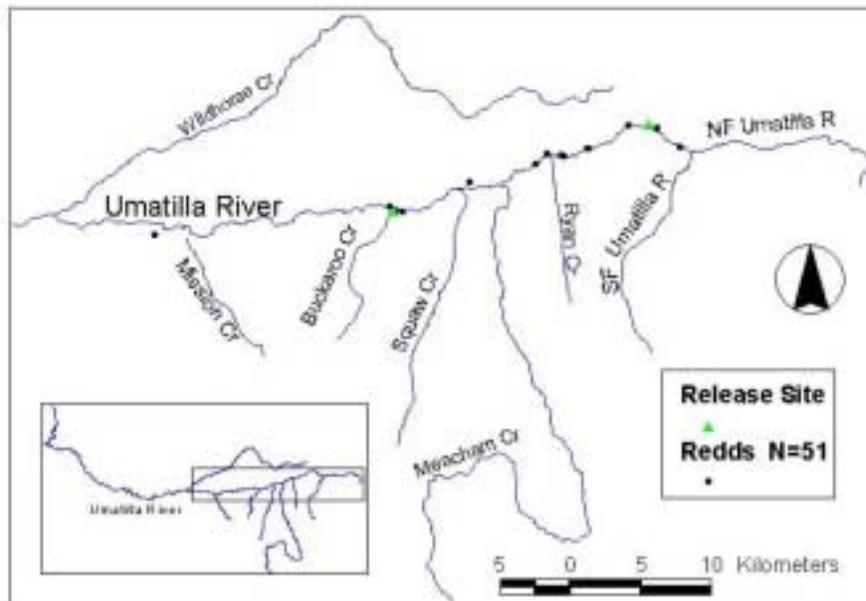


Figure 2. Map of release and spawning locations of adult Pacific lamprey in the mainstem Umatilla River and Meacham Creek.

Outmigrants.- The rotary screw trap captured a total of 133 metamorphosed lampreys and 363 ammocoetes during the sampling period. Metamorphosed lampreys were captured beginning in November 1999 to early March 2000. While a few larvae were captured beginning in October, the peak movement of larval outmigrants occurred in February; however, metamorphosed lampreys peaked in December (Figure 3). Outmigration of metamorphosed and larval lampreys was significantly correlated with river flow ($r_s = 0.315$, $P < 0.001$, and $r_s = 0.378$, $P < 0.001$, $N = 129$, respectively). The mean length for captured metamorphosed lampreys was 149 mm (range 130-172 mm). The mean length of larval lampreys was 154 mm and ranged from 68 mm to 182 mm. Only seven of the captured ammocoetes were less than 130 mm. One recapture of the 129 marked metamorphosed individuals resulted in a trapping efficiency estimate of 0.008, resulting in an abundance estimate of $17,157 \pm 14,902$ (C.I. from the Bootstrap method). No lampreys were caught at the fish collection facility at the West Extension Canal during the collection period.

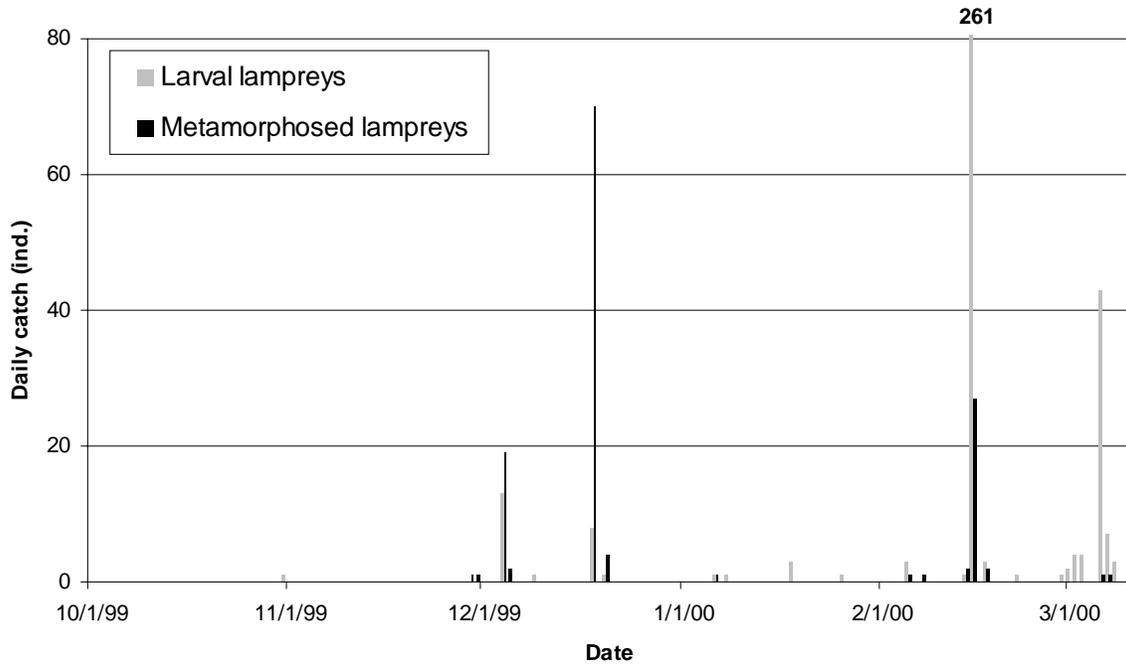


Figure 3. Catch of outmigrant metamorphosed and larval lamprey in the rotary screw trap located at rkm 1.9 in the Umatilla River.

Upstream Adult trapping and video.- During our trapping period, we captured three adult lamprey. One Pacific lamprey was captured 9/16/99. The portable assessment trap placed on the north side opening of the fishway captured two Pacific lamprey adults on April, 28th 2000. Total lengths were 485 mm and 510mm. No recaptures occurred during the sampling period. No adults were observed on video in 2000.

DISCUSSION

Spawning surveys successfully documented the ability of adult lampreys to select nest sites and spawn in the Umatilla River. While some lampreys spawned near the release sites, others dispersed in the mainstem Umatilla River and Meacham Creek. Only a few nests were observed above the upper most release site on the mainstem Umatilla River. It is unclear why lampreys did not move into the upper reaches to spawn. One possibility is that temperature is too cold and limiting movement into the upper drainage. Further research is needed to clarify spawning requirements and selection of nests for Pacific lamprey.

Electrofishing for larval lampreys has shown that natural production is restricted to the lower reaches of the Umatilla River. Even though we expected to capture some larvae resulting from the outplanting of spawning lampreys, we were unable to collect age 0+ larvae. There may be several explanations why we could not detect larvae: 1) spawning was not successful in producing larvae, 2) larvae did not migrate or drift into sampled areas, 3) larvae are not using silty type habitat, or 4) larvae were restricted to the areas near spawning sites. We expect that next year larvae will be detected in the plots.

Outmigrant trapping has shown a low level of metamorphosed and larval lampreys migrating out of the Umatilla River. The natural production is at very low levels; however, we expect increases in numbers of metamorphosed larvae by year 2004 or 2005.

Upstream adult trapping efforts have shown the numbers of adult lampreys entering the Umatilla River are very low. We were unsuccessful in recapturing marked adult lampreys in the Umatilla River. Obviously since we only captured three individuals, there was only a slight chance to recapture those fish. We speculate that with increased numbers of larvae and flows in the Umatilla River, adults will become more attracted to the river and enter to spawn.

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CHAPTER TWO

Habitat Heterogeneity and the Spatial Distribution of Larval Pacific Lamprey in an Oregon Stream

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Introduction

The Pacific lamprey, *Lampetra tridentata*, is an anadromous parasitic lamprey that completes the freshwater phase of its life cycle in streams and rivers from Baja California, Mexico along the northern Pacific Rim to Hokkaido, Japan (Ruiz-Campos and Gonzalez-Guzman 1996). A highly migratory species, the Pacific lamprey spawns in low-gradient streams, often hundreds of kilometers inland in the upper drainages of large rivers in Oregon, Washington, Idaho, and British Columbia (Beamish 1980; Hammond 1979; Richards 1980). Pacific lamprey spend more than half of their 6–10-year life span as filter-feeding larvae buried in the fine sediments of streams and are susceptible, like other species of larval lampreys, to habitat alteration by channelization and flow regulation (Kirchhofer 1995). While most efforts in the United States and Canada have been directed at controlling invasive sea lamprey (*Petromyzon marinus*) populations in the Great Lakes, recent concerns have been raised for the conservation of lampreys in the Northern Hemisphere (Renaud 1997), specifically in the Columbia River Basin (Pacific Northwest) where hydroelectric facilities have impeded migrations of Pacific lamprey (Close et al. 1995). The construction of migration barriers has occurred concurrently with modification of larval rearing habitats in headwater streams, thus a need exists to establish lamprey conservation and restoration programs to evaluate the habitat requirements of larval Pacific lamprey and develop methods for monitoring status and trends in larval abundance.

The response of larval lampreys to environmental heterogeneity is not well understood, but recent work on stream macroinvertebrates has shown that the spatial arrangement of habitat patches at large and small scales influences the distribution and abundance of benthic organisms (Li et al. 2001; Palmer et al. 2000). Previous work on the habitat ecology of larval lampreys has

either been qualitative (Baxter 1957; Hammond 1979; Pletcher 1963) or has focused on larval habitat relationships at one scale only (Beamish and Jebbink 1994; Beamish and Lowartz 1996; Malmqvist 1980; Potter et al. 1986). Although studies of larval habitat have been useful for developing a general understanding of the biology of larval lampreys, conservation and management of lamprey populations require quantitative approaches for evaluating and predicting spatial patterns in larval abundance with respect to management actions. With the increased availability of geographic information systems (GIS) and the development of spatial analysis techniques, statistical models are now being used to predict the distribution of stream fishes at fine (m) and coarse (km) spatial scales (Knapp and Preisler 1999; Torgersen et al. 1999). Similar approaches can be used for lamprey to evaluate the suitability and effectiveness of larval restoration programs and to increase the precision of efforts to control lamprey where they are an invasive species (Fodale et al. 2001). However, spatially explicit larval habitat models will require extensive field data of sufficient resolution to define the scales at which habitat variables influence patterns of larval abundance.

Our goal was to determine whether spatial patterns and habitat relationships of larval lamprey vary with respect to the spatial scale of observation. We hypothesized that (1) habitat heterogeneity at fine and coarse scales influences the measurement and detection of patterns in larval abundance, and (2) stream habitat variables predict patterns in larval abundance but play different roles at different spatial scales. We show that patterns in larval abundance are closely linked to habitat variation at two different scales and that locational factors, such as longitudinal position in the stream section and sample location within the channel unit, explain additional variation in larval abundance that might otherwise be discounted as noise. In addition, we

demonstrate that a nested sampling design is effective for evaluating patterns and habitat relationships of larval lamprey in heterogeneous stream environments.

Methods

Site selection

Larval Pacific lamprey were collected during August 2000 in the upper 55 km of the Middle Fork John Day River, a fourth- to fifth-order stream in northeastern Oregon (Figure 1). Site selection was based on high-resolution, spatially continuous GIS maps of stream habitat created from surveys conducted in 1996 and 1998 (see Torgersen et al. 1999 for study site descriptions and survey methods). Longitudinal profiles of stream temperature, channel gradient, channel unit type and dimensions (i.e., pool–riffle width, depth, and length), substrate composition, and elevation (derived from 10-m digital elevation models) were georeferenced to 1:5,000-scale hydrography and compared with respect to river km (rkm), defined as the distance upstream from the lower boundary of the survey section. Site locations were spaced systematically at 2-km intervals along the survey section and then stratified to capture a full range of habitat conditions for a total of 30 sites (Figure 1). Sites were located in the field with a hand-held global positioning unit (GPS) to within 50 m.

Larval sampling

We used a nested sampling design to evaluate heterogeneity in larval abundance and habitat at two different spatial scales—both within and among sites. Sampling locations (1 x 1-m quadrats, $n = 12$) within a site were distributed in the mid channel and along stream margins in 6 transects spaced every 10 m (Figure 1). Larvae were collected at each sampling location in

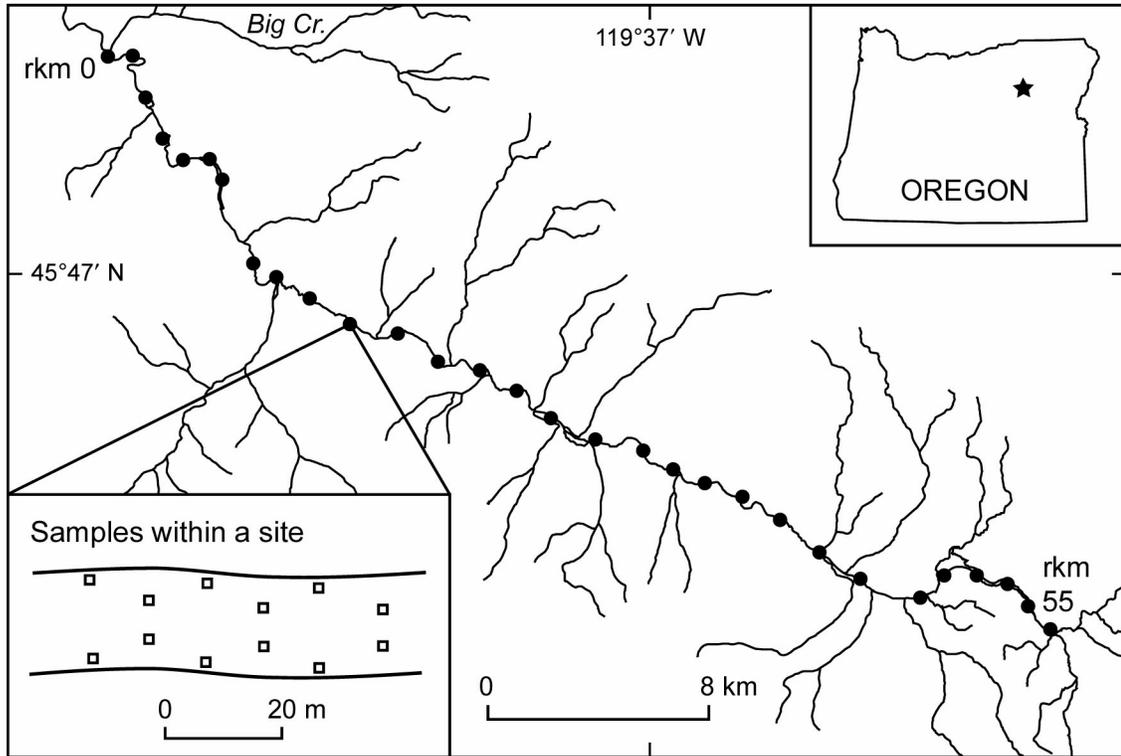


Figure 1. Nested sampling design for a survey of larval Pacific lamprey in the upper 55 km of the Middle Fork John Day River, Oregon.

two 90-s passes with a backpack model AbP-2 larval lamprey electrofishing unit (Engineering Technical Services, University of Wisconsin, Madison, Wisconsin). The electrofishing unit delivered 3 pulses·s⁻¹ (125 volts DC) at a 25% duty cycle, with a 3:1 burst pulse train (three pulses on, one pulse off) to draw larvae from the substrate (Weisser and Klar 1990). Once in the water column, larvae were stunned with 30 pulses·s⁻¹ to facilitate capture (Hintz 1993; Weisser 1994). After collection, larvae were anesthetized in buffered MS-222 (tricaine methanesulfonate at 250 mg·L⁻¹), identified on the basis of caudal pigmentation patterns (Richards et al. 1982), and measured for total length (± 1 mm) before they were returned to the stream. Depletion estimates for two-pass removal were calculated and converted to larval densities per sample (number·m⁻²) with the Capture software program (White et al. 1982; Zippin 1958). Larval abundance was defined as the sum of larval densities per site.

Habitat description

Assessment of larval habitat was conducted at the sample or site level depending upon the nature of stream habitat variables (Table 1). Measurements of water velocity at 60% depth (Model 201D flowmeter, Marsh-McBirney, Inc.) and total water depth were taken once per sample, and dominant substrate and larval habitat type were estimated visually within each 1 x 1-m sampling quadrat. The following definitions were used to classify larval habitat: type I—ideal larval habitat with a mixture of soft sediment particles including silt, clay, fine organic matter, and some sand; type II—suitable habitat similar to type I habitat but with a larger component of sand; type III—unsuitable habitat for burrowing, composed of bedrock, hard clay, cobble, or coarse gravel substrates (Fodale 1999).

Table 1. Explanatory variables evaluated for associations with the abundance of larval Pacific lamprey.

Variable	Units/ category	Data type	Description
Depth	m	continuous	water depth
Organic depth	cm	continuous	depth of organic debris overlying substrate
Velocity ^a	m·s ⁻¹	categorical	design variable based on percentiles
0–0.11			1 st to 33 rd percentile
0.12–0.23			33 rd to 66 th percentile
0.24–1.00			66 th to 100 th percentile
Unit type		binary	channel unit type
Pool	1		
Riffle	0		
Substrate		categorical	dominant substrate type in sample area
Organics	1		organic debris
Silt	2		< 0.1 mm
Sand	3		0.1–3 mm
Small gravel	4		3–10 mm
Large gravel	5		11–100 mm
Cobble	6		101–300 mm
Boulder	7		> 300 mm
Bedrock	8		
Habitat type		categorical	larval habitat classification
Type I	1		ideal
Type II	2		suitable
Type III	3		unsuitable
Position		binary	location of sample in stream channel
Margin	1		stream margin
Mid channel	0		middle of channel
Wetted width ^b	m	continuous	measured at three equally spaced transects
Canopy closure	percent	continuous	measured at three equally spaced transects
pH		continuous	measured once in the middle of each site
Conductivity ^b	µmhos·cm ⁻¹	continuous	measurement taken with pH
Gradient	percent	continuous	channel slope calculated for 50-m site
Temperature	°C	continuous	measured with remote sensing
River km	km	continuous	distance upstream from lower boundary of survey section

Table 1 – continued.

Notes: All variables were measured at the sample level except for wetted width and canopy closure (transect level) and pH, conductivity, gradient, temperature, and river km (site level).

^a Velocity was measured as a continuous variable but exhibited a nonlinear relationship with the logit and was therefore modeled as a categorical design variable.

^b Wetted width and conductivity exhibited a significant ($P \leq 0.05$) linear relationship with river km and were detrended with linear regression.

At the site level, habitat characteristics were expressed either as a proportion of samples within each habitat category (channel unit, substrate, and larval habitat types) or as site means (channel dimensions and water velocity) (Table 1). Measurements of channel gradient (Model RL-HB rotating laser, Topcon Corp.), pH, and conductivity (pH/Con 10, Oakton Instruments) were taken once per site. Percent canopy closure was assessed with a concave spherical densiometer at three equally spaced transects along the length of the site (Platts et al. 1987). Spatially continuous profiles of channel gradient and water depth were generated in a GIS and analyzed longitudinally with a 500-m moving window for gradient calculations and with locally weighted scatterplot smoothing (LOWESS) to identify trends in depth (SPSS 2001; Trexler and Travis 1993).

Statistical analysis

We used multiple logistic regression to describe the relationship between larval abundance and habitat variables within and among sites. Logistic regression has been applied effectively to predict fish–habitat relationships at a variety of scales (Dunham and Rieman 1999; Knapp and Preisler 1999; Torgersen et al. 1999) and was particularly appropriate for modeling larval response to habitat heterogeneity because it requires no assumptions regarding normality or homoscedasticity (Hosmer and Lemeshow 1989; Trexler and Travis 1993). The logistic model uses maximum likelihood estimation and the logit transformation of a binary response variable to predict the probability of occurrence in relation to binary, categorical, or continuous explanatory variables. To evaluate larval habitat relationships within sites (i.e., among samples), we modeled larval occurrence (binary response) with respect to continuous and categorical habitat variables measured at sample and transect levels (Table 1). To assess larval habitat relationships among sites, we compared the spatial correspondence of peaks and troughs in larval

abundance with longitudinal profiles of stream habitat. We created a binary response variable (i.e., peaks and troughs in larval abundance) by relativizing larval abundance with respect to the median. Site-level explanatory variables were also analyzed as binary variables relativized with respect to either the median or the residuals from linear regression if variables exhibited a significant ($P \leq 0.05$) linear relationship with river km (e.g., wetted width and conductivity).

Logistic regression is robust to heterogeneity and non-normality inherent in ecological data, but it is sensitive to multicollinearity among predictor variables and to nonlinear relationships between continuous explanatory variables and the linear predictor (i.e., the logit transform of the fitted response) (Tabachnick and Fidell 2001). We assessed correlations between habitat variables for multicollinearity and graphically evaluated relationships between continuous explanatory variables and the linear predictor. Only one continuous variable, velocity (sample-level), exhibited a nonlinear relationship with the linear predictor and was converted to a categorical design variable based on percentiles. To incorporate spatial structure into the logistic model and account for spatial dependence, we included locational predictors (i.e., river km and sample position in the stream channel) in both site- and sample-level models (Knapp and Preisler 1999). Habitat variables were evaluated individually for significant associations with larval abundance (likelihood ratio χ^2 test, $P \leq 0.05$); variables and combinations of variables were then selected manually and included in the final multivariate model if they contributed to a significant drop in deviance (Ramsey and Schafer 1997). To determine whether the logistic function adequately fitted the observed data, we used the Hosmer–Lemeshow χ^2 test, in which small probability values indicate a significant lack of fit (Hosmer and Lemeshow 1989). The relative explanatory power of respective logistic models was measured with the Nagelkerke coefficient of determination (R^2) (Nagelkerke 1991).

Logistic regression and all other statistical analyses were performed with Statgraphics Plus statistical software (Statistical Graphics 1999).

Results

Spatial distribution of larval lamprey

Larval lamprey occurred throughout the 55-km survey section of the Middle Fork John Day River. A total of 1,414 larvae were collected, and larval abundance for the sampled area (360 m²) was estimated at 1,609 larvae. Variation in larval occurrence was low among sites and high within sites, with larvae present in 28 of the 30 sites but in only 111 of the 360 samples. Mean larval density (\pm SD) was higher in sites (54 \pm 62 larvae) than in samples (4 \pm 13 larvae). Maximum larval density (number·m⁻²) in a 1-m² sample ($n = 118$) was approximately 50% of the maximum number of larvae found in a 12-m² site ($n = 232$).

Identification of larvae at the time of capture indicated that the Pacific lamprey (*L. tridentata*) was the only species of lamprey present in the upper Middle Fork John Day River. Variation in larval length suggested that multiple age-classes were present throughout the survey section. Total length of the larvae ranged between 20 and 160 mm and varied significantly both longitudinally and laterally in the stream channel (Mann-Whitney Wilcoxon test, $P < 0.01$). Median larval length was greater in sites in the upper 27 km of the survey section (76 mm) than in downstream sites (59 mm). Within sites, median larval length was greater in samples collected in the mid channel (70 mm) than along stream margins (61 mm).

Larval abundance was patchy at large scales (5–10 km) and peaked at rkm 10, 26, and 43 (Figure 2a). Reaches with multiple consecutive sites exceeding median larval abundance occurred at rkm 7–18 and 40–45 and were identified as major larval rearing areas. Although

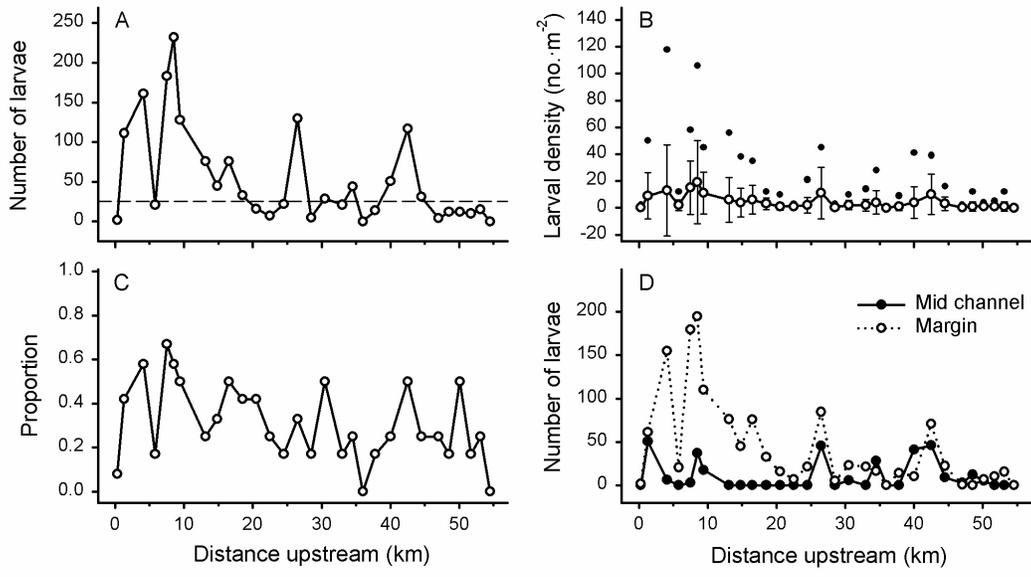


Figure 2. Longitudinal variation in larval abundance within and among sites. Patterns in larval abundance are plotted versus river km, the distance upstream from the lower boundary of the survey section: (a) larval abundance (dashed horizontal line indicates the median), (b) variation in larval density within sites, (c) the proportion of samples containing larvae within individual sites, and (d) the distribution of larvae in mid-channel versus stream margin sampling locations.

larvae were present throughout the survey section, they were 3.4 times more abundant in the lower 27 km of the stream. Peaks in larval abundance among sites corresponded with longitudinal patterns of maximum larval density within sites (Figures 2a and 2b). Variation in larval density within high-density sites was high, indicating that the number of larvae per site was strongly influenced by relatively few samples containing large numbers of larvae (Figure 2b).

The proportion of samples containing larvae within individual sites was low throughout the survey section, even in sites where larvae were abundant (Figure 2c). In 73% of the sites, larvae were present in fewer than 50% of the samples. We evaluated the linear relationship between the proportion of samples containing larvae versus larval density per site and determined that the resolution of the sampling grid adequately captured variation in larval abundance in each 50-m site. Larval density was significantly correlated with the proportion of samples containing larvae and explained 55% of the variation in larval occurrence among samples (positive relationship, $P < 0.01$).

Spatial patterns of larval abundance within sites were heterogeneous, particularly laterally across the stream channel. Over 80% of the larvae were found along stream margins, and the difference in larval abundance between stream margin versus mid-channel habitats was most pronounced in downstream reaches (Figure 2d). Peaks in mid-channel larval abundance at rkm 2, 8, 27, and 40–43 corresponded with peaks in larval abundance in stream margins. Within high-density sites, larval abundance was highest in channel margins and was generally skewed towards the left or right stream bank (Figures 3a, 3b, and 3c). In both high- and low-density sites, more than 40% of the larvae in each site were concentrated in one or two neighboring samples (see Figure 3 for representative sites).

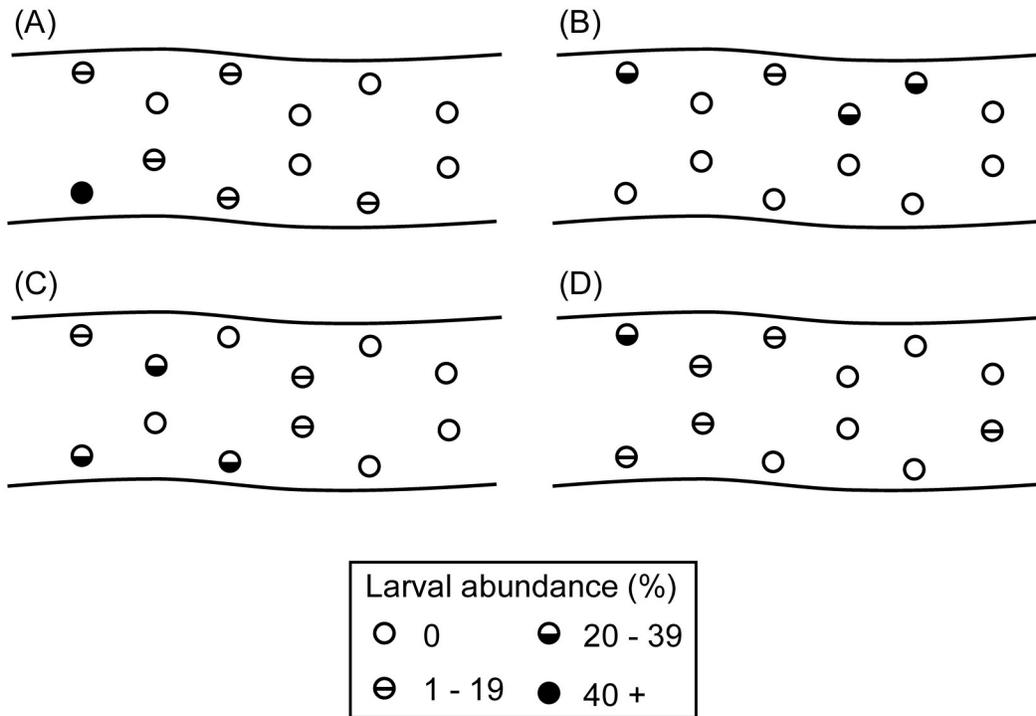


Figure 3. Spatial distribution and percent abundance of larval lamprey within sites. Sites represent high- and low-density reaches in the longitudinal distribution of larval lamprey: (a) rkm 8.5, $n = 232$, (b) rkm 26.5, $n = 130$, (c) rkm 42.5, $n = 117$, and (d) rkm 50.1, $n = 12$.

Habitat heterogeneity at multiple scales

Spatially continuous longitudinal patterns in channel gradient and water depth revealed the complex geomorphic structure of the Middle Fork John Day River survey section (Figure 4). Peaks in LOWESS-smoothed water depth at rkm 10, 30, and 42 indicated the presence of reaches with high frequencies of deepwater habitats (e.g., pools and glides). Reaches high in channel gradient and low in water depth occurred at rkm 2, 15, 24, and 36 and were identified as riffle reaches. A cascade reach characterized by high gradient (3%) and moderate depth (0.4 m) was located at rkm 38. The longest contiguous low-gradient reaches of the survey section coincided with the highest peaks in water depth at rkm 8–12 and 40–43 (Figure 4). Physical characteristics of survey sites reflected spatial trends and heterogeneity in stream habitat in the Middle Fork John Day River study section (Figure 5). Wetted width and conductivity were the only two habitat variables that exhibited linear longitudinal trends (Figures 5a and 5b). Average distance between peaks in longitudinal habitat profiles provided a rough indicator of the varying scales at which habitat heterogeneity was expressed. Longitudinal profiles of water depth and canopy closure reflected stream valley and geomorphic processes occurring over long distances (15–20 km) (Figures 5c and 5d), whereas wetted width and conductivity (detrended), velocity, and channel gradient varied over relatively short distances (5–10 km) (Figures 5a, 5b, 5e, and 5f). Patterns of substrate composition also reflected the influences of fluvial and depositional processes occurring over long (e.g., sand, silt, type I habitat, and organic debris) versus short distances (e.g., cobble and type II habitat) (Figure 6).

Spatial heterogeneity in larval habitat was particularly apparent at small spatial scales within and among adjacent channel units (< 50 m). Cobble and large gravel substrate types varied inversely and dominated the survey sites, typically composing over 60% of the sampled

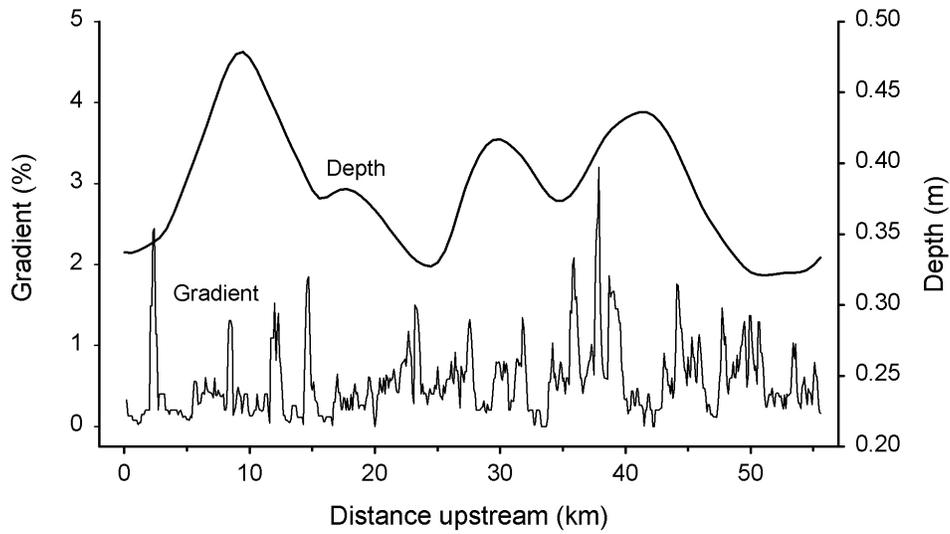


Figure 4. Spatially continuous longitudinal profiles of channel gradient and water depth in the upper 55 km of the Middle Fork John Day River. The longitudinal profile of channel gradient was generated from a 10-m digital elevation model with a 500-m moving window for slope calculations. LOWESS smoothing was used to evaluate spatial patterns in water depth.

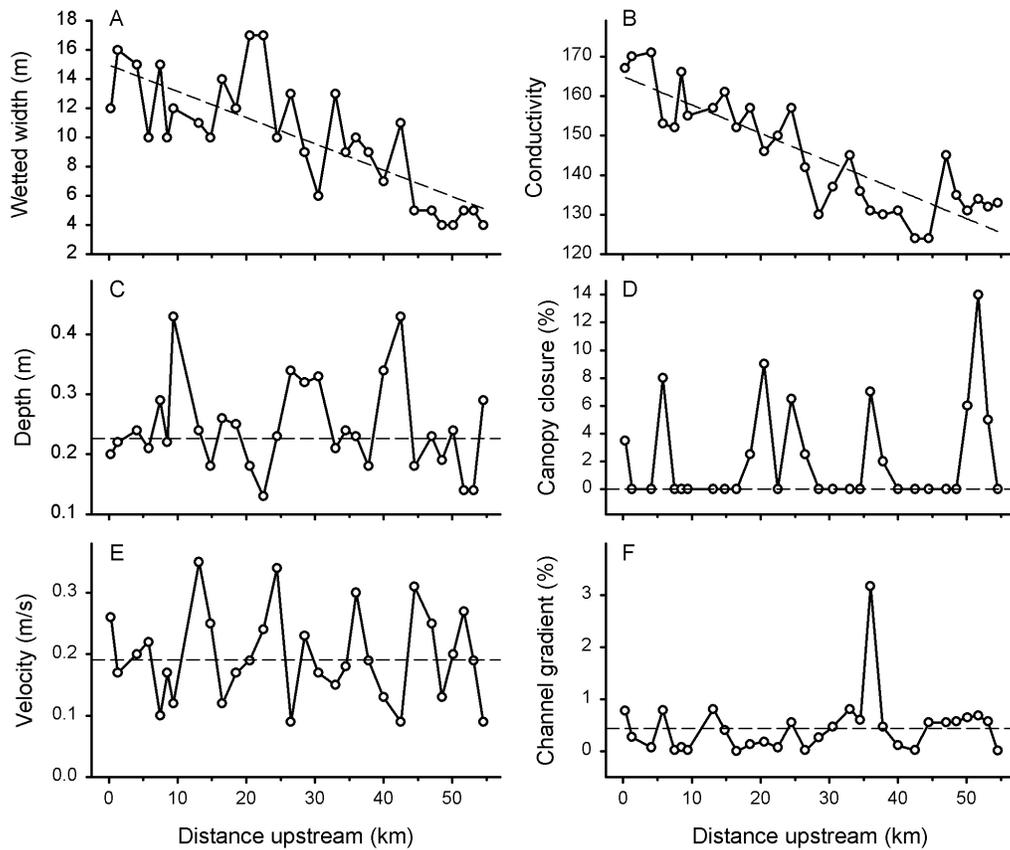


Figure 5. Longitudinal variation in channel morphology and stream habitat among sites. Longitudinal habitat patterns are plotted versus river km, the distance upstream from the lower boundary of the survey section: (a) wetted width, (b) conductivity, (c) water depth, (d) canopy closure, (e) water velocity, and (f) channel gradient. Dashed lines define peaks and troughs with respect to the median (horizontal line) or the residuals from linear regression (trend line).

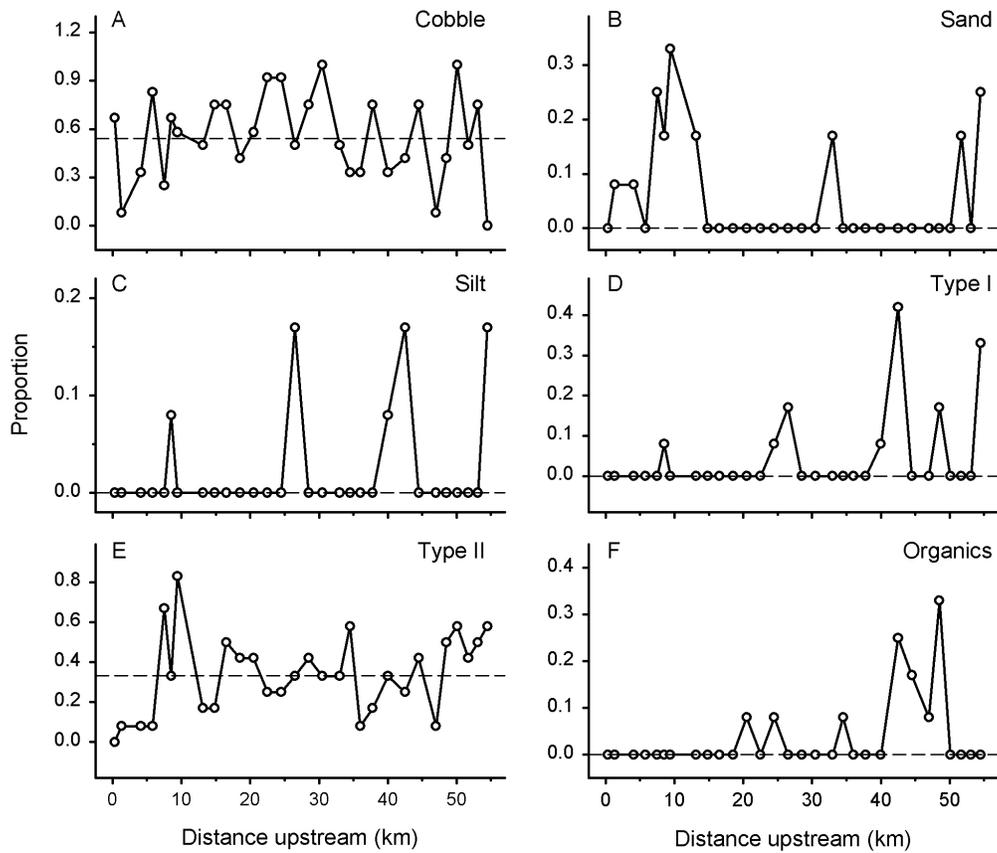


Figure 6. Longitudinal variation in substrate composition among sites. Substrate composition is expressed as the proportion of samples within in each substrate category. Longitudinal patterns are plotted versus river km, the distance upstream from the lower boundary of the survey section: (a) cobble, (b) sand, (c) silt, (d) type I burrowing habitat, (e) type II burrowing habitat, and (f) organic debris. Dashed horizontal lines define peaks and troughs with respect to the median.

area per site (Figure 6a). Sand, silt, and organic debris made up very small proportions (< 0.40) of the sampled area (Figures 6b, 6c, and 6f). The proportion of suitable burrowing habitat (types I and II) within a given site was rarely greater than 0.60 (Figures 6d and 6e). Mean within-site variability (coefficient of variation) in wetted width was low (0.14) compared to water depth (0.51) and water velocity (0.78). Differences in wetted width, water depth, and water velocity within sites ranged 0.4–7.8 m, 0.2–0.8 m, and 0.2–1.0 $\text{m}\cdot\text{s}^{-1}$, respectively.

Multivariate analysis: habitat associations of larval lamprey

Spatial associations between larvae and stream habitat variables varied depending on the scale of statistical analysis. Individual habitat variables explained 14–35% of the variation in the relative abundance of larvae among sites and 2–29% of the variation in larval occurrence among samples (Table 2). Depth, canopy closure, and gradient were the most important predictors of larval abundance at the site level, whereas velocity, burrowing habitat type, and sample position in the channel were the strongest predictors of larval occurrence at the sample level. Velocity, canopy closure, and river km were significantly associated ($P \leq 0.05$) with patterns of larval abundance at both sample and site levels; however, variables that were strong predictors at one scale were relatively weak predictors at the other scale.

After accounting for other site-level explanatory variables, water depth and canopy closure were the only habitat variables that significantly predicted the relative abundance of larval lamprey among sites (likelihood ratio χ^2 test, $P \leq 0.05$) (Table 3). Peaks in water depth and troughs in canopy closure corresponded with peaks in larval abundance and explained 49% of the variation in the relative abundance of larvae. The relative explanatory contribution of each variable, defined as the change in coefficient of determination that resulted from removing the

Table 2. Coefficients of determination from bivariate logistic regression of site- and sample-level variables explaining the abundance of larval lamprey. The coefficient of determination (R^2) indicates the relative explanatory power of variables positively (+) or negatively (-) associated with larval abundance ($P \leq 0.10$).

Variable ^a	Site level	Sample level
Depth (+)	0.35 **	–
Organic depth (+)	0.14	0.03 **
Velocity (-)	0.20 *	0.29 ***
Unit type		
Pool	–	0.06 ***
Substrate		
Organics, silt, and sand	–	0.07 ***
Habitat type		
Type I and II	–	0.26 ***
Position		
Margin	n/a	0.20 ***
Wetted width (+)	–	0.02 *
Canopy closure (-)	0.29 **	0.02 *
Gradient (-)	0.27 **	n/a
River km (-)	0.21 *	0.03 **

* The asterisk symbol indicates the significance level of explanatory variables: $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***).

^a Position (margin) and gradient were modeled only at the levels at which they were collected.

Table 3. Model selection results from multiple logistic regression of site-level habitat variables explaining the relative abundance of larval lamprey among sites.

Variable	Estimated coefficient	Standard error	Likelihood ratio χ^2 test		R^2 ^a
			χ^2	<i>P</i>	
Intercept	2.00	1.30			
Depth	3.15	1.33	8.13	0.004	0.21
Canopy closure	-2.67	1.31	5.43	0.020	0.13
River km	-0.09	0.04	7.11	0.008	0.18

Notes: Regression statistics for the logistic model ($n = 30$) were deviance ($-2 \log L$) = 42 (intercept only) and 21 (intercept and covariates); likelihood ratio χ^2 test (3 df), $P < 0.001$; $R^2 = 0.67$.

^a The R^2 value shown for each variable represents the change in coefficient of determination that resulted from removing that variable from the model.

variable from the model, was greatest for depth (21%), followed by river km (18%), and canopy closure (13%). The site-level model, with river km added to account for spatial autocorrelation, produced a good fit of the factors influencing larval distribution in the Middle Fork John Day survey section ($R^2 = 0.67$). The model passed the goodness of fit test ($P = 0.11$) and correctly predicted 90% of the observations.

Habitat variables associated with the occurrence of larval lamprey among samples differed from variables associated with the relative abundance of larvae among sites. After accounting for other explanatory variables, low water velocity, suitable burrowing habitat (types I and II), and pool habitats were the most important habitat variables explaining variation in larval occurrence at the sample level (Table 4). Locational variables, sample position (margin) and river km, contributed to a significant drop in model deviance and explained 4–6% of the variation in the sample-level model. The full model correctly classified 79% of the observations at the 0.5 cutoff level, passed the χ^2 goodness of fit test ($P = 0.31$), and explained approximately half of the variation in larval occurrence among samples ($R^2 = 0.48$).

Table 4. Model selection results from multiple logistic regression of sample-level habitat variables explaining the occurrence of larval lamprey within sites.

Variable	Estimated coefficient	Standard error	Likelihood ratio χ^2 tests		R^2 ^a
			χ^2	<i>P</i>	
Intercept	-2.91	0.50			
Velocity			13.51	0.001	0.04
0–0.11 m·s ⁻¹	1.56	0.45			
0.12–0.23 m·s ⁻¹	0.81	0.46			
Unit type			10.25	0.001	0.03
Pool	1.10	0.36			
Habitat type			24.76	< 0.001	0.07
Type I	1.60	0.69			
Type II	1.61	0.33			
Position			12.96	< 0.001	0.04
Margin	1.18	0.33			
River km	-0.04	0.01	20.72	< 0.001	0.06

Notes: Regression statistics for the logistic model ($n = 356$) were deviance ($-2 \log L$) = 442 (intercept only) and 294 (intercept and covariates); likelihood ratio χ^2 test (7 df), $P < 0.001$; Nagelkerke $R^2 = 0.48$.

^a The R^2 value shown for each variable represents the change in coefficient of determination that resulted from removing that variable from the model.

Discussion

Patterns in larval abundance were closely linked to variation in habitat structure. Physical gradients in channel morphology established the geomorphic template for larval distribution among reaches and set the context for larval habitat associations at finer scales. Larval abundance was highest in reaches where water depth was high and channel gradient was low. More precise estimates of larval abundance, however, required statistical analysis at progressively smaller spatial scales because larval habitat relationships were scale-dependent. Water depth and an open riparian canopy were positively associated with larval abundance at large scales (5–10 km) but were unrelated to patterns of larval occurrence at small scales (< 50 m). Conversely, low water velocity, suitable burrowing substrate, and pools, explained variation in larval occurrence at small scales but were ineffective at explaining variation in the relative abundance of larvae at large scales. Habitat variables alone explained a large proportion variation in larval abundance, but locational factors such as sample position and river km explained additional variation that might otherwise be discounted as noise. The complexities of larval habitat relationships and spatial heterogeneity in the stream environment have important implications both for our understanding of the biology of larval lampreys and for their management and conservation.

Habitat heterogeneity and larval distribution

A hierarchical model of habitat classification provides a framework for evaluating heterogeneity in streams based on nested geomorphic features at section (10–100 km), reach (0.1–10 km), unit (1–100 m), and subunit (0.01–10 m) levels (Gregory et al. 1991). Environmental heterogeneity in streams can thus be described as patches within patches at

sequentially smaller spatial scales (Kotliar and Wiens 1990). Fishes may respond to habitat heterogeneity differently at each respective scale, but patterns of distribution and abundance are products of the collective spatial structure of the riverine environment (Baxter and Hauer 2000; Montgomery et al. 1999). Investigations of lamprey ecology in streams and rivers have addressed the interplay of macro- and microenvironmental factors and their influence on larval distribution (Baxter 1957; Hardisty and Potter 1971). However, quantitative analysis of such relationships requires sampling approaches that are specifically designed to characterize spatial variance structure at multiple scales (Li et al. 2001). By collecting data with a nested sampling design, we were able to separate the relative influences of habitat heterogeneity on larval abundance patterns at two different spatial scales.

We observed that habitat heterogeneity both within and among sites influences the measurement and perception of patterns in larval abundance and habitat use. Patterns of larval occurrence at the site level indicated that nearly the entire 55-km survey section was suitable for larval rearing, with 93% of the sites containing larvae. Similar analysis of larval occurrence at the sample level, however, revealed that suitable burrowing habitats were much more limited, with larvae present in only 31% of the samples. The perception that suitable rearing habitats were either common or uncommon was largely dependent on the scale of observation. This phenomenon of differences in spatial variance structure at small versus large scales indicated a nested structure in larval abundance patterns and a high degree of heterogeneity in habitat suitability at the unit level. Habitat heterogeneity also influenced spatial variation in larval density, which was high among sites and even higher among samples. Larvae were highly concentrated in small areas; single 1 x 1-m quadrats represented less than 10% of the sampled area and yet often contained 40–50% of the maximum number of larvae found in a site.

Detailed qualitative studies of the distribution of larval Pacific lamprey at small scales confirm our quantitative observations that concentrations of larvae are associated with patchy fluvial features such as stream margins, backwaters, eddies, insides of bends, and downstream ends of sand bars (Hammond 1979; Pletcher 1963). Highly structured larval distribution patterns at small scales are generated both passively with respect to physical gradients and actively through larval movement. Larvae often emerge from their burrows and actively disperse to locate more suitable living and feeding conditions (Potter 1980; Potter et al. 1970). Feeding primarily on suspended material (e.g., diatoms and desmids), larval lamprey have specific flow requirements (Moore and Mallatt 1980). Water velocity over larval habitats must be fast enough to provide a steady influx of food and yet slow enough to promote the deposition of soft sediments needed for burrowing. Thus, in streams with sufficient flow for filter feeding, suitable burrowing habitats may be more limited than is immediately apparent from large-scale habitat patterns.

Spatial context and larval habitat relationships

While many studies have investigated the influence of environmental variables on patterns in larval lamprey abundance, relatively little is known about variation in larval habitat relationships as a function of spatial scale. Broad-scale distribution patterns of larval lamprey have been attributed to variation in channel gradient within and among streams (Baxter 1957; Pletcher 1963; Young et al. 1990). We also observed that patterns in larval abundance follow longitudinal trends in channel gradient; however, the significance of these relationships may depend on the scale over which gradient measurements were taken. Channel gradient is

particularly susceptible to problems of scale because it can be calculated over a range of distances.

In our study of larval distribution in a fourth- to fifth-order stream, channel gradient calculated in a 500-m moving window corresponded with large-scale larval abundance patterns, but channel gradient measured at the site level (50 m) was not a significant predictor of the relative abundance after accounting for water depth and canopy closure. Based on our observations of larval distribution we suspect that the relative influence of channel gradient as a predictor of larval abundance increases at larger spatial scales. An explanation of this phenomenon is complex—at the unit scale, channel gradient is stepped rather than gradual and low-gradient units are often nested within high-gradient reaches. Sediment transport processes during high flow events are not conducive to fine-particulate deposition in high-gradient reaches, so it is unlikely that larvae in high-gradient reaches will find suitable burrowing habitat even though they may be located within relatively low-gradient, low-velocity units.

Larval associations with low water velocity, fine-particulate burrowing substrates, and pool habitats described for other species of lamprey (Beamish and Jebbink 1994; Beamish and Lowartz 1996; Malmqvist 1980; Potter et al. 1986) confirm our observations of habitat selection by larval Pacific lamprey (Hammond 1979; Pletcher 1963; Richards 1980). However, our findings differ substantially from published work on the habitat ecology of larval lampreys because we identified that these habitat variables were only significant at small spatial scales. Moreover, variables we identified as positively associated with larval abundance at large scales (e.g., water depth and an open riparian canopy) were generally considered negative correlates of larval abundance in the published literature (as cited in Potter et al. 1986). Water depth was not a significant predictor of larval occurrence among samples but was highly significant at large

scales. Larvae were more abundant in sites with greater than median depth, but within sites larvae were located along stream margins regardless of depth. At small spatial scales (< 50 m), larvae selected pools over riffles because the morphology of pool margins was more conducive to sediment deposition than riffle margins. The interaction between depth, water velocity, and channel morphology provides a potential explanation for the differential responses of larvae to depth among and within streams and in different seasons (Pletcher 1963; Potter et al. 1986). Suitable burrowing sediments are deposited along stream margins during high flow events, leaving deeper thalweg habitats washed clean of sediments during summer low flow. Water depth at large spatial scales, however, was positively associated with larval abundance patterns because deep reaches were structurally complex and therefore likely to meet the specific velocity and substrate requirements necessary for larval settlement.

We observed that the relationship between larval abundance and riparian vegetation may be related more to geomorphic factors than to larval behavior as has been suggested in the literature. Potter et al. (1986) found that shade was a significant predictor of larval density at small scales and attributed the association to photophobic behavior by larvae. We could not test the association between larval occurrence and riparian canopy at small scales because we did not measure canopy closure or shade at the appropriate scale (i.e., we measured canopy closure at the transect level). However, at the site level we observed exceptionally high larval densities (> 100 larvae·m⁻²) in the most exposed sites and found that an open canopy was an important predictor of larval abundance at large scales. No other quantitative studies of larval lamprey have analyzed large-scale associations with riparian cover, so it is difficult to evaluate this relationship in the context of previous research. However, qualitative observations of larval Pacific lamprey rearing in Oregon coastal streams confirm a negative association with riparian canopy closure

and may indicate differences in habitat selection unique to the species (Kan 1975). Exposed reaches in the Middle Fork John Day River study section occurred consistently in meadow reaches; thus, an open riparian canopy may be an indicator of large-scale habitat factors positively associated with patterns of larval abundance (e.g., low-gradient valley segments). Primary productivity and the availability of larval food sources are also high in exposed meadow reaches and may provide another explanation for the observed concentrations of larvae in exposed sites.

Larval abundance patterns are directly linked to environmental variables, but the spatial context of biological factors such as the spawning distribution of adults also plays an important role in larval distribution. Larvae were much more abundant in downstream versus upstream portions of the study stream even though upstream habitats had greater proportions of suitable burrowing habitat (Figures 6b, 6e, and 6f). The disproportionate distribution of larvae in downstream reaches may be attributable to adult spawning patterns. Pletcher (1963) observed that larval rearing areas were often located within or adjacent to reaches where spawning occurred. It is important to consider the effects of spatial context because some methods of analysis are sensitive to non-normally distributed data. Multiple regression of larval density, as opposed to relativized abundance, does not account for spatial context (e.g., the effect of adult spawning patterns) in larval distribution patterns and may erroneously identify sites with the highest larval densities as optimal habitats. Statistical analyses can be designed to account for spatial context by relativizing the response variable with respect to the median, thereby creating a binary response variable that places peaks in larval abundance on equal footing (Torgersen et al. 1999). Further incorporation of spatial structure in statistical analysis can be achieved by including locational variables (e.g., river km and sample position in the stream channel) in the

regression model (Knapp and Preisler 1999). Direct inclusion of locational variables relaxes the assumption in regression analysis that observations be spatially independent and may explain additional variation in the regression model.

Management implications

The measurement of patterns in larval abundance and the detection of larval habitat relationships are important components of lamprey monitoring programs. Recent technical advancements in larval sampling and habitat assessment methods in lentic environments have shown that high-resolution data can be collected over large areas and provide direct information on variability in larval distribution over a range of spatial scales (Bergstedt and Genovese 1994; Fodale 1999; Fodale et al. 2001). We found that larval habitat assessment methods in small streams can benefit from spatially explicit as opposed to random sampling approaches. While a stratified random sampling design may be effective for obtaining larval population estimates in homogeneous stream habitats (Pajos and Weise 1994), extrapolation of larval abundance in complex stream environments should be based on spatially continuous habitat surveys (Hankin and Reeves 1988). A Hankin–Reeves survey approach has yet to be applied for larval lamprey, but the information provided in this paper on spatial variation in larval abundance will be useful in designing future studies with the objective of obtaining larval population estimates.

The size, number, and arrangement of sample plots have effects on the detection of patterns in larval distribution and habitat relationships. Collecting multiple samples within sites increases precision in distinguishing between suitable and unsuitable habitats and achieves a higher level of reproducibility than by taking fewer large samples (Southwood and Henderson 2000). Given the high degree of heterogeneity in larval abundance patterns at small scales, we

concluded that twelve 1-m² samples distributed over a 50 m of stream were more effective at capturing variability in larval abundance than a single 12-m² sample. Potter et al. (1986) also recommended that sampling area for a given electrofishing quadrat be small (< 1 m²) and that samples be distributed due to the high degree of environmental heterogeneity likely to occur in large samples. In our review of the literature on larval habitat, we found that a nested approach for sampling larval lamprey is generally uncommon and could be applied more frequently in studies of this type, particularly when there has been no a priori assessment of habitat heterogeneity in the environments to be surveyed. Similar guidelines with respect to environmental heterogeneity apply in the selection of larval sampling sites. In choosing the appropriate distribution and number of sites, a systematic design is superior for detecting spatial pattern but is also more labor-intensive. However, spatially continuous stream habitat data and 10-m digital elevation models are often available through natural resource agencies and can be used to stratify site locations based on longitudinal habitat patterns.

Understanding the relationship between habitat heterogeneity and the spatial distribution of larval lamprey is important for establishing conservation and restoration plans and may also be useful in controlling lamprey where they are an invasive species. Simplification of stream habitats through channelization has been identified as a significant cause for the decline of lampreys in Europe (Bohl 1995; Kirchhofer 1995) because larval lampreys in headwaters and low-order streams depend on complex channel structures (e.g., meanders, bars, alcoves, backwaters, and large wood) to create environments suitable for burrowing and filter feeding. Suitable larval habitats may occur throughout an entire stream section but be relatively limited at smaller spatial scales. Lamprey conservation and restoration efforts in rivers and streams need to recognize the importance of habitat heterogeneity at multiple scales and focus on maintaining

and promoting complexity in channel morphology and sediment composition. Increased understanding of habitat heterogeneity and larval abundance also has implications for lamprey management and control, both for the efficient application of lampricide and for the regulation of flow in reservoirs. While managing for habitat heterogeneity is likely to improve habitat conditions for declining lamprey populations in fast-flowing streams, it may actually aid in lamprey control in regulated rivers and reservoirs where channel simplification and flow regulation have decreased water velocities and promoted the homogeneous deposition of fine sediments suitable for larval settlement. In either capacity, for conservation or control, habitat heterogeneity is an important component in the biology of larval lampreys and warrants further descriptive and experimental study.

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CHAPTER THREE

Olfactory Sensitivity of Pacific Lampreys to Petromyzonol Sulfate

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INTRODUCTION

Pacific lamprey (*Lampetra tridentata*) populations are depressed in the Columbia River Basin (CRB) (Close et al. 1995). There are a variety of factors related to the increase in human populations and industrial development of the region that might be responsible for the declines in numbers of Pacific lampreys. Two factors that rank high on this list of potential sources of problems for Pacific lampreys in the CRB are passage constraints at hydroelectric facilities and habitat degradation. To a lesser extent, climatic changes might play a role in the distribution of Pacific lampreys in the CRB. Tribes, states, and federal agencies have joined together to learn more about lampreys in the CRB with the explicit goal of rehabilitating Pacific lamprey populations. Native American treaty tribes in the region have played a pivotal role in bringing attention to this problem. As a result, some studies on lamprey passage at dams, assessment of populations, identification, genetics, and habitat requirements are in progress.

Rehabilitation studies are considered a high priority. A pilot Pacific lamprey rehabilitation study is planned for the Umatilla River Basin. The Confederated Tribes of the Umatilla Indian Reservation (CTUIR) chose this site because: 1) the Umatilla River once had a self-sustaining population of Pacific lampreys but the current population is extremely low; and 2) changes in water management to restore salmon populations may also work to restore Pacific lamprey populations.

Evaluation of the rehabilitation effort is a complex process requiring knowledge of the life history and behavior of Pacific lampreys. Since little is known about Pacific lampreys, research on another species of anadromous lampreys, sea lampreys (*Petromyzon marinus*), was used to help make plans and design studies. Evidence collected in a study of sea lampreys suggests they do not home to natal streams (Bergstedt and Seelye 1995). Instead, they use their

olfactory ability to "sense" the presence of lampreys in a stream as migratory cues. Adult sea lampreys are attracted to the presence of larval lampreys (Bjerselius et al. 2000). This attraction is believed to be a response to bile salts released by the larval lampreys. Adult sea lampreys are attracted most strongly in the early period of upstream migration (Li et al. 1995; Li and Sorensen 1997). As the sea lampreys sexually mature, their olfactory sensitivity to other adult sea lampreys increases. Adult male sea lampreys are attracted to adult female sea lampreys and repulsed by other adult male sea lampreys (Bjerselius et al. 2000).

Preliminary data on Pacific lampreys shows that their larval forms produce bile salts (Sorensen unpublished data). It is possible that these bile salts act as a migratory cue, drawing upstream migrating Pacific lampreys into streams where healthy populations of larval lampreys exist. Pacific lampreys enter fresh water from the ocean over several months (spring through late summer), as much as twelve months before they are mature (Beamish 1980). Sea lampreys enter the streams and spawn within three to four months (Applegate 1950). The longer period Pacific lampreys spend in fresh water while they sexually mature suggests that they may show an extended period of sensitivity to bile salts than sea lampreys. If Pacific lampreys prefer to migrate into tributaries to the Columbia River due to their attraction to larval lampreys producing bile salts, knowledge of this behavior could aid in the rehabilitation of Pacific lamprey populations in the Umatilla River. Therefore, we have proposed to conduct several studies sequentially to determine the role of pheromones in the upstream migration of Pacific lampreys. The objective of this research is to examine the relative sensitivity of upstream migrating Pacific lampreys to bile salts during the freshwater phase of their sexual maturation.

OBJECTIVES: YEAR 1

Design, construct, and test an apparatus to measure olfactory response with electrophysiological techniques (hereafter referred to as "EOG"). Components needed to build

an EOG will be identified, purchased, and assembled at the CRRL. Staff at the CRRL will become familiar with the collection of data from the EOG. Study design for implementation in Year 2 of this project will be refined.

Tasks:

- Review the available literature concerning olfaction in lampreys and fishes
- Contact experts in the field currently working on lamprey olfaction for technical advice; arrange a visit to the CRRL by one of these experts
- Visit a site where an EOG is being applied to lampreys in a similar fashion to our planned activities
- Construct and test an EOG apparatus at the CRRL
- Complete a study plan detailing activities for Year 2

METHODS and MATERIALS

Dr. Peter Sorensen at the University of Minnesota and Dr. Weiming Li agreed to provide technical advice and guidance as we conduct this work. These two scientists are the leading experts on pheromones in lamprey olfaction and behavior. We used the EOG design developed by Drs. Sorensen and Li in their studies as a template for our apparatus. The equipment we purchased is listed in Appendix A

Overview--During the first year, equipment was tested, employees were trained, and techniques were developed. These preliminary experiments were performed to enable us to conduct actual experiments on upstream migrating Pacific lampreys starting early in their migration (as soon after they entered the stream from the ocean as possible) in the second year.

Collection of lampreys--Upstream migrating Pacific lampreys were collected from the Columbia River at Bonneville Dam during June – September 2000. Only lampreys showing no outward signs of the onset of sexual maturity were retained from those collected. These lampreys were held in flow-through tanks provided with surface water (from the Little White Salmon River) at the CRRL.

Source of bile salts--Petromyzonol sulfate was purchased from Toronto Research Chemicals Inc. Arrangements were made with Dr. Peter Sorensen to assay stock solutions of petromyzonol sulfate to be used in experiments.

Description of the EOG apparatus:

A “Faraday box” was constructed to house the EOG apparatus, shielding the electrodes from extraneous radio waves and other interferences. A 1/2 inch thick piece of steel plate, 40"X24" is the base of the box. Steel tubing was used to construct a frame 30" high and 24" deep. Aluminum sheets were cut to fit the top, sides, and back of the box and attached to the steel frame. The entire box was painted and placed on the lab bench on top of a piece of closed-cell foam (to dampen vibrations). A 10 gauge copper wire was bolted to the frame and attached outside to an 8' steel grounding rod driven into the ground.

A recirculating water system was constructed with a temperature controller to maintain temperature within 1°C. The volume of water in this system is approximately 45 l. The 40 l holding tank sits on a small platform built adjacent to the lab bench. A small pump provided

water to the EOG apparatus. The waterbath in the Faraday box will hold about 5 l of water. An overflow from this waterbath runs back into the constant temperature bath on the floor.

A holding trough to contain the lamprey was constructed of acrylic sheeting and lined with nonskid material. The trough was mounted on a frame and suspended in a box that catches the water from the experiment. A tube was connected to an acrylic plate in the holding trough to supply water to irrigate the gills. An overflow drain in the box allowed water to the drain to the sink in the bench top. A grounding electrode wire was connected to the Faraday box and fitted with a clip that will be attached to the lamprey's tail during trials.

A constant temperature waterbath containing approximately 2 l water was constructed from 1/4" acrylic sheeting and located on top of the Faraday box. This waterbath receives water from the reservoir described in the paragraph above and is used to hold beakers to maintain solutions at constant temperature. These beakers supply odorant and fresh water to the animal during in the experiment. The size and configuration of the head box accommodated 100 ml beakers filled with odorant and 500 ml beakers filled with fresh water. Glass siphon tubes supplied sources of water from each beaker to a multi-channel timer and a pneumatic switching valve, which allowed precise control of switching from odorant to fresh water.

The electrodes were made of borosilicate glass capillary tubes, stretched to an opening of about 0.5 mm. They were filled with 8% NaCl in a gelatin matrix. The capillary tube was held in a Ag/AgCl holders (World Precision Instrument MEH 3S). These electrode holders were mounted in an electrode cell holder (World Precision Instruments) that was inserted into a micromanipulator. Individual wires connected each of electrodes to the physiograph.

Olfactory response data collection: The physiograph consisted of a personal computer, DataQ interface board, DC amplifier, acquisition software, and data analysis software. The data were collected and stored on the hard drive. Although details of the data collection design must be adapted as we proceed the following is an outline of our current procedure:

1. Anesthetize the fish with an intramuscular injection of metomidate hydrochloride and immobilize the fish with an intramuscular injection of gallamine triethiodide.
2. Place the fish in the holding trough. Start the flow of river water (flow about 6 ml/min.) to irrigate the gills. Once assured the fish or lamprey is anesthetized and immobilized, place the animal into the EOG apparatus and connect the ground cable to the tail. Using a small scalpel and tweezers, remove the skin around the opening to the nasal cavity. Once the olfactory tissue is visible, start the flow of river water over the olfactory tissue (flow about 6 ml/min.).
3. Place the reference electrode (see electrode preparation below) on the skin of the lamprey, near the nasal opening at a spot thoroughly irrigated by the river water. Place the recording electrode on the olfactory epithelia.
4. After a suitable acclimation period (yet to be determined), hit the timer button to switch the water source to a solution of L-arginine in river water to expose the olfactory to this odor. The response will be noted on the physiograph. If the response is in an acceptable range (yet to be determined), data collection will commence. If the response to L-arginine is too low, connections to the lamprey will be adjusted to optimize the standard response. Each time the standard concentration of L-arginine is measured for the first 10 lampreys and the results are in

the proper range, an average response will be calculated. An acceptance/rejection criterion will be developed. The response to L-arginine will be measured at the beginning and end of the test period for each lamprey. We will also establish a quality control program where periodic measurements of L-arginine are taken that represent from 10 to 20% of the total measurements taken. Each time an acceptable measurement of L-arginine is made, that reading will be added to the running average for quality control. This information should be plotted and posted near the EOG apparatus.

5. Measurement of responses to petromyzonol sulfate solutions will be made using the technique described above. After an acceptable response to L-arginine has been measured, the olfactory tissue will be rinsed with river water for a minimum of 3 minutes or until the recording electrode channel returns to a stable baseline. An odorant solution will be administered for 5 seconds and the response will be recorded. Ideally, a peak area will be recorded. This will be the area under the curve during the 5 seconds the odorant is administered.

Data reduction and analysis: Peak height measurements from the standard L-arginine solution and from the material being tested will be examined using published techniques (Li et al 1995). The response data are presented as a ratio of the peak height obtained from the odorant to the average peak height obtained for L-arginine for that exposure period. If L-arginine was applied five times with acceptable responses during the testing of a lamprey, all five values are averaged and used to calculate the response ratio. These responses can be compared using analysis of variance and appropriate mean contrast procedures. As an alternative method of "standardization", we will try another method for adjusting the odorant responses. The L-

arginine measurement will be made on a fish. If the response meets the quality assurance guidelines, petromyzonol sulfate will be administered. L-arginine will be measured at the beginning and end of the testing period for a fish and it will be measured after each three-odorant samples. After all of the measurements have been collected, the application of a "correction factor" will facilitate comparisons of responses to petromyzonol sulfate from time to time and fish to fish. The correction factor will be calculated by dividing each arginine measurement by the largest for that time and lamprey. This could be the average L-arginine response divided by the largest L-arginine response. Each odorant measurement for that fish and that time period would be multiplied by the corresponding correction factor.

RESULTS AND DISCUSSION

Initial measurements with EOG—To test the operation of the system, we started our experiments with teleost fishes. Northern pikeminnow, rainbow trout, and coho salmon were used to evaluate the performance of the EOG apparatus. EOG measurements were conducted according to methods described by Li et al. (1995). Data were collected using a PC-based physiograph connected to a DC amplifier, in turn connected to a reference electrode and a recording electrode (as described above). Fish were anesthetized, placed in a holding device, and the electrodes inserted into the olfactory epithelia. The gills and nose were perfused with flowing fresh water at 15° C.

Each test started with an exposure of the fish's olfactory tissue to a solution containing amino acids or water that we had put our hands into (Li and Sorensen 1992). The resulting responses were recorded. These preliminary experiments have provided guidance on quality assurance criteria necessary for the apparatus and techniques we are using. Coho salmon were

tested for response to finger washings, serine, and glutamine. Coho response to finger washings had a magnitude of 4.13 mV (tested on 4/27/2001); average response magnitude was 4.00 mV (n = 5, range 3.41 mV to 4.33 mV; from 3 exposures to 1 fish on 4/27/2001 and 2 exposures to 1 fish on 5/24/2001). Coho response magnitude to a serine (10^{-4} M concentration) was 2.34 mV from same fish tested above on 5/24/2001; average response magnitude was 2.41 mV (n = 2, range 2.34 mV to 2.48 mV; from 2 exposures to 1 fish on 5/24/2001) (Figure 2). Coho response magnitude to glutamine (10^{-4} M concentration) was 2.48 mV from same fish tested above on 5/24/2001; average response magnitude was 2.59 mV (n = 2, range 2.48 mV to 2.69 mV; from 2 exposures to 1 fish on 5/24/2001) (Figure 3).

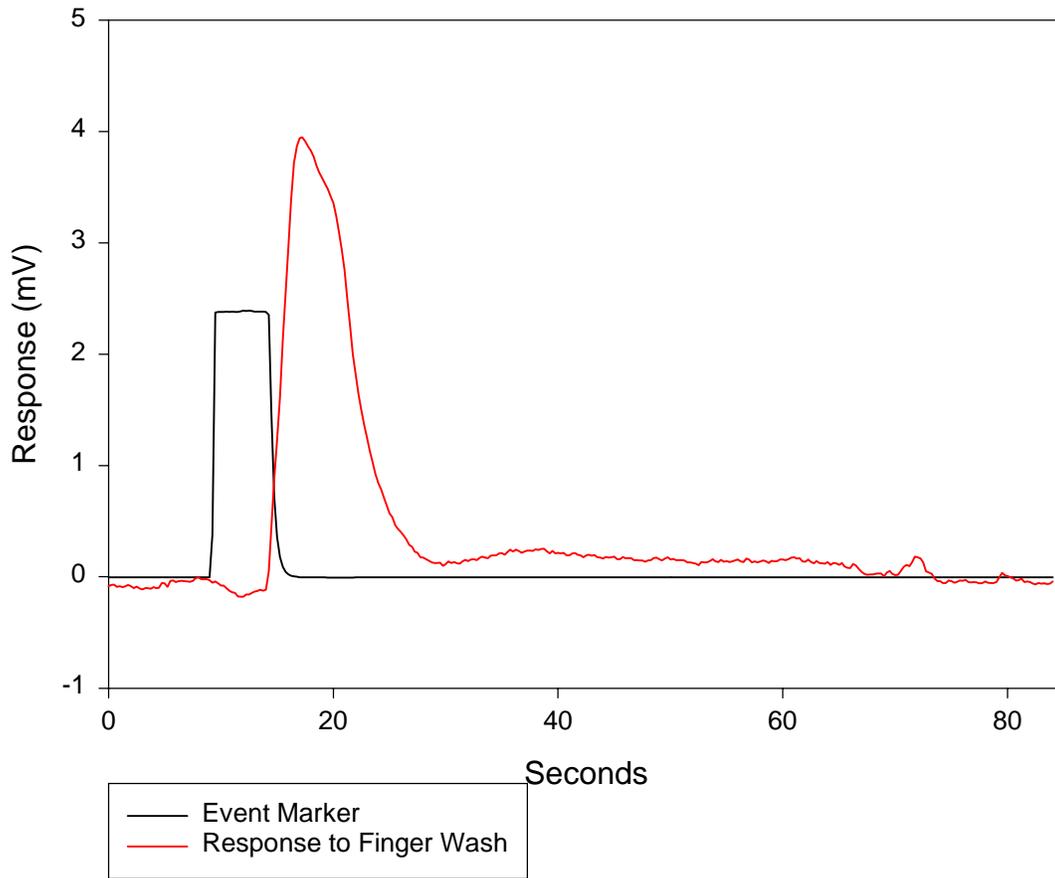


Figure 1. Olfactory response of coho salmon to finger washings as measured with electro-olfactogram. Response magnitude is 4.13 mV from a single fish tested on 4/27/2001. Event marker denotes time of release of odorant.

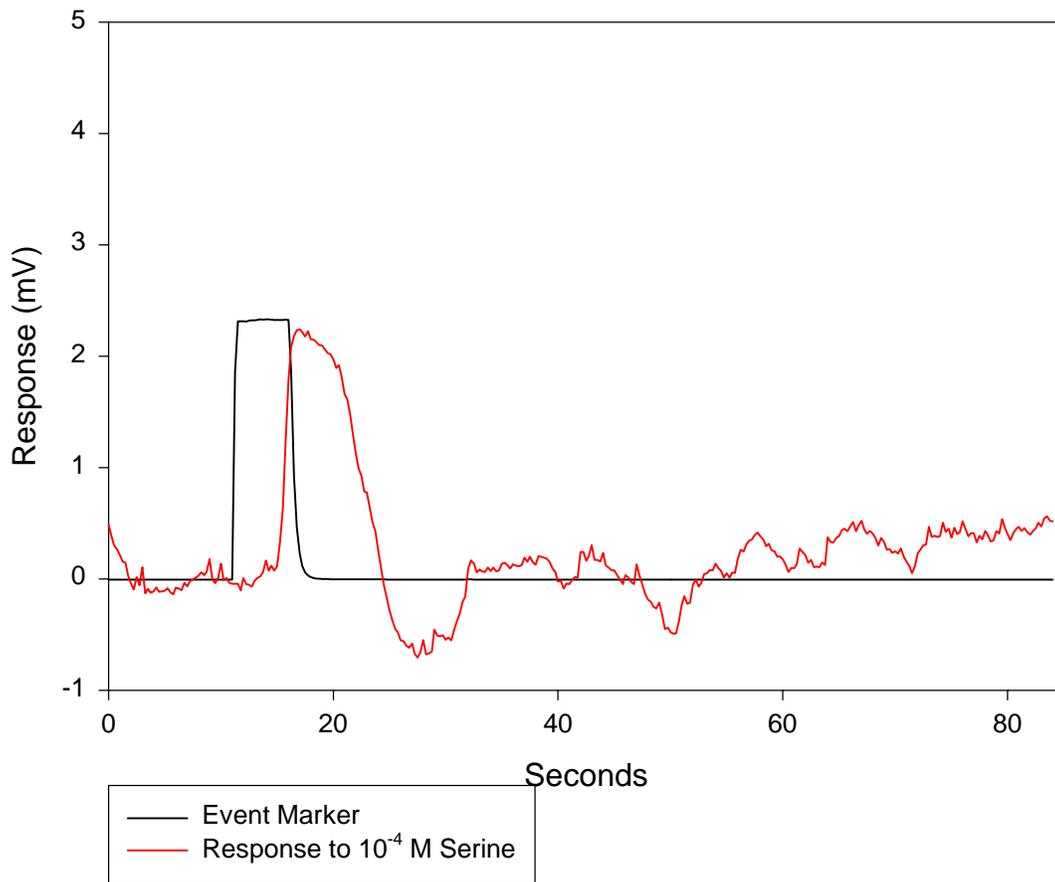


Figure 2. Olfactory response of coho salmon to serine (10^{-4} concentration) as measured with electro-olfactogram. Response magnitude is 2.34 mV from a single fish tested 5/24/2001. Event marker denotes time of release of odorant.

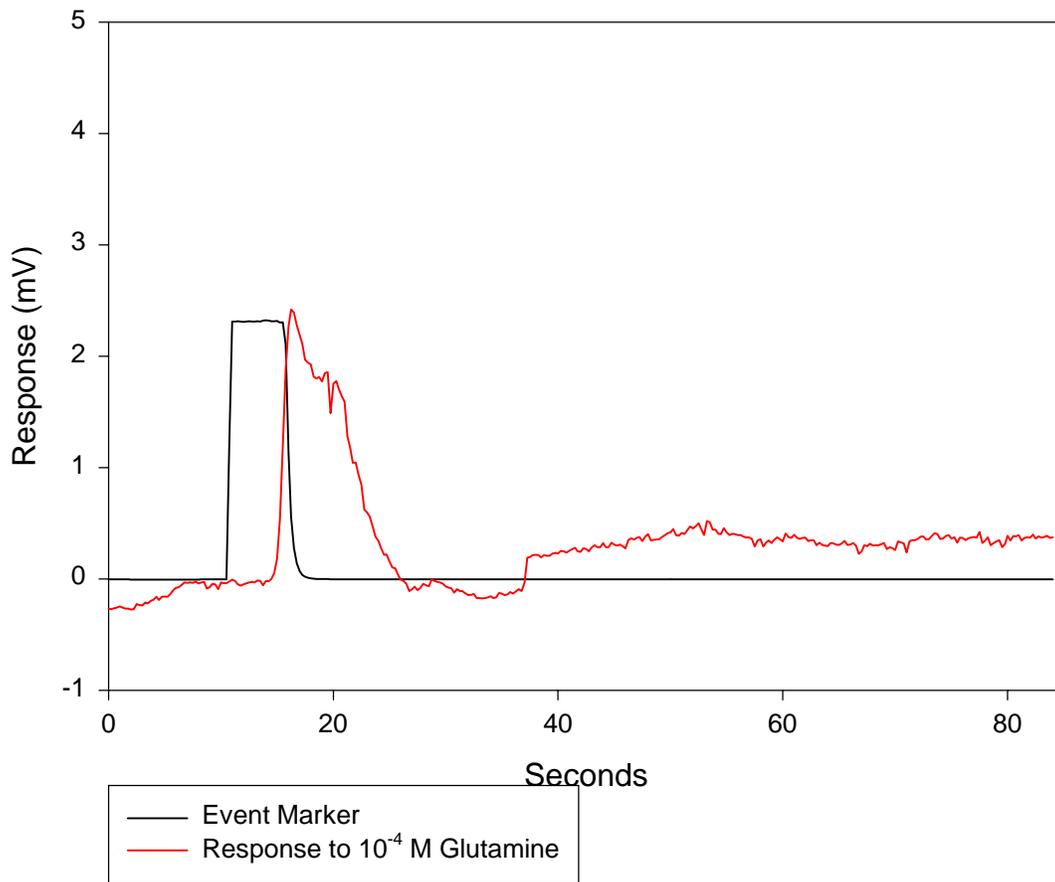


Figure 3. Olfactory response of coho salmon to glutamine (10^{-4} concentration) as measured with electro-olfactogram. Response magnitude is 2.48 mV from a single fish tested on 5/24/2001. Event marker denotes time of release of odorant.

An EOG apparatus at the USGS Hammond Bay Biological Station, set up by Dr. Weiming Li from Michigan State University was photographed and described by one of Dr. Li's students. Dr. Peter Sorensen provided a list of equipment and recommendations for components of the system. One CRRL employee visited the Hammond Bay Biological Station in 2000 to observe the operation of their EOG. On numerous occasions, Dr. Sorensen and Dr. Li provided guidance on how to design and construct our EOG. Dr. Li visited the CRRL to provide on-site

technical advice. Pacific lampreys were collected from the Columbia River and held at the CRRL for these preliminary experiments.

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Appendix A. Supplies required for EOG.

Item	Model Number	Purchased/constructed/ previous CRRL property
DC Amplifier / Kynet	BMA-931	Purchased
Physiograph /Data Q	DI 400	Purchased
2-Micromanipulators	55133	Purchased
2-Magnetic stand / Flex Bar	None	Purchased
Odorant water bath	None	Constructed
Valve for odorant supply / Asco	8320 G 202	Purchased
Timer (2-Channel) / GraLab	545	Purchased
Electrode Puller /Sutter Instrument Co.	P 87	Purchased
Electrode Cell - Glass Capillary tubing	None	CRRL
Microelectrode Holder/ World Precision Instr.	MEH 810	Purchased
Faraday cage	None	Constructed
Silicone tubing	None	CRRL
Valves	None	CRRL
Microscope stand w/ arm / Leica	None	Purchased
Fiber optic light tubes & Illuminator/Dolan-Jenner	MI-150	Purchased
Petromyzonol sulfate m.w. 474.65 / TRC	P293525	Purchased
KCl / Sigma	None	CRRL
NaCl ACS / Sigma	S-9888	Purchased
Gelatin /Sigma	232-554-6	Purchased
Arginine Fisher Scientific	BP370-100	Purchased
Glassware	None	CRRL
Pipetor	None	CRRL
Fish holder with water receptacle	None	Constructed
20 gallon tall aquarium All-Glass Aquarium Inc.	20H	Purchased
Anesthetic/ water tank and valve	None	Constructed
Computer / Gateway	TB3/ GP7-650	Purchased
Water Chiller / Aqua Logic, Inc.	Nema Type 4X	Purchased

Aquarium heater / Penn Plax	15"	CRRL
Water Pump/ Little Giant Pump Co.	977446	Purchased
Microscope/ Leica	MZ 6	CRRL
Battery Backup/ A.P.C.	8P4205	Purchased
Keyboard/ Gateway	G9900	Purchased
Monitor / Gateway	VX 720	Purchased
Foot Switch/ GraLab	560	Purchased
Personal Scope / Velleman	HPS 5	Purchased
Scalpel Blades/ Medi Source	Size 10	Purchased
Universal Adapter/ Radio Shack	4029312693	Purchased
