Pacific Lamprey Research and Restoration Project

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Bonneville Power Administration
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PACIFIC LAMPREY RESEARCH AND RESTORATION PROJECT

2002 ANNUAL REPORT
(January 1, 2002 to December 31, 2002)

Prepared by:

David A. Close
and
Kimmo K. Aronsuu

Confederated Tribes of the Umatilla Indian Reservation
Department of Natural Resources
Fisheries Program
Pendleton, Oregon 97801, USA

Prepared for:

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

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EXECUTIVE SUMMARY

Pacific lamprey (*Lampetra tridentata*) has significantly declined along the Oregon coast and in the Columbia River Basin (Downey et al. 1993, Close and Jackson 2001). Declines in adults can be partially attributed to hydroelectric dams, which have impeded passage of adult Pacific lamprey in the Columbia and Snake rivers (Moser et al. 2002), thus effecting larval recruitment in the basin (Moser and Close in press). Adult Pacific lamprey also declined in numbers in the Umatilla River, a tributary of the Columbia River (Close and Jackson 2001). In addition to hydro power dams in the Columbia River, habitat alterations and chemical treatments have been involved in the collapse of Pacific lamprey populations in the Umatilla River (Close 1999). To initiate the restoration effort, CTUIR began developing a restoration plan in 1998. 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 level. This report is summarizing the studies and restoration efforts concluded in 2002.

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

A total of 491 adult lampreys were collected at the John Day Dam fish ladder in the winter of 2001-2002. Fish were then transported and maintained in a raceway at Three Mile Falls Dam facility. Pacific lamprey were outplanted into the upper Umatilla River and Meacham Creek in late April. Nest surveys were conducted to determine number and location of nests and spawning temperature and time of outplanted adult lampreys. Electrofishing was conducted in 31 index sites in the Umatilla River and three sites in Meacham Creek to monitor larval abundance. In addition, ages of 153 larvae were determined. A rotary screw trap and fish collection facility at the West Extension canal were used to study the number and size distribution of outmigrating larval and metamorphosed lampreys. The number of upmigrating lampreys was estimated by collecting them with portable assessment traps and fyke net in the lower end of the Umatilla River. The migration timing of upmigrating adult lampreys in the Columbia River at the John Day Dam was compared to the Umatilla River hydrograph.

Outplanted adult lampreys found spawning sites and constructed nests successfully in the mainstem Umatilla River and Meacham Creek. In total 67 viable nests and in addition 118 test nests were located during the surveys (05/16-07/18). All nests were above river kilometer 96 in the mainstem Umatilla River and approximately 2/3 was located in Meacham Creek. In the mainstem Umatilla River, nests were more widely distributed than in Meacham Creek. In Meacham Creek, most of the nests were within one kilometer of the release site. Majority of lampreys spawned during the first two weeks of June when the daily mean temperature in the river varied from 8.8 to 13.1 ^{o}C.

Larval densities in index sites above river kilometer 100 in the mainstem Umatilla and Meacham Creek (sites 20-34) was high (18.0 ind.m^{-2}). Larval length and age distributions showed that almost all larvae were year classes 2000 and 2001 and most likely the offspring of adults outplanted in those years. The growth rate of larvae has been high. Below rkm 100 the mean larval density at the index sites 1-19 was only 0.2 ind.m^{-2} showing that larvae of year classes 2000 or 2001 had not yet dispersed into the lower part of the river and natural reproduction has been low.

The total number of captured outmigrating metamorphosed and larval lampreys was only 25 and 58 individuals. The total number of outmigrating metamorphosed or larval lampreys was not estimated. At the end of catching season, it was found that lampreys were able to escape from the collection box of the rotary screw trap.

No adult lampreys were captured by fyke net or portable assessment traps during the catching season showing that the number of adult lampreys entering the Umatilla River was very low even though a few tens of thousands lampreys passed the mouth of the river. During the peak adult migration in the Columbia River, flow in the Umatilla River is very low due to irrigation which lowers the attractiveness of the river for upmigrating adult lampreys. In addition, the majority of larvae are still over 100 km upriver from the mouth of the river and we speculate that pheromones released by larvae do not reach the mainstem Columbia River before dissociation.

Chapter II: Olfactory Sensitivity of Pacific Lampreys to Petromyzonal Sulfate

Pacific lampreys (*Lampetra tridentata*) are in decline or have been extirpated from much of their historical range in the Columbia River Basin. The U.S. Geological Survey, in consultation with researchers from the University of Minnesota and Michigan State University, modeled the current study of olfaction in Pacific lampreys after similar studies of sea lampreys (*Petromyzon marinus*) in order to support the research and restoration efforts of the Confederated Tribes of the Umatilla Indian Reservation. Ultimately, the purpose of this study is to investigate the role of olfaction in the migration and spawning behaviors of adult Pacific lampreys. This report examines data collected in 2002, and compares these data to findings from Pacific lamprey studies conducted in 2001. Additionally, we will compare our results to studies of sea lamprey olfaction, which is a well-understood model of pheromones as migratory and spawning cues in lampreys.

Olfactory responses of captive adult Pacific lampreys to larval lamprey bile acids, petromyzonol sulfate (PS) and allocholic acid (ACA), and an adult lamprey bile acid, 3-keto petromyzonol sulfate (3-keto PS), were measured by electro-olfactogram (EOG) from the time of capture during upstream migration (June) through the time of natural spawning (May to June the following year). In order to demonstrate that our EOG apparatus was functioning properly, we also tested olfactory responses of adult sea lampreys (*Petromyzon marinus*), another agnathan species, to these same bile acids and to L-arginine. Between January and December 2002, 94 adult Pacific lampreys were tested on the EOG apparatus, with 24 adults from the 2001 upstream migration and 70 adults from the 2002 upstream migration. The olfactory system of migrating adult Pacific lampreys was sensitive to PS and 3-keto PS, but showed few measurable responses to ACA. Adult Pacific lampreys were less sensitive to bile acids than the sea lamprey and showed an atypical positive potential response to L-arginine. In addition, the limits of detectability for these compounds appeared to be at greater concentrations for adult Pacific lampreys than for sea lampreys. However, the duration of Pacific lamprey sensitivity to bile acids was much longer, reflecting their prolonged period of freshwater migration to spawning grounds. By the time of spawning, Pacific lamprey sensitivity to lamprey bile acids was immeasurable. These results from 2002 confirm

results from 2001 and indicate a possible role for larval and adult lamprey bile acids as pheromone cues for Pacific lampreys.

Chapter III: HPLC and ELISA analyses of larval bile acids from Pacific and western brook lampreys

Comparative studies were performed on two native lamprey species, Pacific lamprey (Lampetra tridentata) and western brook lamprey (Lampetra richardsoni) from the Pacific coast along with sea lamprey (Petromyzon marinus) from the Great Lakes, to investigate their bile acid production and release. HPLC and ELISA analyses of the gall bladders and liver extract revealed that the major bile acid compound from Pacific and western brook lamprey was petromyzonol sulfate(PZS), previously identified as a migratory pheromone in sea lamprey. An ELISA for PZS has been developed in a working range of 20 pg – 10 ng/well. The tissue concentrations of PZS in gall bladder were 127.40, 145.86, and 276.96 μg/g body mass in sea lamprey, Pacific lamprey, and western brook lamprey, respectively. Releasing rates for PZS in the three species were measured using ELISA to find that western brook and sea lamprey released PZS 20 times higher than Pacific lamprey did. Further studies are required to determine whether PZS is a chemical cue in Pacific and western brook lampreys.

Chapter IV: Habitat suitability criteria for larval Pacific lamprey

The Physical Habitat Simulation System (PHABSIM) is part of a broad conceptual and analytical framework for addressing stream flow management issues called the Instream Flow Incremental Methodology (IFIM). PHABSIM is used in Columbia River basin to predict the effects of flow alternations on fish habitat availability. However, the lack of suitability criteria has prevented fisheries managers from including ammocoete habitat in the models. In 1999, Torgersen and Close (2002) collected data to predict the relative abundance of larvae among different scales in the Middle Fork John Day River. The data was used to calculate suitability and the Jacobs' electivity index for six of the sample level habitat variables which significantly ($p< 0.05$) explained the abundance of larval Pacific lamprey in bivariate logistic regression (Torgersen and Close 2002). Indices were calculated for all larvae and for two separate

size classes: total length 30-69 mm and 70-109 mm. Larvae preferred areas where soft sediments were dominant. The habitats where silt was the dominant substrate were most preferred. The next highest suitability was found for sand and then organic detritus. Larvae avoided coarse mineral sediments from small gravel to boulders. Suitability of burrowing habitat increased when depth of the organic material increased. Larvae showed preference for low, under 0.1 m/s, current speed and strong avoidance for current speed higher than 0.19 m/s. Larvae preferred pool to riffle area and channel margins to mid channel. The subjective habitat typing seemed to work well. Habitat type 1 was strongly preferred, habitat type 2 preferred and habitat type 3 strongly avoided. The indices showed some differences between size groups. The larger larvae did not avoid cobble substrate and mid channel as strongly as the smaller larvae. Furthermore, the most preferred current speed for larger larvae was higher than that of smaller larvae. All the index values are given in the table for future use in PHABSIM.

CHAPTER ONE

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

Kimmo K. Aronsuu, Aaron D. Jackson, and David A. Close

Tribal Fisheries Program, Department of Natural Resources, Confederated Tribes of the Umatilla Indian Reservation, Pendleton, Oregon, U.S.A.

INTRODUCTION

The Pacific lamprey (*Lampetra tridentata*) is an anadromus fish, which is distributed in rivers from California along the Pacific Rim to Hokkaida, Japan (Simpson and Wallace 1982, Ruiz-Campos and Gonzales-Guzman 1996). 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). It is an important component of the ecosystem both as a predator and prey (Close et al. 2002). Furthermore lampreys are culturally important to Native peoples along the West Coast of the United States (Keim 2000, Close et al. 2002).

Pacific lamprey has significantly declined along the Oregon coast and in the Columbia River basin (Downey et al. 1993, Close and Jackson 2001). Declines in adults can be partially attributed to hydroelectric dams, which have impeded passage of adult Pacific lamprey in the Columbia and Snake rivers (Moser et al. 2002), thus effecting larval recruitment in the basin (Moser and Close 2003). Adult Pacific lamprey has also declined in numbers within the Umatilla River, a tributary of the Columbia River (Close and Jackson 2001). In addition to hydroelectric dams in the Columbia River, habitat alterations due to loss of beaver, overgrazing, agriculture, logging, irrigation, together with chemical treatments performed in the 1960s and 1970s have contributed in the collapse of Pacific lamprey populations in the Umatilla River (Close 1999).

The tribes raised awareness regarding declines of Pacific lamprey along the Oregon coast and interior Columbia River basin (Downey et al. 1993, Close et al. 1995). The NPPC approved the Status Report in 1995 that initiated CTUIR's lamprey research and restoration project in the Columbia River basin. The main goal of the project is to restore natural production of Pacific lamprey to self-sustaining and harvestable levels in traditional fishing areas in the Umatilla River and within the whole Columbia River basin. To initiate the restoration effort, CTUIR began developing a restoration plan in 1998. Because the basic knowledge of the Pacific lamprey is limited, part of the project has been to produce new information about the biology, ecology, and restoration possibilities of Pacific lamprey.

This report is summarizing the studies and restoration efforts concluded in the Umatilla River in 2002.

THE STUDY AREA

General Description of the Umatilla basin

The Umatilla River Subbasin Plan (CTUIR and ODFW 1990) describes the basin in detail. "The Umatilla River in Northeast Oregon originates on the west slope of the Blue Mountains east of Pendleton (Figure 1). The river flows northwesterly across the Umatilla Plateau for about 185 km to its confluence with the Columbia River at river kilometer (rkm) 465. Virtually all of the 5931 km^2 drainage is within Umatilla County."

"The basin is comprised of two major physiographic regions. Multiple basalt flows formed the Deschutes-Umatilla Plateau, a broad upland plain that slopes northward from the Blue Mountains to the Columbia River. Elevations range from about 82 m at the Columbia River to about 914 m along the toe of the Blue Mountains. Faulting and folding of a variety of volcanic, sedimentary, and metamorphic rocks created the Blue Mountains region. The mountains stretch along the southern and eastern boundary of the basin. Elevations of the mountains range from 914 to 1829 meters. Multiple flows of lava known as the Columbia River basalt underlie nearly the entire Umatilla River basin. Older volcanic, sedimentary, and metamorphic rocks are exposed along the crest of the Blue Mountains. Sedimentary deposits cover the basalt throughout much of the basin. Alluvium deposited by modern rivers and streams is common in valleys and floodplains. Windblown silt and fine sand cover much of the basin. Annual precipitation ranges from less than 25.4 cm in a band along the Columbia River, up to 114.3 cm in the Blue Mountains. Annual temperatures for the lower elevation areas average from 10 degrees to 13 degrees Celsius."

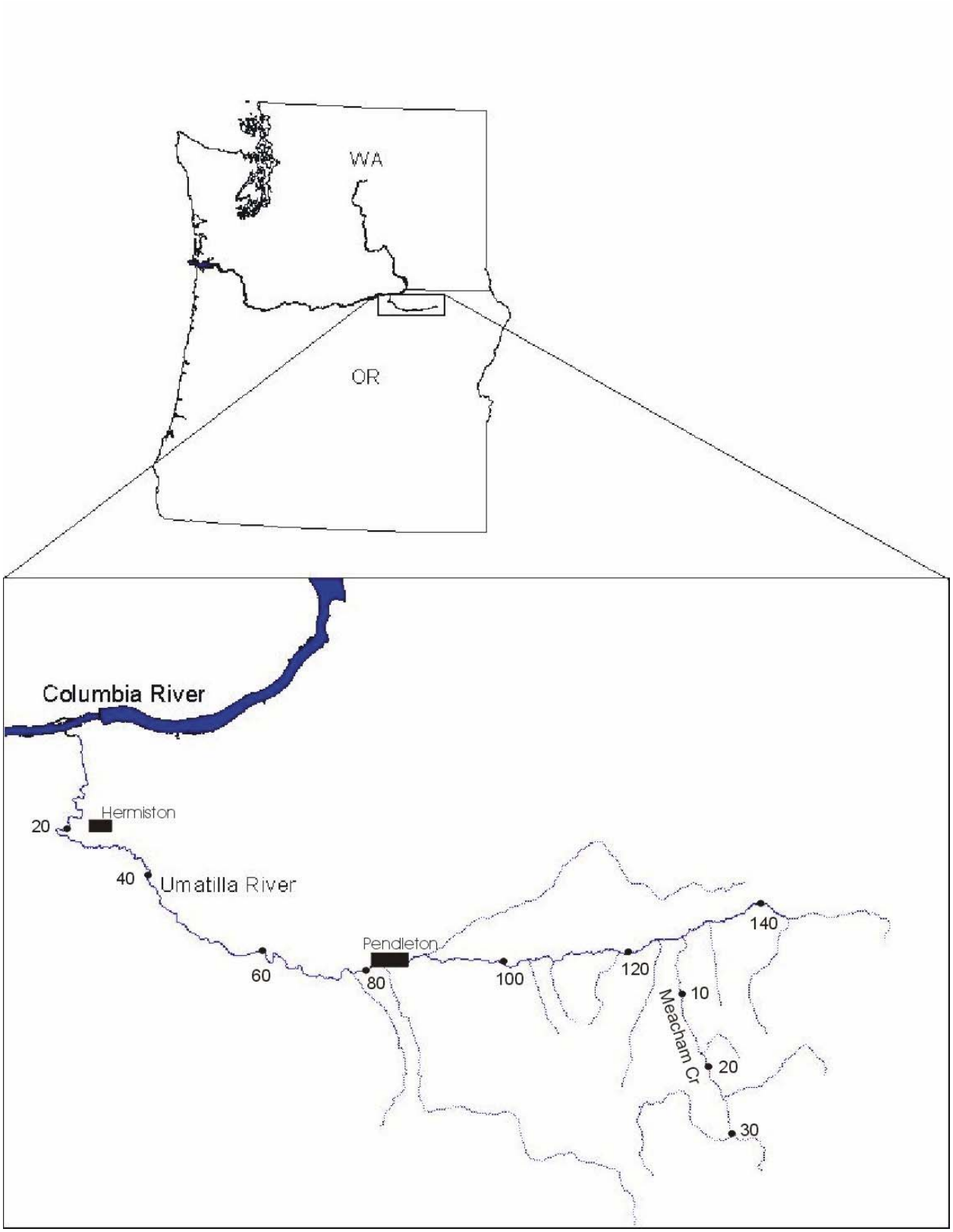

Figure 1. The Umatilla River basin in Northeast Oregon. Numbers in the map represent river kilometers.

Flow and water temperature during the study period

During the study period from October 2001 to September 2002, the daily mean discharges in the Umatilla River at rkm 131, 90 and 4.5 were 11.0, 13.6 and 12.0 m^3s^{-1}, respectively. In the late February and early April there were relatively high flow peaks (Figure 2). Discharge was highest in the middle of April, and exceeded 130 m^3s^{-1} at all observation stations (Figure 2). Discharge decreased by late April. In the lowermost station (near the river mouth) discharge dropped quickly in early July and varied between 0.03 and 0.14 m^3s^{-1} (mean 0.05 m^3s^{-1}) from July 11th through August 15th (Figure 2).

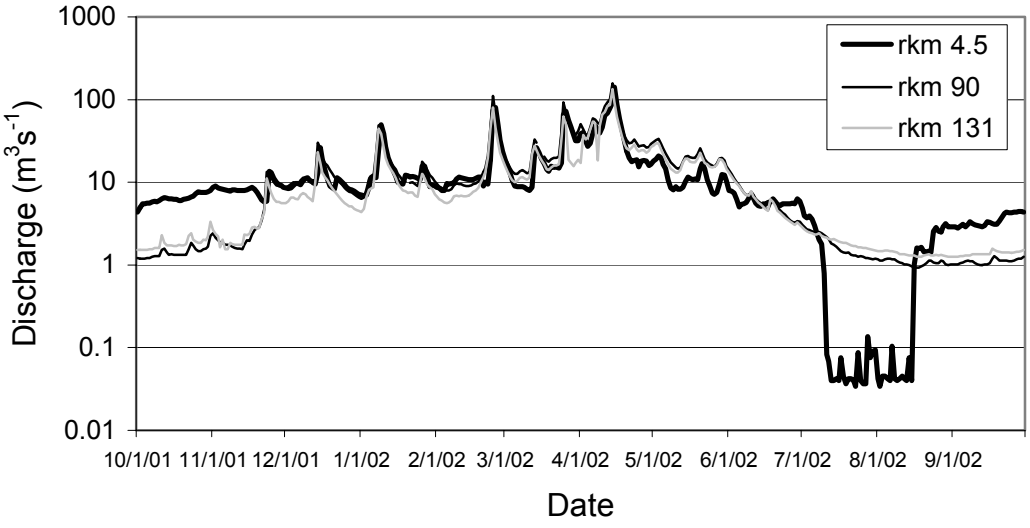

Figure 2. Daily mean discharge in the Umatilla River at river kilometers 131, 90 and 4.5 from October, 2001 to September, 2002.

Water temperature in the upper reaches is much lower than just above McKay Creek confluence (rkm 81.7, Figure 3). The mean water temperatures during the summer season (06/01/02-09/15/02) collected by stream thermographs at rkm 131.5, 117.3 and 81.7, were 15.5, 17.6 and 21.2 °C, respectively. Mean temperatures during same period in Meacham Creek at rkm 8.4 and 3.3 were 16.7, and 17.5 °C, respectively. Maximum temperatures at rkm 81.7 exceeded 30 °C (Figure 3).

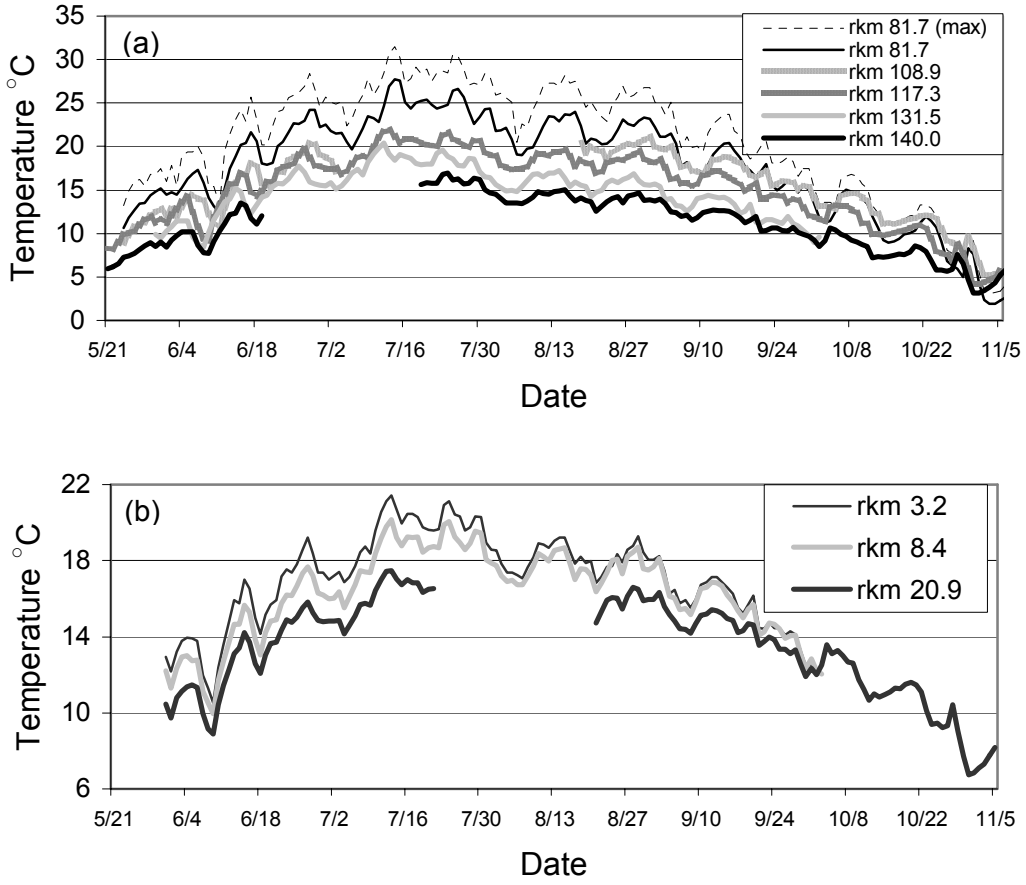

Figure 3. Daily mean temperatures at five locations in the Umatilla River (a) and three locations in Meacham Creek (b) from 05/21/02 to 11/05/02.

ADULT OUTPLANTINGS

Objective

The main goal of adult outplantings initiated in 2000 has been to re-establish larval abundance by outplanting mature Pacific lamprey close to spawning time in the Umatilla River. The purpose has also been to gain knowledge about maintaining Pacific lamprey and to collect information about the release timing and locations.

Collecting and maintenance

In total 510 adult lampreys were collected during the winter dewatering and maintenance at the John Day Dam fish ladder on January 8th, 2002. Lampreys were transported to the Three Mile Falls Dam fish facility on the Umatilla River, where they

were treated with oxytetracycline at a dose of 10 mg kg^{-1} for bacterial infections. Total length and weight of all individuals was measured. The mean length of the adults was 627 mm (range 515-782 mm) and the mean weight 379 g (range 209-782 g). Fish were maintained in a raceway at the Three Mile Falls Dam facility. During holding period the mean temperature in the raceways was 7.0°C and ranged between 2.3 and 13.2°C. Lampreys were checked weekly then daily for ripeness. In 04/03/02 sixteen lampreys were examined for disease by ODFW fish pathology in La Grande, Oregon. All assays for virus or any other replicating agents were negative.

Releasing

In the late of April (4/25-4/30) in total 491 lampreys were transferred in aerated tanks to four different releasing sites: 150, 100 and 141 adults were released into the main stem Umatilla river at rkm 98.8, 118.4 and 139.9, respectively. In addition, 100 adults were released into Meacham Creek (rkm 17.5), a major tributary of the Umatilla River. The daily mean temperature in the raceways on the releasing days varied between 10.3 and 14.5°C. Water temperature at releasing sites was from 10.9-13.8°C. On releasing days the daily mean flows at rkm 131 varied from 22.5 to 24.2 m^3s^{-1} (Figure 2).

NEST SURVEYS

Objective

The objective of nest surveys during the spring of 2002 was to record all viable and test nests and observed spawning activity in the Umatilla River and Meacham Creek to assess the distribution and the number of nests. In addition, extra attention was directed to surveys in Meacham Creek to determine spawning time and water temperature of spawning lampreys.

Methods

The mainstem Umatilla River (rkm 90.0-144.3) North Fork Umatilla (rkm 0-4.3), South Fork Umatilla (rkm 0-5.6) and Meacham Creek (0-25.1) were surveyed to locate lamprey nests. In addition, surveys were conducted in the lowermost 4 km of Camp Creek which enters Meacham Creek adjacent to the releasing site.

Surveyors walked downstream along the margins or in the river and traversed from bank to bank checking the tail out of each pool and above each riffle. To maximize the ability to view nests or spawning lampreys, surveyors used polarized sunglasses and walked the stream only if visibility was good. Once a viable nest, test nest or spawning lampreys were located, surveyors recorded approximate location with a hand held GPS unit and coded the observation. Pink fluorescent flagging was then placed in the vicinity of the nest. In later observations, the same code was used to identify the nest. In the final nest count the last observation of each nest was used to determine if the nest contained eggs (viable) or a test nest.

Nest surveys were conducted from 05/16/02 through 07/18/02. In the mainstem Umatilla, 1.6 km sections around all outplating sites were surveyed on the 5th and 10th of June. After that, surveyors walked 119.4 km between rkm 90 and 144.3 and after the 19th of June all the possible spawning areas of this section were surveyed at least once. South Fork Umatilla was surveyed on the 24th of June and North Fork Umatilla on the 25th of June. In Meacham Creek most of survey effort was directed to the area, where lampreys were known to spawn in earlier years i.e. rkms 114.3-119.1. In this area surveys were started on the 20th of May and last surveys were conducted on the 8th of July. All together surveyors walked 28 km in this 4.8 km long section. The lowermost section of Meacham Creek (rkm 0-14.3) was surveyed from the 18th of June through the 20th of June and the uppermost section (rkm 19.1-25.1) on the 11th of July. Surveys in Camp Creek were conducted on the 10th of July.

To study timing of spawning and temperature during spawning activity, the number of new nests were observed between rkm 17.1 and 18.0 in Meacham Creek. The section was surveyed on the 20th, 28th, 29th, and 30th of May and on the 3rd, 6th, 13th of June and 3rd of July. When both male and female were present at the nest, the observation was recorded as spawning activity. A nest without adults and with viable eggs was recorded as a viable nest and it was assumed that spawning took place between the date the section was last surveyed and the date when the nest was located. To determine temperature at the study section we used temperature data collected by stream thermograph at rkm 20.9 in Meacham Creek. Before 05/30 temperature at rkm 20.9 was

estimated by using liner correlation ($P<0.001$, $R^2= 0.991$) of temperatures at rkm 20.9 in Meacham Creek and at rkm 140 in the Umatilla River.

Results

During the surveys, 21 viable nests were located in the mainstem Umatilla River and 46 viable nests in Meacham Creek. In the Umatilla River, two of the detected nests were at rkm 96.2, 2.6 km below the lowermost releasing site. Two viable nests were between lowermost (rkm 98.8) and middle (rkm 118.4) releasing sites and four between the middle releasing site and Meacham confluence (rkm 126.8). The rest of the located viable nests (13) in the Umatilla river were above the uppermost releasing site (rkm 139.9) between rkm 140.6 and143.7 (Table 1). In Meacham Creek, 30 of 46 located viable nests were situated no more than 0.5 kilometer away from release site between rkm 17.1 and 18.0 (Table 1). Nineteen of 30 nests were above the releasing site, one at the releasing site, and 9 below the releasing site. In the lower part of Meacham Creek, three viable nests were found at rkm 4.3, one at rkm 7.2, two at rkm 10.1 and four at rkm 10.5. In addition, five nests were located in the uppermost part of Meacham Creek: one at rkm 19.0, three at rkm 19.1 and one at rkm 21.9.

In addition to 67 viable nests, surveyors found 118 test nests which were not classified as a viable nest (Table 1). Thirty one of located test nests were in the Umatilla River and 87 in Meacham Creek. A cluster of test nests were located between rkm 10.1 and 10.5 in Meacham Creek, where 32 test nests were found, but only 6 viable nests were counted.

Table 1. Number, location, and date of localization of new viable nests in the mainstem Umatilla River (a) and in Meacham Creek (b).

(a)	Number of nests																	
Nest type	rkm 96.2	rkm 107	rkm 113.6	rkm 118.3	rkm 119.1	rkm 119.9	rkm 121.5	rkm 123.1	rkm 124.7	rkm 126.1	rkm 126.3	rkm 126.8	rkm 140.6	rkm 141.6	rkm 143.2	rkm 143.4	rkm 143.7	tot.
Viable	2	1	1	0	0	0	0	0	1	0	1	2	7	2	2	1	1	21
In const.	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2
Test	3	0	5	1	2	1	1	1	0	2	0	0	3	0	6	3	3	31

(b)	Number of nests																						
Nest type	rkm 4.3	rkm 7.2	rkm 9.2	rkm 9.5	rkm 9.7	rkm 10.1	rkm 10.3	rkm 10.5	rkm 16.9	rkm 17.1	rkm 17.2	rkm 17.5	rkm 17.7	rkm 17.9	rkm 18.0	rkm 18.3	rkm 18.5	rkm 18.8	rkm 19.0	rkm 19.1	rkm 21.9	rkm -	tot.
Viable	3	1	0	0	0	2	0	4	0	7	2	1	1	7	12	0	0	0	1	3	1	1	46
In const.	0	0	0	0	0	2	0	0	1	3	0	1	0	3	4	2	1	0	0	0	0	0	17
Test	1	1	4	1	1	24	3	5	0	9	1	0	1	10	11	1	0	1	0	9	2	2	87

In the intensively surveyed section (rkm 17.1-18.0) in Meacham Creek spawning activity was detected on the 28th of May and the first viable nest was found at the same site on the 29th of May. All of the 14 spawning activity observations in this section were made between the 28th of May and the 13th of June. A total of 28 viable nests were constructed between the 28th of May and the 13th of June (Figure 4). Only two nests were found to be constructed after that. Temperature during the main spawning period (05/28-06/13) varied between 7.5 and 14.9 ^{o}C and daily mean temperature varied between 8.8 and 13.1 ^{o}C. The mean temperature of the time period was 10.5 ^{o}C.

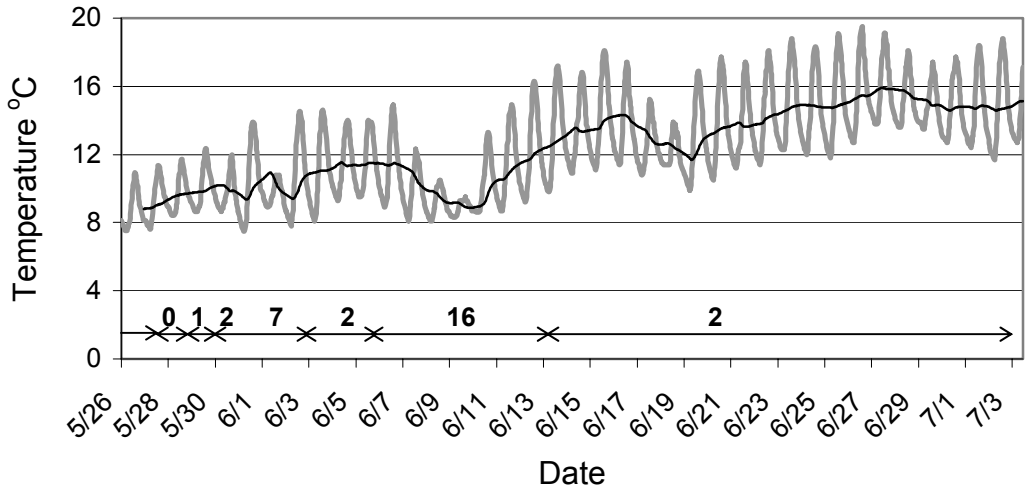

Figure 4. Hourly water temperature (grey line) and moving average of 24 hours (black line) at rkm 20.9 in Meacham Creek and number of new viable nests (numbers above arrows) between river kilometers 17.1 and 18.0. Arrows show the time periods nests have been constructed.

Conclusions

The total number of nests located in 2002 was high compared to the number of outplanted adults. Farlinger and Beamish (1984) found that the total nest count in Eel Creek in British Columbia was approximately 20 % of total number of spawning adults. Since the total number of nests we found in 2002 was 37 % of the number of outplanted lampreys we assume that a high proportion of outplanted lampreys found spawning sites and constructed nests successfully. Egg survival studies were not conducted in summer 2002. However, there is no reason to believe that egg survival would have been lower than in 2001, when survival was 86 % (Aronsuu et al. 2002). By using the same rough estimates as in 2001 (Aronsuu et al. 2002) we estimate that outplanted adult lampreys released 46 millions eggs and 5.5 millions prolarvae hatched in the nests during the summer of 2002.

In 2002, the percentage of viable nests located in Meacham Creek was 69 % of all the nests. The high number of located nests in Meacham Creek was partly due to higher surveying intensity, however, it is rather obvious that for some reason adults prefer Meacham Creek over the mainstem Umatilla as a spawning area. The same phenomenon was observed in surveys conducted in 2001 (Aronsuu et al. 2002). The preference is even more pronounced when we take into account that only 20 % of the brood stock was outplanted into Meacham Creek. Based on nest distribution we believe that a high portion of 250 adults outplanted into the mainstem below Meacham Creek confluence migrated into Meacham Creek to spawn. Upstream migration in spring time after winter holding period (Bayer et al. 2001) and spawning in the upper reaches (Potter 1980) may be natural behaviors explaining why lampreys left the two lowermost releasing sites. Of course we can't rule out the possibility that the fish just couldn't find appropriate spawning sites. It seemed that at the Meacham Creek releasing site, lampreys did not have a need to migrate.

Substrate, current, water depth, water quality and temperature are known to have an effect on selected spawning areas (Mattson 1949, Pletcher 1963, Kan 1975, Russel et al. 1987). In addition, pheromones released by larvae and other adult lampreys may have effect on migration behavior (Robinson et al. 2003) and they may also affect

selected spawning areas. More information is needed to find out why lampreys select one but reject other spawning area and the best release locations.

Water temperature is an important triggering factor for lamprey spawning (Applegate 1950, Manion and McClain 1971). Lampreys in Meacham Creek spawned about a month later than along the coast of Oregon (Kan 1975), but spawning temperatures were consistent with observations made by Kan (1975). In coastal rivers, Pacific lamprey was observed to begin spawning in April when the water temperature reached about 8.5 ^{o}C. Most spawning fish from these watersheds were collected in May with water temperature ranging from 10 to 12.5 ^{o}C (Kan 1975). Most of the lampreys spawned in Meacham Creek within two weeks. Temperature during migration and winter holding affect timing of ripeness and spawning. All lampreys that spawned in Meacham Creek in 2002 were collected during winter holding at the same fish ladder and held before releasing at the same fish holding facility, and consequently, they had rather similar migratory/temperature history. It is likely that in natural spawning populations, the migratory/temperature history of spawning adults is more heterogeneous, which may lead to more variation in the timing of ripeness and a longer spawning period than what we found in our study.

LARVAL ABUNDANCE AND DENSITIES

Objective

The approach has been to follow the development of larval densities and length distribution in the index sites by conducting larval lamprey electrofishing surveys selected in 1998. In 1998-2000 we studied larval densities before and after outplanting adult lampreys. In 2002, larval densities were detected for the second time after outplantings.

Material and Methods

In 1998, a total of 42 sites were selected in the Umatilla River for detecting change in larval densities. Some of sites had two different sampling locations (a and b). Since 2000, the number of index sites has been reduced. We sampled 30 sites and reduced sites with plots (a or b) to one or the other. In 2001, we added one index site to

the mainstem Umatilla River and three index sites to Meacham Creek. In 2002, we studied the same 34 index sites as in 2001. All the selected sites were habitat type 1 (i.e. silty substrate characteristics) where larvae are typically most abundant. From some index sites, the silty substrate material has been flushed away. In those cases the site has been moved to the closest possible habitat type 1 area, which is large enough to properly conduct electroshocking.

Each 7.5 m^{-2} site was measured and larvae were collected in two 11.25 minute passes with a backpack model Abp-2 electrofishing unit (Engineering Technical Services, University of Wiscosin, Madison, Wisconsin). If the catch of the second pass was over 70 % of the first catch a third pass was conducted. If no larvae were detected in the first pass, only one pass was conducted. The electrofishing unit delivered 3 pulses per second (125 volts DC) at 25 % duty cycle, with a 3:1 burst train (three pulses on, one pulse off) to remove larvae from the substrate (Weisser and Klar 1990). Once larvae emerged from the substrate, 30 pulses per second were applied to stun and capture larvae. Voltage and pulse rates selected were based on electrofishing studies on sea lamprey larvae (Hintz 1993, Weisser 1994). Following collection, larvae were anaesthetized in MS-222 (50 mg l^{-1} tricane methanesulfonate), identified by tail pigmentation (Richards *et al.*1982) and measured for total length ($\pm$ 1 mm). After recovery, larvae were returned to the river. The electrofishing survey was conducted from 08/06/02 through 08/21/02.

A population estimate was conducted within each plot. The Two Catch method (Seber and LeCren 1967) was used to estimate density and its' variance in area sampled excluding the site 32, where the Three Catch method (Junge and Libovarsky 1965) was used. To get variance of total number larvae caught variances of sites were summed. Confidence intervals (95%) for the estimated densities were calculated by multiplying square root of variances by 1.96.

On the 27th and 28th of August, 160 larvae were collected from the index sites 22, 30, 31, 34 for aging (40 indivuals/site). The length of larvae were measured ($\pm$ 1 mm) and weighed ($\pm$0.01 g). Larvae were frozen (-80^{o} C) until extraction. Statoliths were extracted from 01/15/03-02/12/03. First, otic capsules were removed and cleaned under the dissecting microscope. Otic capsules were cut open and statoliths were removed, cleaned, and transferred to the coded well filled with immersion oil. Later (02/28/03-

05/07/03) larvae were aged by counting the number of bands with a dissecting scope (Volk 1986). Statoliths were coded numerically so that estimation of age was not biased by the knowledge of length of the individual or by an age previously assigned. Each larva was aged twice by an experienced reader and once by an inexperienced reader. Only the aging results of the experienced reader were used in the analysis of the data. The larvae with unreadable statoliths (3 individuals) and the larvae which were aged differently between two agings (4 individuals) were not included in the final data.

Results

A total of 1,346 ammocoetes were collected, and the estimate of the total amount of larvae (± 95% confidence interval) for the sampled area was 2,042 ± 218 individuals. The estimated mean density for the sampled area was 8.0 ± 0.9 ind.m^{-2}. Ammocoetes were detected in all but one of the index sites in Meacham Creek (sites 32-34) and in all index sites above rkm 102.2 in the mainstem Umatilla River (sites 20-31, Figure 5). The mean density estimate for sites 20-34 was 18.0 ± 1.9 ind.m^{-2}. The highest density was detected at site 28 with 60 ± 22 ind.m^{-2}. Below rkm 102.2 ammocoetes were only abundant in four sites between rkm 4.0 and 36.8 (Figure 5) and the mean density for sites 1-19 was only 0.2 ind.m^{-2}.

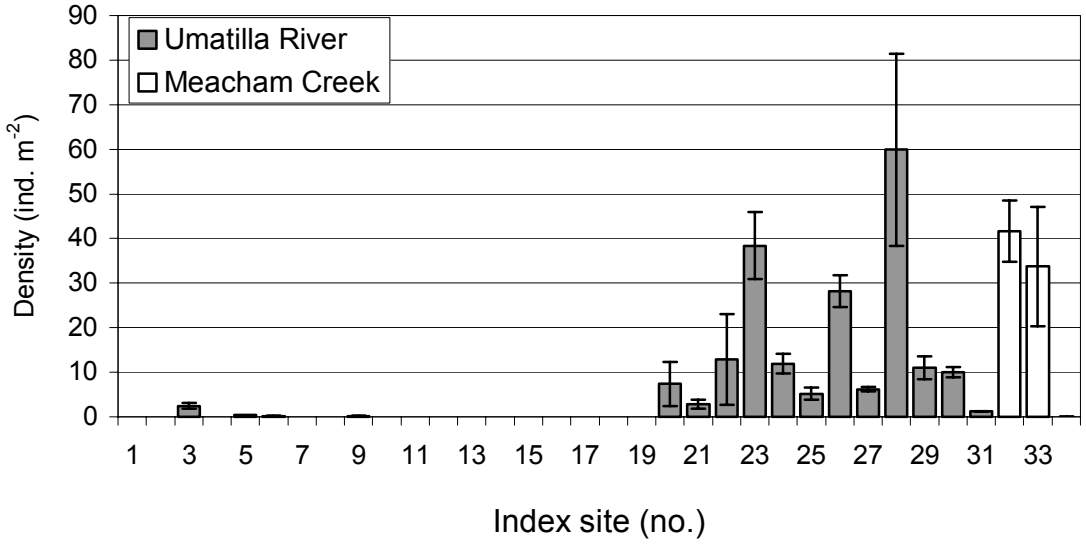

Figure 5. Estimated mean densities (± 95 % confidence interval) for index sites in the Umatilla River and Meacham Creek in 2002.

The median length of all captured larvae was 76 mm. The size distribution of all collected larvae was bimodal (Figure 6). Larvae were smallest in Meacham Creek (Figure 6) where median length of larvae was only 52 mm. Larvae caught in the lower part of the river (sites 3-9) were much longer than in the other sites. The median length of larvae was 146 mm and only two of 22 larvae were under 100 mm long (Appendix 1). Only larvae at ages 0+, 1+, and 2+ were found at sites 22, 30, 32 and 34 (Figure 7). The length of the larvae at age 1+ differed statistically between sites (Kruskall-Wallis test, p= 0.005). At the site 22 (rkm 109.1) the mean length of 1+ larvae was 81 mm, while at the sites 30 (rkm 124.9), 32 (rkm 2.4) and 34 (rkm 17.5) varied between 55 and 63 mm (Table 2) . The mean length of 2+ larvae at 22 was 111 mm (Table 2). In the lowermost site the majority (59 %) of aged larvae lived their third summer while in the three other sites the proportion of 2+ larvae was only 8-21%.

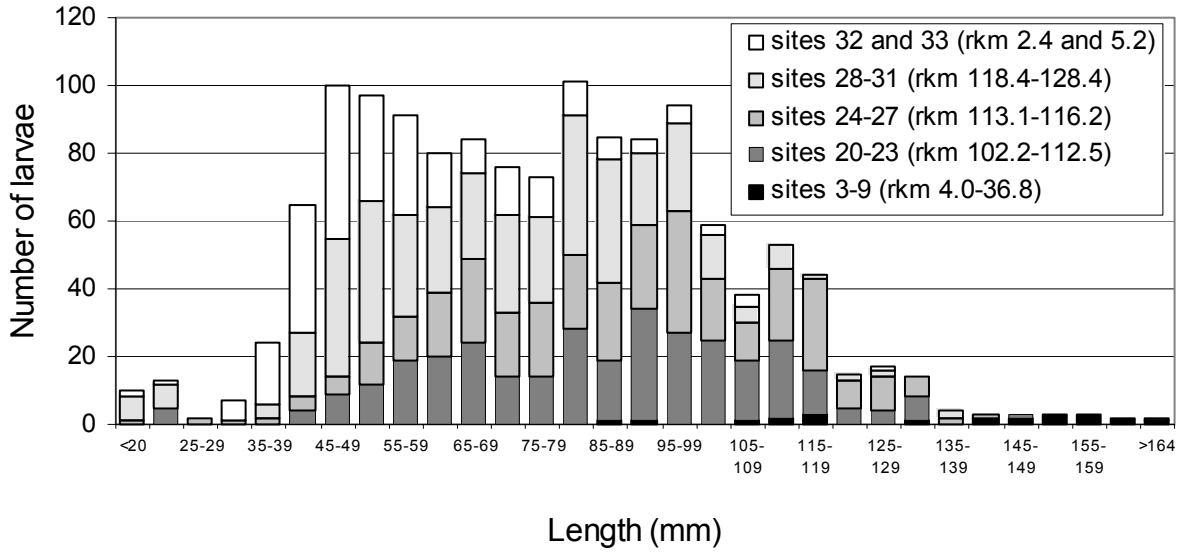

Figure 6. Length distribution for index sites in the Umatilla River (sites 3-9 and 20-31) and Meacham Creek (sites 32-33) in 2002.

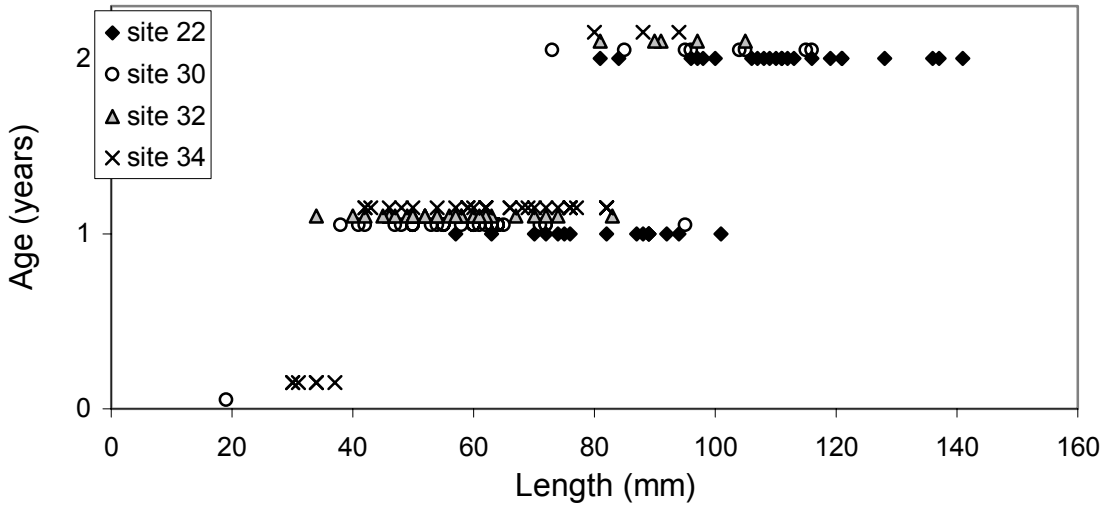

Figure 7. Length at age distribution for index sites 22 (rkm 109.1) and 30 (rkm 124.9) in the Umatilla River and for index sites 32 (rkm 2.2) and 34 in Meacham Creek (rkm 17.5) in 2002.

Table 2. The number and the mean length of larvae at ages 0+, 1+ and 2+ collected at two sites in the Umatilla River and two sites in Meacham Creek.

Location	River kilometer	Age (years)	Number of larvae	Mean length ±sd (mm)
Umatilla R	109.1	0+	0	-
Umatilla R	109.1	1+	16	81±12
Umatilla R	109.1	2+	23	111±15
Umatilla R	124.9	0+	1	19
Umatilla R	124.9	1+	30	58±11
Umatilla R	124.9	2+	8	99±15
Meacham Cr	2.4	0+	0	-
Meacham Cr	2.4	1+	31	55±11
Meacham Cr	2.4	2+	5	93±9
Meacham Cr	17.5	0+	6	33±3
Meacham Cr	17.5	1+	30	63±12
Meacham Cr	17.5	2+	3	87±7

Conclusions

Larval surveys have shown that outplanting mature lampreys (starting in the spring of 2000) have been successful. In larval surveys from 1998 through 2000, no larvae were found in the index sites near the detected spawning areas of outplanted adults (sites 20-31) (Close 2000, Close et al. 2002, Moser and Close 2003) and all the aged

larvae were two years or less showing that almost all larvae found in sites 19-34 were offspring of adults outplanted in 2000 and 2001.

Our data has shown that the number of larvae was very low in the middle and lower part of river. It seems that the larvae of year classes 2000 and 2001 haven't yet dispersed to the lower portion of the river. The very low density and high median length of larvae caught in the lowermost section of the river reflects that natural reproduction has been very low during the last few years.

Lamprey larvae move downstream during their life cycle. It is believed that this downstream movement disperses larval lamprey throughout the watershed (Potter 1980). Before outplanting adults in the upper part of the river, there was an empty niche for the larvae, therefore the lack of competition may be one reason for low dispersal downriver. In addition, larvae may not have had enough time to disperse to the lower river. For example, when sea lampreys were introduced to the Garlic River in Michigan, larvae remained near the spawning areas for two years. The main downstream migration occurred in the third year after the introduction (Manion and McClain 1971). The degree of dispersion within a river system depends on hydrological and physiographic characteristics of the river (Potter 1980). Ammocoetes were observed to disperse more widely in rivers where the gradient and flow is relatively high (Potter 1980). Even though the winter and spring 2002 peak flows in the Umatilla River were relatively high (Figure 2), lampreys did not disperse below rkm 100. However, aging showed that a proportion of 2+ larvae were highest in the lowermost site. The uppermost sites contained only a few 2+ larvae. This may reflect the downriver migration of larvae; however, more aging results are needed to verify this behavior. After a few more years we will be able to determine how effectively and at what age larvae will colonize the middle and lower part of the Umatilla River.

However, we cannot rule out the possibility that larvae have already dispersed to the middle and lower part of the river. Survival of the larvae could be low due to high temperature, poor water/sediment quality, predation, or some other reason. In earlier surveys, mainly large larvae have been found in the lower reaches of the river and in some index sites the densities has been 5.0 ind.m^{-2} (Moser and Close 2003). In surveys conducted from 1998-2002 no larvae have been found between rkm 36.8 and 102.2

(Close 2000, Close et al. 2002, Aronsuu et al. 2002, Moser and Close 2003). If larval densities do not increase through time in the middle and lower sections of the river, we should investigate why.

As shown in previous annual reports, growth of larvae was very high during they first year and growth was related the river temperature/elevation (Aronsuu et al. 2002). The same phenomenon was apparent in 2002 length distribution data. However, overlapping of two year classes and probable downward migration of ammocoetes makes length distribution data difficult to interpret. Aging verified the high growth rate of larvae. Larvae caught at rkm 109.1 have grown fast.

OUTMIGRANT ABUNDANCE

Objective

The number of outmigrants is the final measure of larval production in the river. Lampreys leave the river as larvae or recently metamorphosed lampreys called macrophthalamia. The scope of this objective is to determine if trends increase for larvae or recently metamorphosed lampreys migrating out of the Umatilla River.

Material and Methods

The outmigration of larval and metamorphosed lampreys was monitored from 10/31/01-03/09/02 using a 1.5 m diameter rotary-screw trap (RST). Due to high flows the trap was pulled up 02/24 and deployed 02/26. The trap had revolving 3.5 mm mesh cone mounted on aluminum pontoons. The trap was located 1.9 km upriver from the mouth. Total river width at this location was approximately 75 m. The river bottom was mainly bedrock with carved channels. A 1.8 m wide and 1.5 m deep channel on the west bank of the river served as the trap location. The trap was operated 24 hr d^{-1} by the personnel of ODFW. The trap was checked and the catch was enumerated twice a day. Lampreys were anaesthetized in MS-222 (50 mg l^{-1}) tricane methanesulfonate and measured for total length (± 1 mm). After recovery, lampreys were returned to the river.

From March 7, 2002 to July 12, 2002, outmigrant lampreys were collected at the fish collection facility (FCF) at West Extension Canal on the west side of Three Mile Falls Dam by ODFW. The facility has fish trapping and bypassing capability (Knapp et

al. 1996) and generally operates from late March through mid-October with a maximum canal flow of 5.1 $m^3 s^{-1}$ and a bypass flow of either 0.14 or 0.7 $m^3 s^{-1}$. Description of the juvenile fish trapping facility is provided in Knapp et al. (1996). The daily catch of outmigrant lampreys were counted and the total length of lampreys was measured.

Results

During the study period, only 25 metamorphosed and 58 larval lampreys were caught with RST. No larval or metamorphosed lampreys were caught at the FCF at the West Extension Canal, but in late June two adults were captured. The great majority of individuals were caught on 12/16/02 (Table 2). The mean length of captured metamorphosed lampreys was 147 mm (range 132-170 mm). For larval lampreys mean length was 155 mm (range 79-174 mm).

Table 3. Daily catch of metamorphosed and larval lampreys during the collection period 10/31/01-05/09/02.

Date	Number of captured individuals	
	metamorhosed lampreys	larval lampreys
11/6/01	1	0
11/25/01	1	0
11/28/01	1	0
12/13/01	1	0
12/16/02	17	50
12/17/02	0	1
12/21/02	2	0
1/9/02	1	0
1/11/02	0	1
2/27/02	1	2
2/28/02	0	2
3/3/02	0	1
3/4/02	0	1
total	25	58

Conclusions

Because of technical problems with the rotary screw trap the results of catching period 2001-2002 are unreliable. Furthermore, peak flows during caching period were so high that trapping efficiencies must have been very low during the peak flows when it is

likely that the main part of lampreys left the river (see Close et al. 2002, Aronsuu et al. 2002). It is also probable that some lampreys migrated during the highest flow in February when the trap was pulled up. However, no larval or metamorphosed lampreys were captured at FCF in 2002 when in 2001 FCF catch of metamorphosed lampreys was 161 individuals reflecting that number of outmigrating lampreys in 2002 may have been much lower than in 2001.

UPMIGRATION OF ADULT LAMPREYS

Objective

The final goal of the project is to restore natural production of Pacific lamprey to self-sustaining and harvestable levels in traditional fishing areas in the Umatilla River and within the whole Columbia River basin. The number of upmigrating lampreys into the Umatilla River depends on number of lampreys reaching the mouth of the river and the attractiveness of the river during the time they pass the river. The objectives of this study have been to determine the number adult lampreys entering the Umatilla River and the amount of lampreys available in the Columbia River.

Material and methods

In the Columbia River timing and relative number of migrating lampreys have been estimated based on visual counts made as lamprey pass through fishways at hydropower dams during pre-spawning migrations (Starke and Dalen 1995). Typically, counting is conducted during two consecutive 8-hr shifts from 0500 to 2100 (Moser and Close 2003). Number of daily counts of Pacific lamprey at the John Day dam 5/1/02-10/31/02 was collected from the website of Fish Passage Center (FBC) (http://www.fpc.org/adult.html). The daily number of counted lampreys was plotted with daily mean discharge at the lowermost end of the Umatilla River to evaluate the attractiveness of the Umatilla River compared to the migration pattern of lampreys in the mainstem Columbia River.

In 2002, the number of upmigrating adult lampreys entering into the Umatilla River was estimated by trapping adult lampreys by portable assessment traps and fyke net.

Portable assessment traps are widely used for catching sea lampreys in Great Lakes region (Schuldt and Heinrich 1982). Two portable assessment traps were fished 75 nights from August16th, 2002 to October 30th, 2002. Traps were placed just below water surface on both sides of the entrance of the Three Mile Falls Dam fish ladder (rkm 6.4, Figure 7). Trap dimensions were: height 60 cm, length 116 cm, and width 49 cm. The trap had a 40 cm deep mouth in both ends with an opening of 12x10 cm. In the openings were two vertical metal bars to prevent bigger fish like salmonids from entering the trap (Figure 7). Adult traps were checked daily and lampreys captured in the trap were supposed to be measured and marked for release and recapture.

Figure 8. Portable assessment trap

Fyke netting is the most common way to catch River lampreys (*Lampetra fluviatilis*) in Finland (Personal communication, E. Ojutkangas). The fyke net we used in the Umatilla River had larger mesh size and openings than in traps used by commercial fishermen in Finland. However, everything else in the trap design was similar (Figure 8). The length of the wings was 30 m and the height was 2.5 m and mesh size was 20 mm. Floats were attached every 0.8 m in the upper end of wings and lead string (1000 g/m) was attached to the lower end of the wings. The width of the opening of the porch (the part between wings and collection box) was 2.75 m and the height was 2.5 m. The length of the porch was 2.5 m and it narrowed down when approaching the collection box. The mesh size of the netting covering all four sides of the porch was 12 mm. The collection box was constructed of 6 plastic tube hoops (diameter 55 cm) and 10 mm mesh size netting. The total length of the box was 6 m and its' diameter was 55 cm. The collection box had two throats; the third and the third hoop from the opening of the collection box (Figure 8). The diameter of the opening of the first throat was 15 cm and the diameter of the opening of the second throat was 5.5 cm.

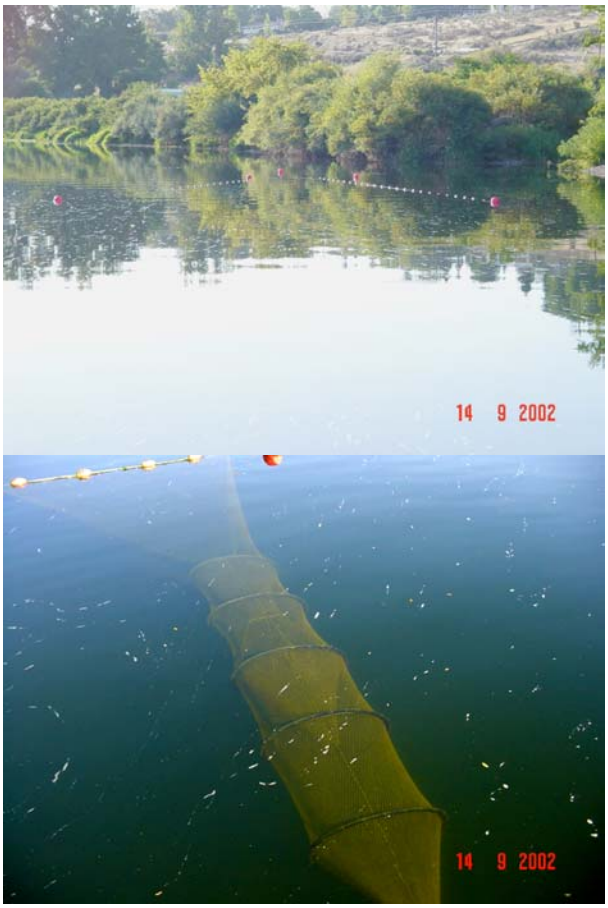

Figure 9. Fyke net

The fyke net was placed to the Umatilla River at rkm 0.5 on 29th of August. Weights were used to anchor and tighten up the wings, porch opening and the collection box. The angle between the wings was approximately 70 ^{o} and so the distance between the down river end of the wings was approximately 18 m (Figure 8). The trap was checked daily and lampreys captured in the trap were supposed to be measured and marked then released about 300 meters below the trap to estimate trapping efficiencies. The fyke net was fished until 27th of September i.e. a total of 29 nights.

Results

In 2002, a total of 26,830 lampreys were counted at the John Day Dam and 11,280 lampreys at the McNary dam. The peak migration occurred in July and August which accounted for 78 % of all lampreys passing the John Day Dam (Figure 9). Approximately 53 % of lampreys were counted from 07/11-08/15, when discharge in the

Umatilla River was very low (range 0.03 - 0.14 $m^3 s^{-1}$). The number of counted lampreys at the John Day Dam during assessment trap trapping was 10,174 and during fyke net trapping 4,901 individuals.

No lampreys were captured by portable assessment traps nor by fyke netting during the catching period.

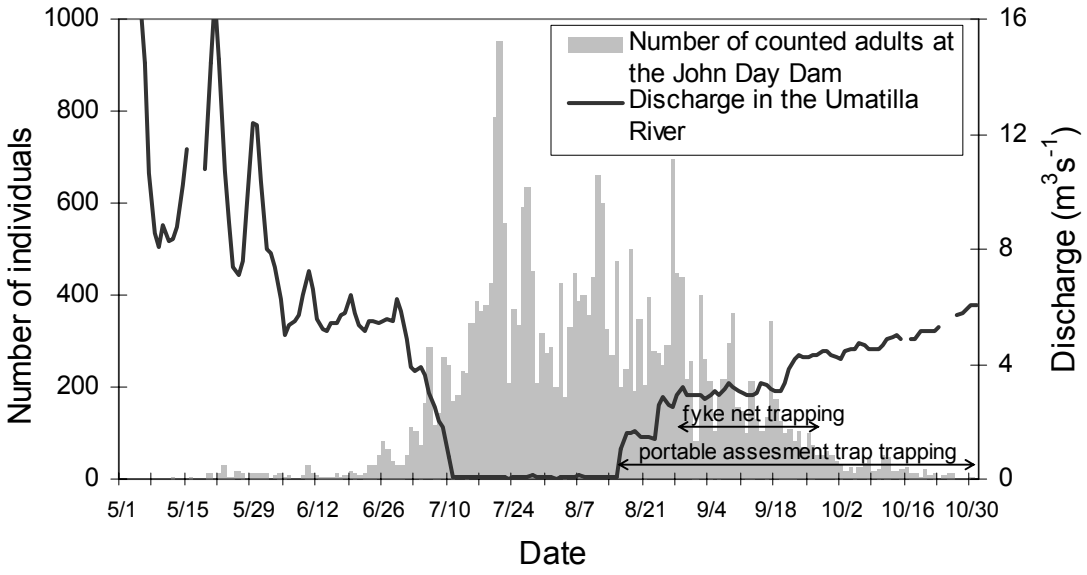

Figure 10. Daily counts of lampreys at the John Day Dam and discharge at rkm 4.5 in the Umatilla River.

Conclusions

In many studies the spawning migration of lampreys has been found to be induced by increases in the river flow (Asplund and Södergren 1975, Abou-Seedo and Potter 1979, Aronsuu et al. 2002). The unidirectional flow is a strong orientation cue for migratory animals. Another factor influencing migration besides flow could be pheromones such as petromyzonol sulfate released by larvae and carried by the water (Li et al. 1995, Robinson et al. 2002). It's obvious that low flows during the best migration season decreases attractiveness of the Umatilla River for upmigrating lampreys. Due to present flow regulation during the peak adult migration, pheromones are mainly diverted into the irrigation channels and flow at the mouth is almost zero. Because the number of

migrating adult lampreys in the Columbia River is currently low (Close 1999) it is very important to have moderate flows in the Umatilla River during the whole migration period to attract lampreys into the river.

However, almost 50 % of lampreys passed the Umatilla River, when discharge in the Umatilla River was higher than it has been historically (Close 1999) and we had an effort to capture upmigrating lampreys part of that time without any success. We weren't able to estimate trapping efficiencies of the traps, but the trap types we used have been used successfully to catch other lamprey species. We believe that the reason we did not capture any adults was due to very low number of lampreys entering the River Umatilla, not low efficiency of the traps. It seems that the Umatilla River does not attract lampreys to enter it even when the flow is moderate.

The latest study results dealing with larval pheromones have showed that it's possible that larval Pacific lamprey releases less and adult Pacific lamprey is less sensitive to pheromones than Sea lamprey (Robinson et al. 2003,Yun et al. 2003). We speculate that the reason for the low number of upmigrating adults is still a lack of attractive pheromones. Even though larval densities have increased significantly in the upper reaches no changes have occurred below rkm 100. The half life of known pheromones attracting adult lampreys is less than a week at 20-22 ^{o}C and it speeds up when temperature rises (personal communication, W. Li, MSU). Due to high temperature and low flow during the migration period (figures 2 and 3) it is probable that almost all pheromones released by larvae above rkm 100 are degraded before reaching the mainstem Columbia River. If our hypothesis is correct, the reason for very low numbers of upmigrants is lack of pheromones. The dispersion of larval lampreys to the lower and middle section of the river and/or successful reproduction in the lower reaches of the river should increase the number of upmigrating adults into the Umatilla River.

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CHAPTER TWO

Olfactory Sensitivity of Pacific Lampreys to Petromyzonol Sulfate

T. Craig Robinson, Jennifer M. Bayer, and James G. Seelye

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

INTRODUCTION

Pacific lampreys (*Lampetra tridentata*), once plentiful in the Columbia River Basin (CRB), are in decline or have been extirpated from much of their historical range. Tribal entities first noted this decline as their traditional fishery for lampreys yielded smaller catches (Close et al. 1995). State agencies such as the Oregon Department of Fish and Wildlife (ODFW) were also concerned, and in 1993 the Pacific lamprey was listed as an Oregon State sensitive species (OAR 635-044-0130), with additional protection granted in 1996 (Kostow 2002). The Northwest Power Planning Council's Fish and Wildlife Program (1994) requested a status report on Pacific lampreys to identify research needs. The resulting report (Close et al. 1995) recognized that many of the factors that may be affecting Pacific lamprey populations in the CRB, such as hydrosystem passage, declining habitat quality and quantity, reduction in prey base during the ocean phase, and water pollution, are the same issues affecting salmon populations in the Northwest. Due to the lack of basic biological information on Pacific lampreys in the CRB, Close et al. (1995) called for studies on lamprey abundance, evaluation of their current distribution, and determination of habitat limiting factors.

Mounting concerns for the status of the Pacific lamprey in the CRB led to the inception of a Pacific lamprey research and restoration program by the Confederated Tribes of the Umatilla Indian Reservation (CTUIR). Since 1996, this program has been gathering data on historical and current distribution of lampreys within the ceded lands of the CTUIR and collaborating with the Columbia River Inter-Tribal Fish Commission (CRITFC) to examine trends in lamprey passage at hydroelectric facilities in the CRB (Jackson et al. 1997a, Jackson et al. 1997b, Close 2000, Close 2001). Additionally, the CTUIR has been working with researchers at Oregon State University (OSU), University of Minnesota (UMN), University of Idaho (UI), and the U.S. Geological Survey (USGS) to study lamprey physiology related to passage, migration, and spawning (Jackson et al. 1997a, Jackson et al. 1997b, Close 2000, Close 2001). In 2000, the CTUIR began a pilot study of Pacific lamprey rehabilitation in the Umatilla River Basin, OR, which has been tracking the reproductive success of outplanted spawning-phase adults in the upper Umatilla River (Close 1999, Close et al. 2002, Aronsuu et al. 2002).

To better understand the migration and spawning behaviors of Pacific lampreys, the USGS and CTUIR examined studies of pheromone cues used by sea lampreys (*Petromyzon marinus*) in the Great Lakes region. Great Lakes researchers reported reduced returns of adult sea lampreys to streams where lamprey larvae had been eradicated by chemical treatment with larvicides in the effort to control populations of the exotic species (Moore and Schleen 1980). Mark-recapture studies suggested that sea lampreys do not exhibit homing behavior to natal streams (Bergstedt and Seelye 1995). Instead, olfactory studies of sea lampreys showed that adult upstream migrants are attracted to streams containing larval lampreys because of pheromones excreted by these larvae. Specifically, two unique larval lamprey bile acids, petromyzonol sulfate (PS, Table 1) and allocholic acid (ACA, Table 1), have been shown by electro-olfactogram (EOG) studies to be potent olfactory stimulants in adult sea lampreys early in their upstream migration (Li et al. 1995, Li and Sorensen 1997). Later in their migration, as spawning approaches, adult sea lampreys lose their sensitivity to these larval pheromones (Bjerselius et al. 2000) and sexually mature females become highly sensitive to a bile acid, 3-keto petromyzonol sulfate (3-keto PS, Table 1), excreted through the gills of spermiating males (Li et al. 2002). Although EOG studies showed olfactory sensitivity to these compounds in sea lampreys, only behavioral studies could determine whether lamprey bile acids were attractants to migrating adult sea lampreys. In behavior studies using two-choice mazes, early migrating adult sea lampreys were attracted to waters containing larval pheromones (Teeter 1980, Bjerselius et al. 2000, Vrieze and Sorensen 2001), and sexually mature females were attracted to waters containing the 3-keto PS emitted by spermiating males (Li et al. 2002).

The current USGS study of olfaction in Pacific lampreys is modeled after these pheromone studies of sea lampreys, and was developed in consultation with researchers from UMN and Michigan State University (MSU) to support the research and restoration efforts of the CTUIR. Yun et al. (in preparation) have confirmed preliminary findings by Sorensen and Close (2000) showing that larval Pacific lampreys produce PS and do not produce ACA. In 2000, the USGS built and tested an EOG apparatus (Seelye and Bayer 2002), and in 2001 confirmed that the olfactory organ of upstream migrating adults responds to stimulation by PS, but not ACA, when measured by EOG (Robinson et al. 2002). Additionally, we found that upstream migrating adult Pacific lampreys respond to olfactory stimulation by 3-keto PS (Robinson et al. 2002). Pacific lampreys enter fresh water from the ocean over several months (spring through late summer) and remain in the riverine environment as much as a year before spawning (Beamish 1980, Bayer et al. 2001), whereas sea lampreys enter streams over a 3 to 4 month period and spawn within 2 to 4 weeks (Applegate 1950). In 2001, we confirmed that upstream migrating Pacific lampreys exhibit a longer period of sensitivity to larval and adult bile acids than sea lampreys (Robinson et al. 2002), perhaps due to their extended residence in fresh water during upstream migration through spawning. In 2002, we continued to use EOG to track the olfactory sensitivity of adult Pacific lampreys from their capture in the CRB during their upstream migration in the spring through spawning a year later. This report examines data collected from January through December 2002, completing the tracking of the olfactory sensitivity of 2001 upstream migrants through their spawning in June 2002, and repeating these experiments for 2002 upstream migrants. We compare these data to findings from 2001 as well as studies of sea lamprey olfaction, which is a well-understood model of pheromones as migratory and spawning cues in lampreys.

METHODS

Study Animals

Upstream migrating adult Pacific lampreys were collected from fish ladders on the Willamette and Columbia Rivers, as well as from the mouth of the Klamath River. In 2001, lampreys were collected in June from Willamette Falls in Oregon and Bonneville

Dam in Washington. In 2002, lampreys were collected in April from the mouth of the Klamath River in California, in June from Bonneville Dam in Washington, and in November from John Day Dam in Oregon. Collected lampreys were transported to the Columbia River Research Laboratory (CRRL) and held in the onsite wetlab facility. Lampreys were maintained on a simulated natural photoperiod with temperature-controlled, sand-filtered, flow-through water from the Little White Salmon River. Water temperature was near ambient Columbia River values, as measured at Bonneville Dam, from January through May and during November and December, and was 15°C from June through October (Figure 1). A natural barrier to anadromous lampreys exists downstream of the CRRL and no resident species of lamprey were found in spot-check surveys of suitable habitat upstream of the facility. Thus, no naturally occurring lamprey bile acids were known to be present in the water source for these experiments.

Electro-olfactogram (EOG) recording

Lampreys were anesthetized and immobilized with intramuscular injections of metomidate hydrochloride (3 mg/kg body weight) and gallamine triethiodide (150 mg/kg body weight). They were then placed in a holding trough with their body submerged and their gills and naris constantly perfused with fresh water (5.7 ml/min). Water for perfusion, odorant preparation, and odorant temperature maintenance was the same as that used to maintain lampreys in the wetlab facility.

The olfactory organ was surgically exposed and EOG responses were recorded following methods of Li et al. (1995). The skin and adipose tissue above the olfactory organ and the dorsal portion of the olfactory organ were removed, exposing the ventral lamellae. Electrodes consisted of a glass capillary (mean (± standard deviation) tip diameter = 347.5 ± 28.0 μm, n=20) filled with 0.9% saline/ 8% gelatin, bridged to an Ag/AgCl electrode holder (MEH8, WPI, Sarasota, FL) filled with 3 M KCl. The tip of the reference electrode was placed on the skin surface near the olfactory organ, and the tip of the recording electrode was placed near the base of the ventral lamellae. Placement of the recording electrode was optimized by changing the position of the electrode until a standard 10^{-4} M L-arginine solution produced an acceptable response and baseline noise

was minimized. L-arginine was chosen as a standard because of its olfactory potency in sea lampreys (Li et al. 1995, Li and Sorensen 1997) and results from screening Pacific lamprey sensitivity to 20 L-amino acids and a bile acid, taurolithocholic acid 3-sulfate, in 2001 (Robinson et al. 2002). An odorant delivery device similar to one used by Li et al. (1995) minimized temperature and flow differences between blank perfusion water and test solutions, and provided a near instantaneous switch between these sources. Electro-olfactogram responses, indicated by voltage potential peaks with magnitudes greater than baseline values, were magnified 1000X on a bio-amplifier (BMA-931, CWE Inc., Ardmore, PA) and recorded on a PC-based physiograph (DI-400-PGH, Dataq Instruments, Akron, OH). Due to the invasive nature of the procedure, each lamprey was used only once. Lampreys that showed no response to the standard were not tested further and were excluded from analysis.

Once a satisfactory standard response and baseline were established, the olfactory organ was exposed to a series of concentrations of each test odorant and the results were recorded. Each series was tested by performing 5-second exposures to odorants in the following order: standard, blank water, test odorant series (in order of increasing log molar concentration), blank water, and standard. A 3-minute interval of blank water between odorant administrations was used to allow the olfactory receptors to recover. Exposures to the L-arginine standard were performed approximately every 30 minutes. A methanol blank was periodically used as well. Each odorant exposure was replicated twice, and if the second response was not similar to the first, further replicates were performed until two repeatable measurements were made. At the conclusion of each experiment, length, weight, anterior girth (body circumference posterior to seventh gill pore), mid girth (body circumference anterior to first dorsal fin), and posterior girth (body circumference between first and second dorsal fins) of lampreys were measured, sex was determined, and the gonad was removed and weighed for computation of the gonadal-somatic index (GSI).

Stock solutions of standard and test odorants were prepared prior to experiments. A 10^{-2} M L-arginine stock (Sigma-Aldrich, St. Louis, MO) was prepared biweekly in deionized water and stored at 4°C for a maximum of two weeks. Bile acid stocks were prepared in methanol as needed at a concentration of 10^{-3} M. Petromyzonol sulfate (PS,

Toronto Research Chemicals, North York, Ontario, Canada) and 3-keto PS (a gift from Dr. Weiming Li, MSU) were stored at -80°C, and ACA (Toronto Research Chemicals, North York, Ontario, Canada) was stored at -20°C. Dilutions of stock solutions were prepared on the day of the experiment using the same river water source as was used for perfusion of the naris and gills.

Recording EOG responses of sea lampreys

Olfactory responses of sea lampreys to L-arginine, PS, 3-keto PS, and ACA, were measured during their upstream migration in order to examine the performance of another lamprey species on our EOG apparatus. The technique of recording EOG responses is well established in a number of teleost species (Caprio 1980, Kitamura and Ogata 1989, Baatrup et al. 1990, Crnjar et al. 1992, Morin et al. 1997) and in the sea lamprey (Li et al. 1995, Li and Sorensen 1997). Recording EOGs from adult sea lampreys enabled us to examine whether our equipment was producing data comparable to previous studies. During May and June, a total of 6 sea lampreys were shipped from the USGS, Hammond Bay Biological Station, Millersburg, MI by priority overnight in chilled well water under 100% oxygen. When sea lampreys were received at the CRRL, they were acclimated to the temperature regime in effect for Pacific lampreys at the time of their arrival. Individuals were then either prepared for EOG recording the same day of arrival or held overnight and prepared the following day. Electro-olfactogram recording was performed using the same methods described above.

Tracking changes in olfactory sensitivity during Pacific lamprey migration and spawning seasons

Olfactory responses of captive adult Pacific lampreys were measured at intervals from the time of capture during upstream migration through the time of natural spawning. From January through June, we attempted to record EOG responses for five consecutive days every fourth week from lampreys caught in 2001 and April 2002. From June through September, we attempted to record EOG responses from lampreys caught in June 2002 for 10 days during two-week periods, with a two-week gap between periods. From

October through December, we returned to the schedule of five consecutive days every fourth week that was followed the previous winter and spring, measuring EOG responses of lampreys caught in June and November 2002.

Data analysis

Peak heights of olfactory responses were measured in millivolts (mV) and expressed as a percent of standard responses, following the methods of Li et al. (1995) with some modifications. Electro-olfactogram responses were measured from the baseline immediately prior to the response, to the maximum height of the response. Acceptance criteria were developed for response measurements because steady baselines were often difficult to attain with Pacific lampreys. An EOG response was considered measurable if it appeared in the same time interval as other typical EOG responses (3 to 6 seconds after the initiation of odorant exposure) and was at least three times the range of the baseline noise during the odorant administration. Potential responses that did not meet these criteria were classified as "no measurable response" and assigned a value of zero. For odorants that were replicated more than two times, the two responses that were most similar in size and shape were selected for analysis. Responses that were not repeatable were assigned a value of zero. Duplicate responses for each test odorant were averaged and expressed as a percent standard response by dividing the value of the average test response by the average magnitude of the nearest standard responses. Expression of EOG responses as a percentage of the standard response controlled for inter-experimental differences in electrode placement and individuals tested. If the responses to the standard before and after a series of test odorants were not within ± 20% of the mean of those standard responses before and after that series, the responses to the test odorants were considered uncontrolled and discarded from analysis. Plots of dose-response curves were made for each test interval and changes in olfactory responsiveness of Pacific lampreys over time were described.

RESULTS

Olfactory sensitivity of adult Pacific lampreys

In 2002, 94 adult Pacific lampreys were tested on the EOG apparatus for olfactory sensitivity to larval and adult lamprey bile acids, with 24 adults from the 2001 upstream migration and 70 adults from the 2002 upstream migration (Tables 2 and 3). Lampreys caught in 2002 and tested on EOG from April through December 2002 (Table 3), showed similar morphometric statistics of length, weight, and girths, when compared to lampreys caught in 2001 and tested in January 2002 (Table 2), as well as those tested from June through December 2001 (Robinson et al. 2002). Lampreys caught in 2001 showed a steady decrease in length and weight from February through spawning time in June (Table 2). Gonadal-somatic index was constant in 2002 migrants from May through December, with slightly greater variation from September through December (Table 3). Gonads were not removed and weighed from 2001 upstream migrants. Sex ratios among test groups varied, with an overall distribution of 33% females and 67% males for the 2001 upstream migrants (Table 4), and 37% females and 63% males for the 2002 upstream migrants (Table 5). The rate of successful EOG recordings varied among test groups, with 18 lampreys (75%) from 2001 and 61 lampreys (87%) from 2002 producing valid EOG recordings (Tables 4 and 5). The rate of successful EOG recordings did not vary by sex.

Adult Pacific lampreys responded to bile acids with typical negative potential EOGs and responded to L-arginine with atypically positive potential EOGs. Figure 2 shows representative EOG recordings from adult Pacific lampreys exposed to PS, 3-keto PS, ACA, and the L-arginine standard. Similar to EOG recordings from previous studies of olfaction in fish (Caprio 1980, Kitamura and Ogata 1989, Baatrup et al. 1990, Crnjar et al. 1992, Li et al. 1995, Li and Sorensen 1997, Morin et al. 1997), Pacific lamprey olfactory responses to PS and 3-keto PS were classic EOG recordings with sharp negative potential peaks that gradually returned to baseline, increasing in magnitude with each 10-fold increase in odorant concentration (Figures 2 A and 2 B). Exposure to ACA produced little or no measurable olfactory response by Pacific lampreys. Although a slight response is evident at higher concentrations of ACA, most peaks were not

discernable from background noise (Figure 2 C). Exposure of Pacific lampreys to 10^{-4} M L-arginine produced EOG recordings with a classic shape but atypically positive potential (Figure 2).

Olfactory responses of adult Pacific lampreys to L-arginine were consistent through time, confirming its use as a standard despite its atypical positive potential. Regression analysis performed on mean olfactory responses of adult Pacific lampreys to 10^{-4} M L-arginine through time revealed no relation for 2001 upstream migrants and a very weak relation for 2002 upstream migrants (Figure 3). Mean responses to 10^{-4} M L-arginine that were used to calculate percent standard response values for test odorants were plotted over time and a regression line was fit to these data points. Mean olfactory responses to L-arginine for 2001 upstream migrants measured from June 2001 to June 2002 ($n = 214$) had a median of 2.89 mV, ranged from 1.07 mV to 5.80 mV, and revealed no significant relation ($r = -0.108$, $p = 0.1141$). Mean olfactory responses to L-arginine for 2002 upstream migrants measured from April to December 2002 ($n = 361$) had a median of 2.78 mV, ranged from 1.24 mV to 7.59 mV, and revealed a weakly positive relation that was significant ($r = 0.209$, $p < 0.0001$). However, the regression could only account for 4.4% of the variation ($r^2 = 0.044$) present in the data set. Thus, olfactory responses of adult Pacific lampreys to the L-arginine standard have been considered consistent through time and further test results are reported relative to this standard.

Adult Pacific lampreys responded to olfactory stimulation in a dose-dependant manner. Increasing concentrations of PS and 3-keto PS from 10^{-9} M to 10^{-6} M produced logarithmic dose-response curves (Figures 4 and 5). The lower threshold of detectable olfactory responses varied between 10^{-9} M and 10^{-8} M for PS and between 10^{-9} M and 10^{-7} M for 3-keto PS. Variability in these detection thresholds was most influenced by baseline stability. Increasing concentrations of ACA produced no measurable increase in most EOG tests of adult Pacific lampreys (Figure 6).

Olfactory responses of adult Pacific lampreys to larval and adult bile acids were greater upon first entry into the riverine environment in the spring, declined to stable levels for a year during the period of freshwater migration, and became immeasurable at the spawning phase the following spring. Olfactory responsiveness of 2001 upstream migrants to 10^{-6} M PS ranged between means of 22% to 86% L-arginine standard from

January to April and were then immeasurable in June during the spawning phase (Figure 7 A). Likewise, responses of 2001 upstream migrants to 10^{-6} M 3-keto PS had means of 54% and 49% L-arginine standard in February and April and were then immeasurable in June during the spawning phase (Figure 8 A). Exposure to ACA produced no measurable olfactory responses during this same interval of time (Figure 9 A). Responses of 2002 upstream migrants to 10^{-6} M PS ranged between means of 27% and 187% L-arginine standard from entry into fresh water in April to June, and declined to stable levels between 23% and 51% L-arginine standard from July to December (Figure 7 B). Similarly, responses of 2002 upstream migrants to 10^{-6} M 3-keto PS ranged between means of 83% and 91% L-arginine standard from April to June, and declined to stable levels between 22% and 50% L-arginine standard from July to December (Figure 8 B). Exposure to ACA produced few measurable olfactory responses during this same interval of time (Figure 9 B). When compared by sex, there were no significant differences (t-test, $p > 0.05$) in adult Pacific lamprey responses to 10^{-6} M PS and 3-keto PS for either 2001 or 2002 upstream migrants (Table 6). Thus, results from 2002 confirm findings reported by Robinson et al. (2002) for 2001 (Figures 10 to 12).

EOG recordings from adult sea lampreys

In May and June 2002, 6 upstream migrating adult sea lampreys were tested on the EOG apparatus for olfactory sensitivity to L-arginine, PS, 3-keto PS, and ACA. Lengths, weights, and girths were not significantly different (t-test, $p > 0.05$) between sexes (Table 7). Mean GSI, however, was significantly greater ($t = 4.69$, $p < 0.05$) for females (18.8%) than males (2.2%). The rate of successful EOG recordings was 50% overall (Table 8).

EOG recordings from responses of adult sea lampreys to lamprey bile acids and L-arginine were similar to recordings from adult Pacific lampreys. Figure 13 shows EOG recordings from adult sea lampreys exposed to PS, 3-keto PS, ACA, and L-arginine in June 2002. Adult sea lampreys responded to 10^{-6} M PS, 3-keto PS, and ACA with classic sharp negative potential peaks that gradually returned to baseline (Figure 13). However, 10^{-4} M L-arginine exposure elicited atypical positive potential EOGs, while exposure to

10^{-5} M L-arginine resulted in little or no measurable responses (Figure 13). The mean response of adult sea lampreys to PS was greater than the mean responses for 3-keto PS and ACA (Figure 14), but there were no clear differences in responses by sex despite significant differences in GSI.

DISCUSSION

Results from the current study confirm that the olfactory system of Pacific lampreys is sensitive to larval and adult lamprey bile acids, specifically PS and 3-keto PS, throughout the migratory phase of the adult life stage. The olfactory sensitivity of adult Pacific lampreys to select bile acids and L-amino acids is similar to that of adult sea lampreys (Li et al. 1995, Li and Sorensen 1997), and is consistent with findings in a preliminary study of Pacific lamprey olfaction performed by Sorensen and Close (2000). However, there are a number of apparent differences between the olfactory systems of Pacific lampreys and sea lampreys in response magnitudes, response thresholds, and duration of sensitivity.

Adult Pacific lampreys are less sensitive to bile acids than sea lampreys from olfactory studies in the Great Lakes region. Comparing magnitudes of responses in mV, olfactory responses of adult Pacific lampreys to 10^{-6} M PS (Table 5) were about one sixth of the values reported for sea lampreys (approximately 9 to 10 mV, Li et al. 1995, Li and Sorensen 1997). Responses to 10^{-6} M ACA were non-measurable in Pacific lampreys compared to approximately 3 mV in sea lampreys (Li et al. 1995, Li and Sorensen 1997). Likewise, when compared to sea lampreys, Sorensen and Close (2000) found greatly reduced responses to ACA in Pacific lampreys that were likely below the detection level of our EOG system. A possible explanation for this reduced sensitivity to ACA may lie in the preliminary findings that larvae of Pacific lamprey do not produce ACA (Sorensen and Close 2000, Yun et al., in preparation), and therefore adults do not use this bile acid as a pheromone cue. To date there are no published results of sea lamprey EOG responses to 3-keto PS. However, unpublished data shows that responses to this bile acid in Pacific lampreys are also less than those recorded in sea lampreys (personal communication, W. Li, MSU). Additionally, throughout the migratory phase of the adult

life stage of Pacific lampreys, there does not appear to be sexual dimorphism in responses to 3-keto PS as reported for spawning phase sea lampreys (Li et al. 2002).

Adult Pacific lampreys show an atypical response to L-arginine. Positive potential responses of Pacific lampreys to 10^{-4} L-arginine in both 2001 (Robinson et al. 2002) and 2002 studies are unlike results from sea lampreys (Li et al. 1995, Li and Sorensen 1997). However, in 2002 we recorded positive potential EOGs from sea lampreys on our EOG apparatus (Figures 13 and 14) and have hypothesized that these results may reflect an unknown interaction between our water supply and the olfactory system of agnathan species. Despite the novelty of lamprey responses to L-arginine on our EOG apparatus, we conclude that it is still a viable standard due to its consistent measurability.

In addition to a reduced sensitivity to larval and adult lamprey bile acids, the limits of detectability for these compounds appear to be at greater concentrations for adult Pacific lampreys than for sea lampreys. In contrast to measurable olfactory responses in sea lampreys to concentrations as low as 10^{-12} M PS and ACA, EOG responses of Pacific lampreys to PS and 3-keto PS were only measurable to concentrations of 10^{-9} M and 10^{-8} M. In our experiments, the threshold of detectable responses was highly influenced by baseline stability. Nonetheless, detection limits 1,000 to 10,000 times greater than those of a similar species are noteworthy when considering whether Pacific lampreys can use these compounds as pheromones. Polkinghorne et al. (2001) estimated that sea lamprey larvae released PS in sufficient quantities to result in river concentrations of 10^{-12} M, a level detectable by adults of that species, but not necessarily detectable by Pacific lampreys as measured by EOG in the current study. However, Vrieze and Sorensen (2001) showed that, in behavioral tests, adult sea lampreys responded to larval lamprey washings with PS and ACA concentrations approximately 10 times less than the lower detection limits measured by EOG. Behavioral studies have also shown that sea lamprey attraction to streams containing lamprey larvae cannot be fully accounted for by attraction to PS and ACA alone (Vrieze and Sorensen 2001). Thus, future behavioral trials with Pacific lampreys using larval lamprey washings may give a clearer picture of the true lower limits of pheromone detection in this species than our EOG experiments were capable of.

Although adult Pacific lampreys appear to be less sensitive to larval and adult lamprey bile acids than sea lampreys, the duration of their sensitivity to these compounds is much longer. Olfactory responses of Pacific lampreys to PS and 3-keto PS were measurable by EOG throughout their migratory phase into Spring 2002, when olfactory responses to all lamprey bile acids were immeasurable during the spawning phase. This is in stark contrast to the 2 to 4 week duration of behavioral sensitivity to PS and ACA seen in sea lampreys (Bjerselius et al. 2000). Presumably this longer period of olfactory sensitivity to larval bile acids seen in Pacific lampreys reflects the longer period of time (up to 12 months, Beamish 1980, Bayer et al. 2001) spent by this species migrating upriver searching for appropriate spawning habitat. However, the loss of sensitivity to all lamprey bile acids in the spawning phase, including 3-keto PS, leaves the role of these odorants at this phase in question. Adult sea lampreys of both sexes lose their sensitivity to larval pheromones as spawning approaches (Bjerselius et al. 2000), and sexually mature females become highly sensitive to 3-keto PS excreted through the gills of spermiating males (Li et al. 2002). In a study of annual sex steroid profiles of upstream migrating adult Pacific lampreys, Mesa et al. (2003) found that levels of estradiol 17-β and progesterone increased dramatically during the spawning phase in late April through May 2001, indicating a period of rapid reproductive-related endocrine activity. Electro-olfactogram testing of spawning phase adult Pacific lampreys from 2001 was conducted in late April and early June 2002 (Tables 2 and 4), and thus we may have failed to observe a change in sensitivity during this interval of rapid physiological change. In order to examine the possibility of an endocrine-pheremone sensitivity connection, a more intensive examination of EOG responses by spawning phase Pacific lampreys to lamprey bile acids is planned for Spring 2003.

To summarize findings from 2002, the olfactory system of early migrating adult Pacific lampreys was sensitive to the larval lamprey bile acid, PS, and the adult lamprey bile acid, 3-keto PS, but showed few measurable responses to the larval lamprey bile acid, ACA. Adult Pacific lampreys were less sensitive to bile acids than the sea lamprey and showed an atypical positive potential response to L-arginine. In addition, the limits of detectability for these bile acids appeared to be at greater concentrations for adult Pacific lampreys than for sea lampreys. Although adult Pacific lampreys appeared to be less sensitive to larval and adult lamprey bile acids than sea lampreys, the duration of their sensitivity to these compounds was much longer, reflecting their prolonged period of freshwater migration to spawning grounds. Pacific lamprey sensitivity to lamprey bile acids was immeasurable during the spawning phase. These results from 2002 confirm those from 2001 (Robinson et al. 2002) and indicate that, similar to sea lampreys, larval and adult lamprey bile acids have the potential to act as pheromone cues for migration and perhaps spawning of adult Pacific lampreys. Further data collection is needed, and thus, EOG studies of adult Pacific lamprey olfaction will continue into 2003. In 2002, we began to design and test experimental systems for behavioral studies of Pacific lamprey olfaction that will begin in 2003.

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TABLES

Table 1. Bile acids tested by electro-olfactogram (EOG) in 2002 for olfactory responsiveness of adult Pacific lampreys.

Common name (Abbreviation)	Chemical name
Petromyzonol sulfate (PS)	3α, 7α, 12α, 24-Tetrahydroxy-5α-cholan-24-sulfate
3-keto Petromyzonol sulfate (3-keto PS)	7α, 12α, 24-Trihydroxy-5α-cholan-3-one 24-sulfate
Allocholic acid (ACA)	3α, 7α, 12α-Trihydroxy-5α-cholan-24-oic-acid

Table 2. Morphometric data from adult Pacific lampreys caught in 2001 and tested by electro-olfactogram (EOG) in 2002. Data arranged by test interval as mean ± standard deviation (SD).

Dates	n	Length ± SD (mm)	Weight ± SD (g)	Anterior girth ± SD (mm)	Mid girth ± SD (mm)	Posterior girth ± SD (mm)
2001-2002 Upstream Migrants:						
1/20 to 1/26/2002	5	667.8 ± 49.0	473.46 ± 56.28	113.8 ± 4.1	110.6 ± 6.0	89.4 ± 3.0
2/17 to 2/23/2002	4	579.8 ± 38.2	306.83 ± 36.21	96.8 ± 3.3	93.3 ± 3.8	77.0 ± 2.2
3/24 to 3/30/2002	6	588.5 ± 36.3	358.50 ± 78.86	105.3 ± 12.2	101.2 ± 8.4	82.7 ± 6.3
4/21 to 4/27/2002	4	557.0 ± 34.4	269.30 ± 35.87	92.0 ± 4.2	89.3 ± 3.8	74.0 ± 1.8
6/2 to 6/8/2002	5	460.0 ± 52.4	214.92 ± 108.10	89.8 ± 18.5	87.8 ± 20.7	68.0 ± 11.9
Total:	24					

Table 3. Morphometric data from adult Pacific lampreys caught and tested by electro-olfactogram (EOG) in 2002. Data arranged by test interval as mean ± standard deviation (SD).

Dates	n	Length ± SD (mm)	Weight ± SD (g)	Anterior girth ± SD (mm)	Mid girth ± SD (mm)	Posterior girth ± SD (mm)	Gonadal-somatic index (%)
2002-2003 Upstream Migrants:							
4/14 to 4/20/2002	5	610.2 ± 39.3	398.34 ± 94.53	114.0 ± 11.7	105.2 ± 9.8	85.0 ± 7.6	--
5/19 to 6/1/2002	5	600.2 ± 17.0	365.12 ± 52.69	109.2 ± 7.0	103.4 ± 6.2	83.4 ± 4.4	3.1 ± 0.7
6/9 to 6/22/2002	10	688.5 ± 45.2	503.98 ± 116.32	121.0 ± 11.1	115.0 ± 8.5	90.9 ± 6.5	2.8 ± 0.7
7/7 to 7/20/2002	10	671.6 ± 42.2	462.25 ± 92.37	116.1 ± 8.9	110.7 ± 9.1	88.7 ± 6.3	2.7 ± 0.6
8/4 to 8/17/2002	10	674.9 ± 30.2	481.21 ± 54.49	118.3 ± 5.0	113.4 ± 5.5	90.4 ± 4.3	3.2 ± 0.6
9/1 to 9/14/2002	9	627.8 ± 33.6	376.57 ± 55.22	106.7 ± 8.6	104.1 ± 6.1	83.3 ± 5.3	4.2 ± 2.4
9/29 to 10/5/2002	5	671.6 ± 21.6	463.36 ± 47.89	115.4 ± 5.5	112.2 ± 5.6	90.8 ± 2.8	2.7 ± 1.0
10/27 to 11/2/2002	4	653.3 ± 37.4	430.10 ± 37.14	115.3 ± 3.5	111.3 ± 3.5	89.0 ± 0.8	3.2 ± 1.2
11/17 to 11/26/2002	7	632.1 ± 15.1	370.60 ± 61.12	106.3 ± 10.6	103.7 ± 8.6	83.3 ± 6.1	2.8 ± 1.5
12/15 to 12/21/2002	5	621.8 ± 42.2	365.28 ± 57.80	105.4 ± 7.8	102.2 ± 5.5	82.4 ± 5.4	3.4 ± 2.3
Total:	70						

Table 4. Electro-olfactogram (EOG) recording success rates by sex from adult Pacific lampreys caught in 2001 and tested by EOG in 2002. Data arranged by test interval.

Dates	n	Successful EOG recording (% n)	Female (% n)	Successful EOG Female (% female)	Male (% n)	Successful EOG Male (% male)
2001-2002 Upstream Migrants:						
1/20 to 1/26/2002	5	3 (60%)	5 (100%)	3 (60%)	0 (0%)	--
2/17 to 2/23/2002	4	4 (100%)	0 (0%)	--	4 (100%)	4 (100%)
3/24 to 3/30/2002	6	2 (33%)	1 (17%)	1 (100%)	5 (83%)	1 (20%)
4/21 to 4/27/2002	4	4 (100%)	1 (25%)	1 (100%)	3 (75%)	3 (100%)
6/2 to 6/8/2002	5	5 (100%)	1 (20%)	1 (100%)	4 (80%)	4 (100%)
Total:	24	18 (75%)	8 (33%)	6 (75%)	16 (67%)	12 (75%)

Table 5. Electro-olfactogram (EOG) recording success rates by sex from adult Pacific lampreys caught and tested by EOG in 2002. Data arranged by test interval.

Dates	n	Successful EOG recording (% n)	Female (% n)	Successful EOG Female (% female)	Male (% n)	Successful EOG Male (% male)
2002-2003 Upstream Migrants:						
4/14 to 4/20/2002	5	3 (60%)	2 (40%)	0 (0%)	3 (60%)	3 (100%)
5/19 to 6/1/2002	5	3 (60%)	3 (60%)	2 (67%)	2 (40%)	1 (50%)
6/9 to 6/22/2002	10	6 (60%)	4 (40%)	3 (75%)	6 (60%)	3 (50%)
7/7 to 7/20/2002	10	10 (100%)	4 (40%)	4 (100%)	6 (60%)	6 (100%)
8/4 to 8/17/2002	10	10 (100%)	4 (40%)	4 (100%)	6 (60%)	6 (100%)
9/1 to 9/14/2002	9	9 (100%)	4 (44%)	4 (100%)	5 (56%)	5 (100%)
9/29 to 10/5/2002	5	5 (100%)	2 (40%)	2 (100%)	3 (60%)	3 (100%)
10/27 to 11/2/2002	4	3 (75%)	1 (25%)	1 (100%)	3 (75%)	2 (67%)
11/17 to 11/26/2002	7	7 (100%)	1 (14%)	1 (100%)	6 (86%)	6 (100%)
12/15 to 12/21/2002	5	5 (100%)	1 (20%)	1 (100%)	4 (80%)	4 (100%)
Total:	70	61 (87%)	26 (37%)	22 (85%)	44 (63%)	39 (89%)

Table 6. Olfactory responses of adult Pacific lampreys to petromyzonol sulfate (PS) and 3-keto petromyzonol sulfate (3-keto PS) by sex from January to December 2002. Responses expressed as percent 10^{-4} M L-arginine standard response (% L-arg std). Data arranged as mean ± standard deviation (SD).

	10^{-6} M PS				**10^{-6} M 3-keto PS**			
Sex	**n**	**Mean Response ± SD (% L-arg std)**	**Min.**	**Max.**	**n**	**Mean Response ± SD (% L-arg std)**	**Min.**	**Max.**
All 2001-2002 Upstream Migrants:								
Female	23	44.5 ± 25.1	0	95.1	12	25.4 ± 26.3	0	82.2
Male	30	45.1 ± 31.9	0	103.6	19	31.5 ± 21.1	0	78.5
2001-2002 Upstream Migrants measured in 2002:								
Female	6	23.6 ± 19.9	0	48.7	2	15.6 ± 22.0	0	31.1
Male	10	59.5 ± 38.7	0	103.6	5	34.2 ± 34.3	0	78.5
2002-2003 Upstream Migrants measured in 2002:								
Female	19	68.2 ± 95.6	0	426.2	17	33.2 ± 38.4	0	136.4
Male	36	48.7 ± 33.7	0	158.5	38	42.3 ± 30.0	0	111.5

Table 7. Morphometric data from adult sea lampreys (*Petromyzon marinus*) caught and tested by electro-olfactogram (EOG) in 2002. Data arranged as mean ± standard deviation (SD).

Sex	n	Length ± SD (mm)	Weight ± SD (g)	Anterior girth ± SD (mm)	Mid girth ± SD (mm)	Posterior girth ± SD (mm)	Gonadal-somatic index (%)
Female	3	477.0 ± 19.3	226.30 ± 25.13	97.3 ± 2.3	90.0 ± 5.6	72.7 ± 5.1	18.8[a] ± 6.1
Male	3	496.0 ± 46.8	230.23 ± 62.87	92.7 ± 7.2	85.3 ± 6.7	70.0 ± 6.2	2.2[a] ± 0.5
Total:	6						

[a] Significant difference ($t = 4.69$, $p < 0.05$)

Table 8. Electro-olfactogram (EOG) recording success rates by sex from adult sea lampreys caught and tested by EOG in May and June 2002.

n	Successful EOG recording (% n)	Female (% n)	Successful EOG Female (% female)	Male (% n)	Successful EOG Male (% male)
6	3 (50%)	3 (50%)	1 (33%)	3 (50%)	2 (67%)

FIGURES

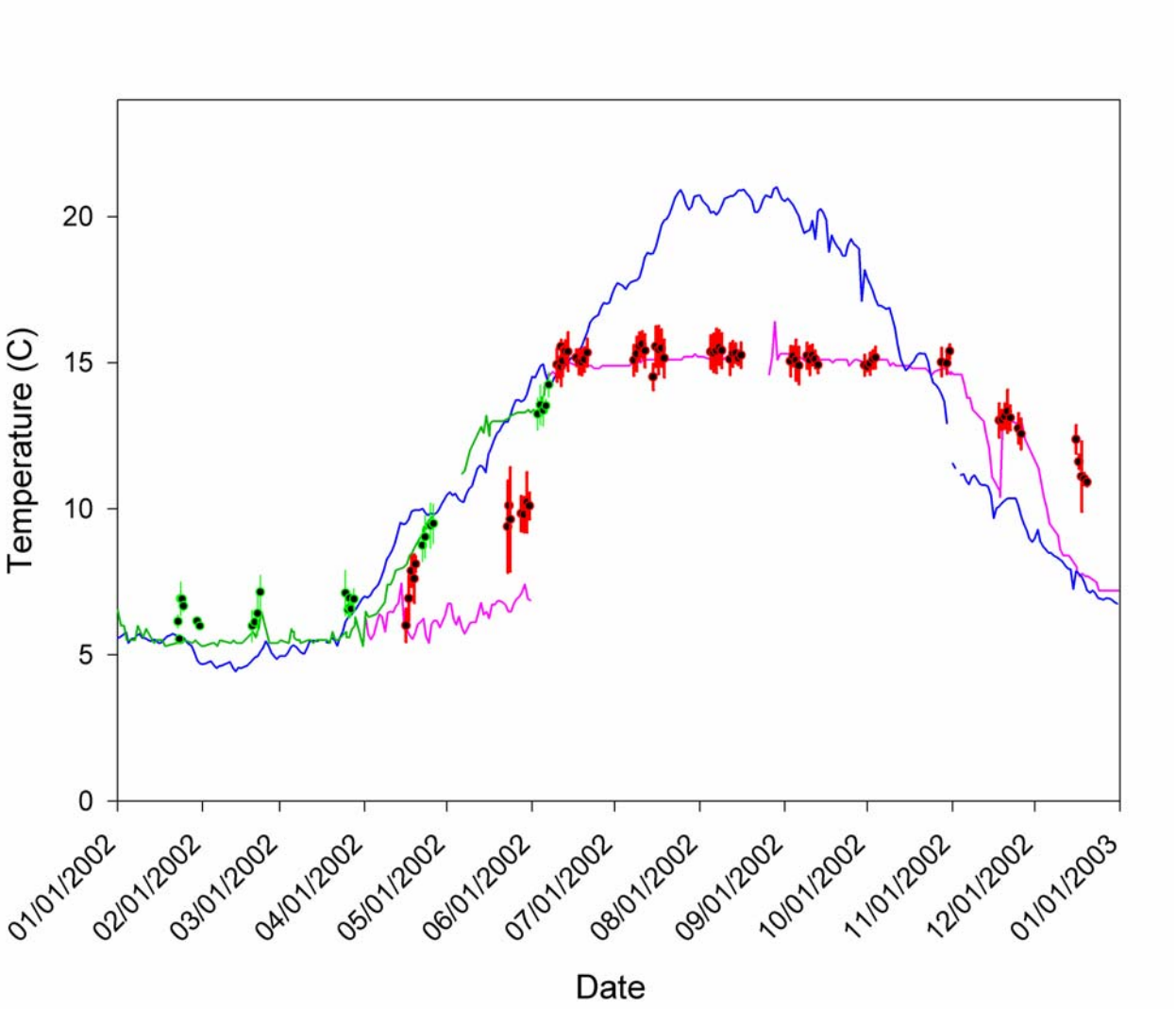

Figure 1. 2002 daily temperatures of the Columbia River at Bonneville Dam (RKM 234, blue line), lamprey holding tanks at the Columbia River Research Lab (2001 – green line, 2002 - pink line), and electro-olfactogram (EOG) apparatus (black dots, 2001 – green error bars, 2002 - red error bars). Columbia River temperature data are from DART (http://www.cqs.washington.edu/dart/dart.html). Holding tank temperature data are from daily single point readings. EOG temperature data are mean ± 2 standard deviations of temperature during each experiment.

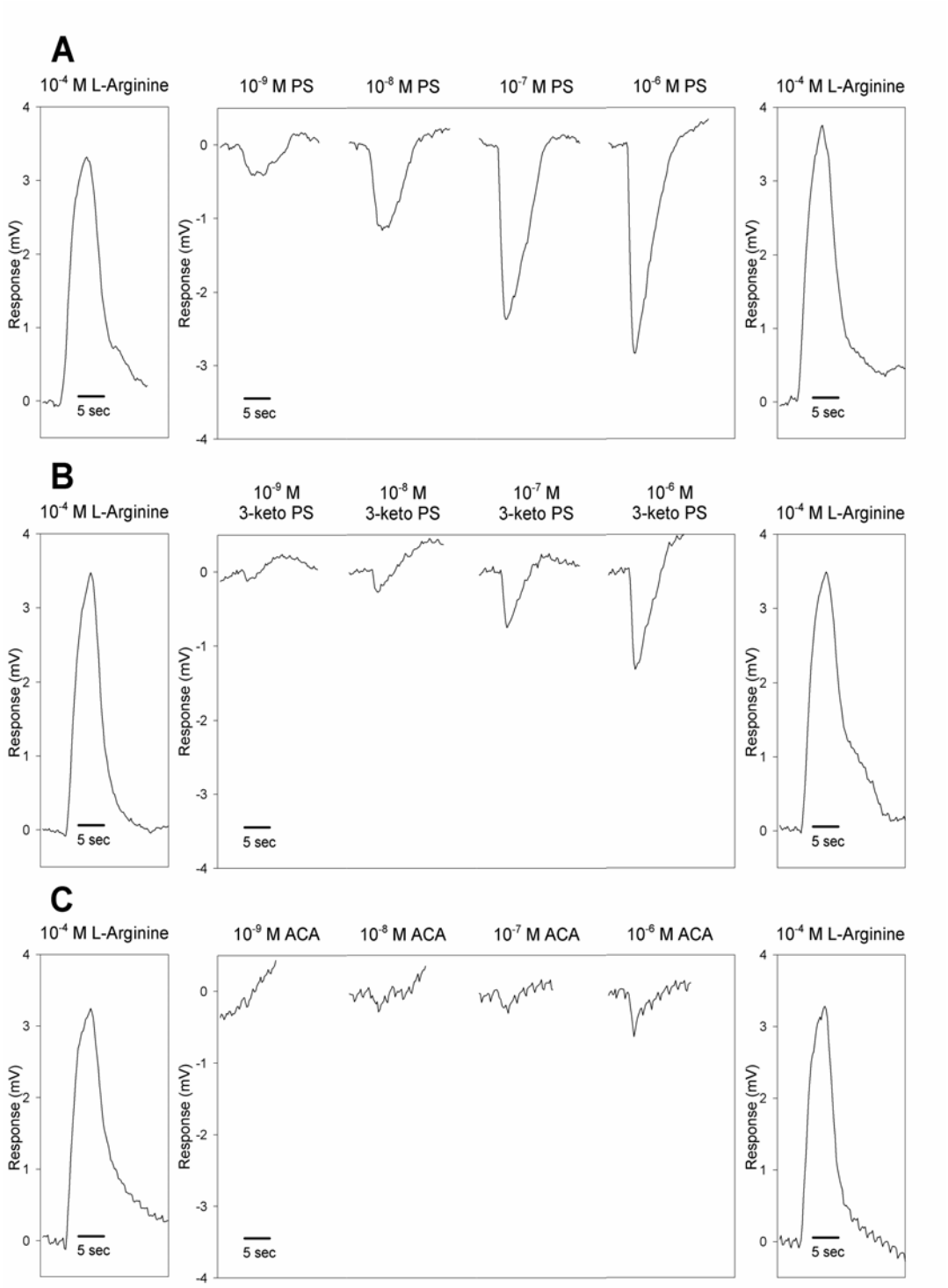

Figure 2. Electro-olfactogram (EOG) recordings from olfactory responses of adult Pacific lampreys to bile acids and 10^{-4} M L-arginine standard in 2002. A) petromyzonol sulfate (PS) in October 2002, B) 3-keto petromyzonol sulfate (3-keto PS) in September 2002, and C) allocholic acid (ACA) in September 2002.

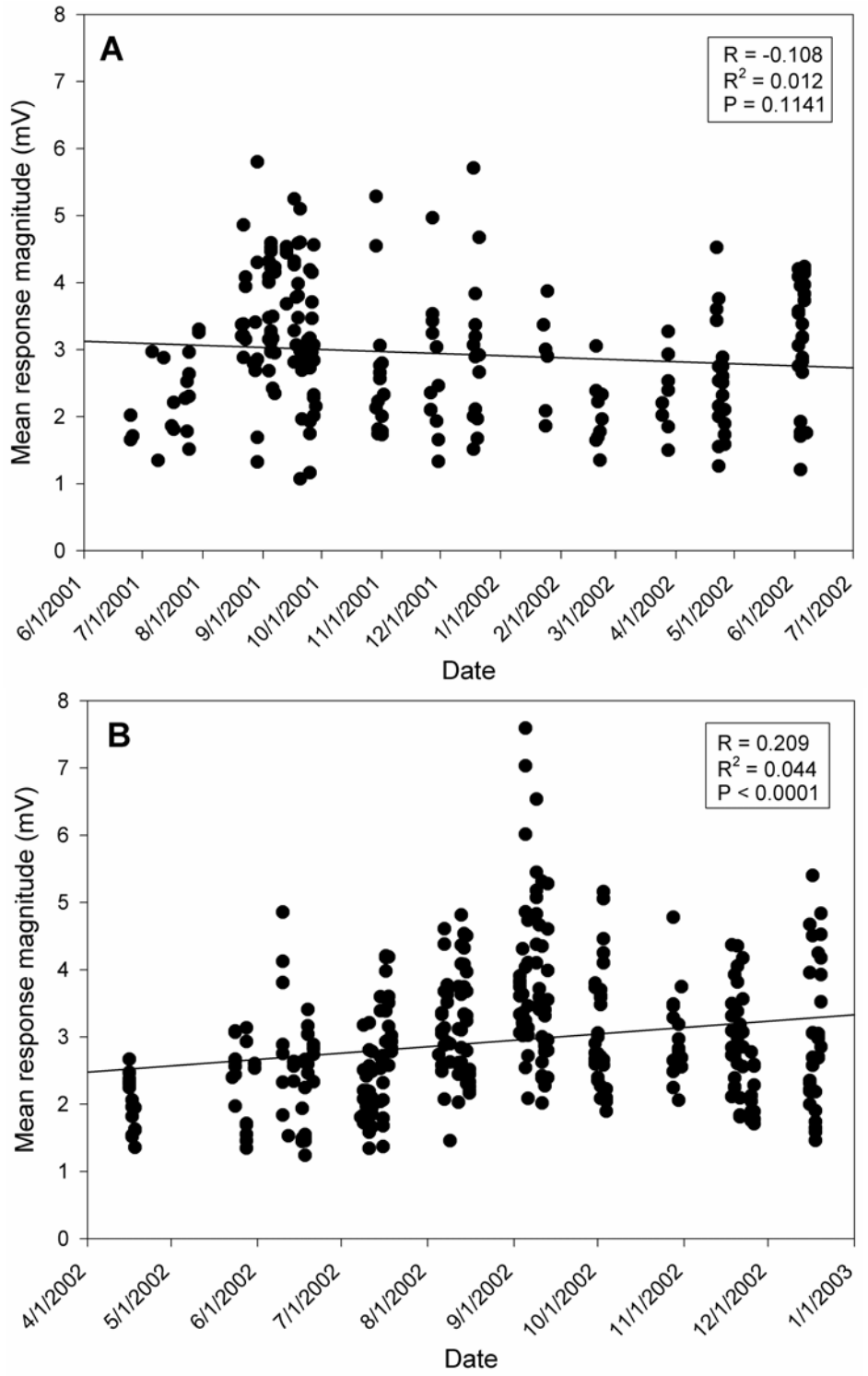

Figure 3. Scatter plots and regression lines of mean olfactory responses of adult Pacific lampreys to 10^{-4} M L-arginine through time. A) 2001 upstream migrants from June 2001 to June 2002. B) 2002 upstream migrants from April to December 2002. Response magnitudes in millivolts (mV).

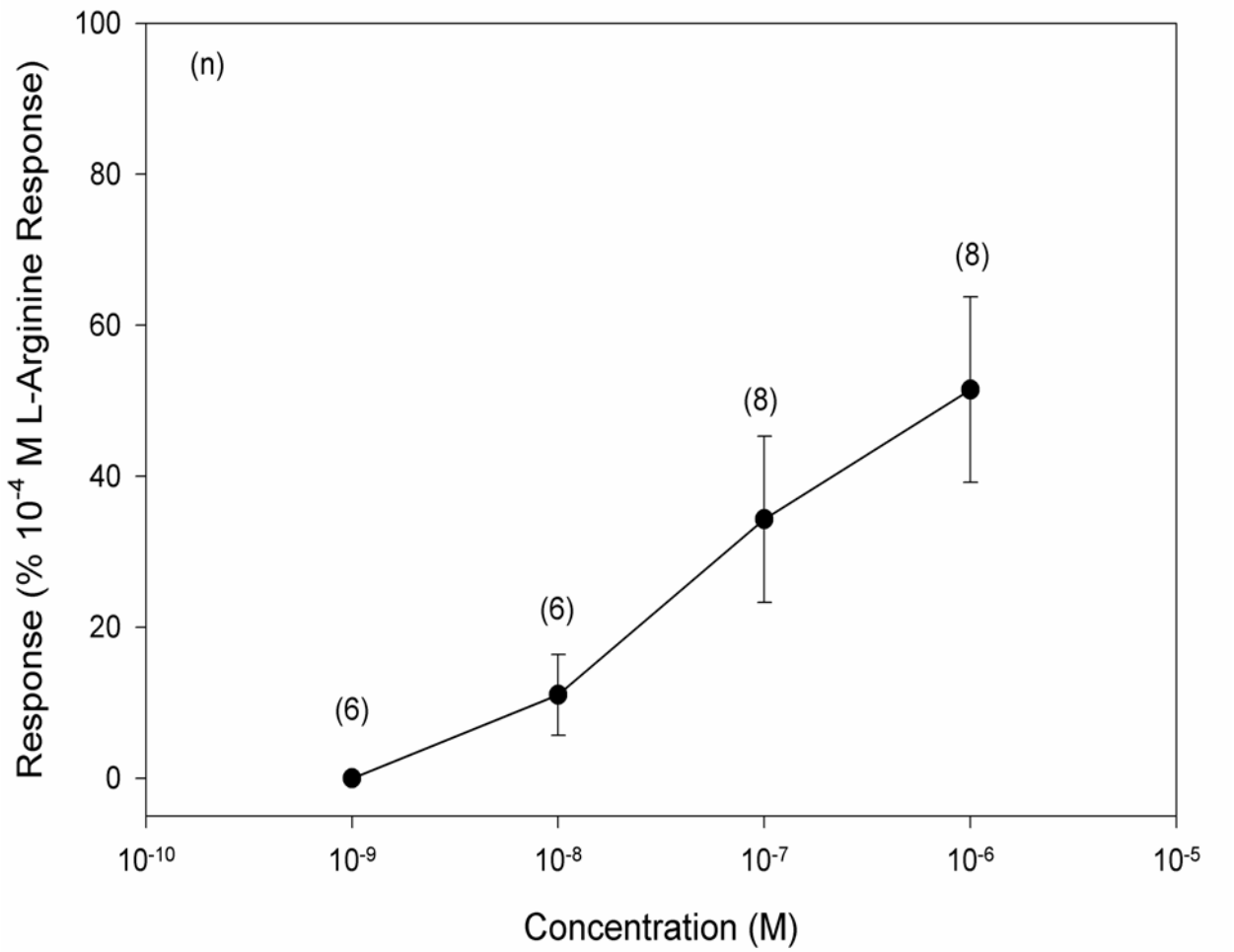

Figure 4. Representative graph of relation between the molar concentration (M) of petromyzonol sulfate (PS) and olfactory responses of adult Pacific lampreys in July 2002. Numbers above points are the sample sizes for each point. Error bars are ±1 standard error.

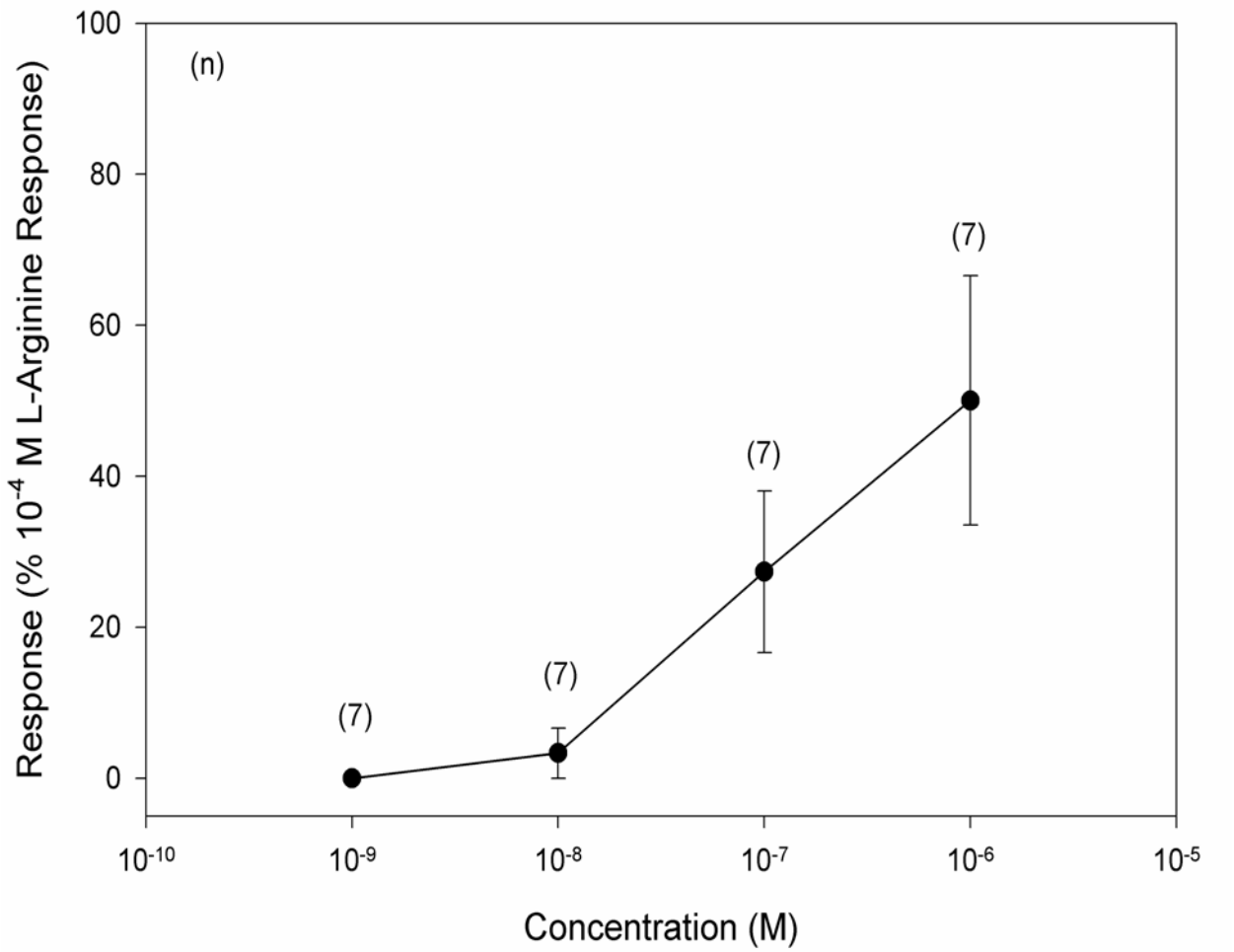

Figure 5. Representative graph of relation between the molar concentration (M) of 3-keto petromyzonol sulfate (3-keto PS) and olfactory responses of adult Pacific lampreys in November 2002. Numbers above points are the sample sizes for each point. Error bars are ±1 standard error.

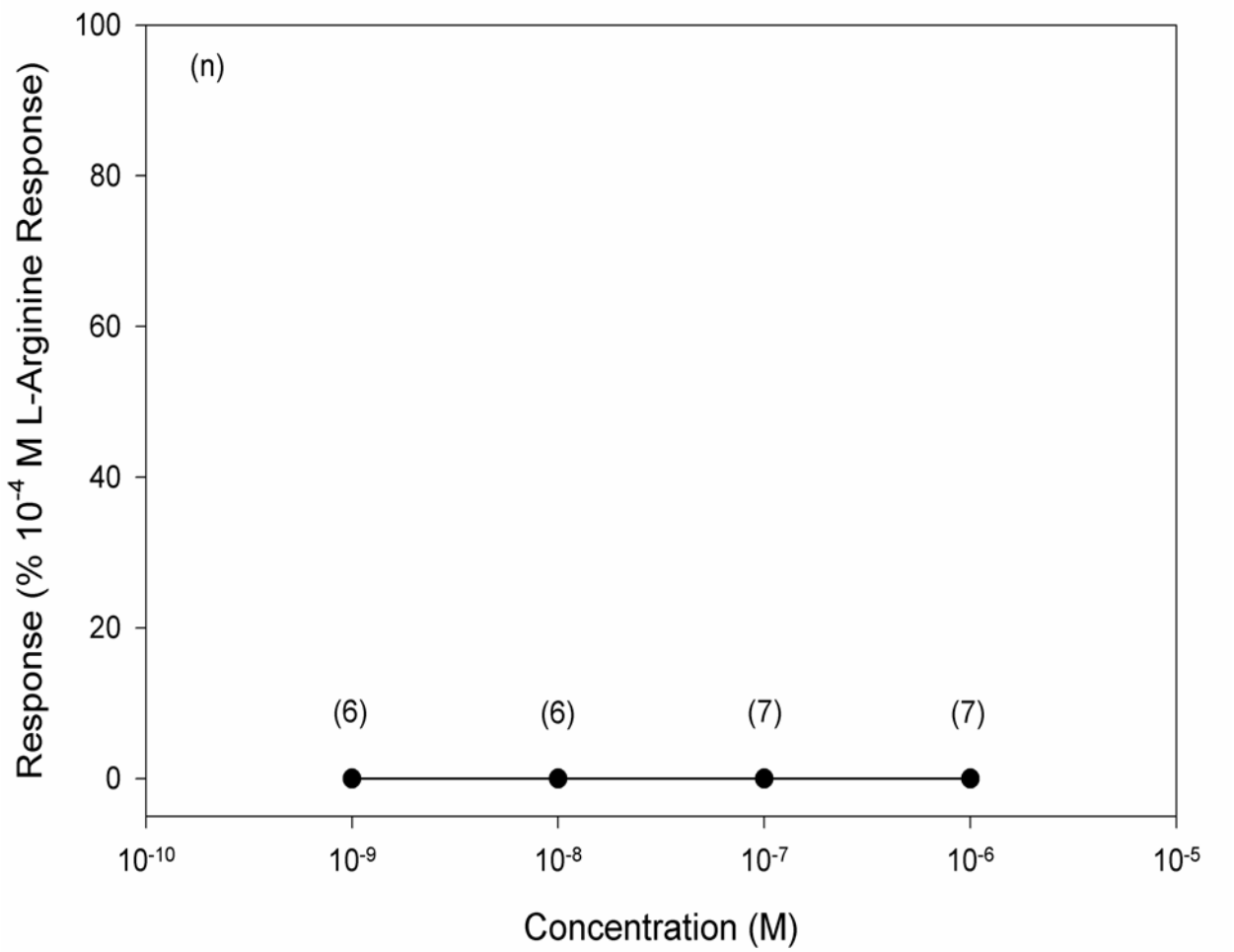

Figure 6. Representative graph of relation between the molar concentration (M) of allocholic acid (ACA) and olfactory responses of adult Pacific lampreys in November 2002. Numbers above points are the sample sizes for each point. Error bars are ±1 standard error.

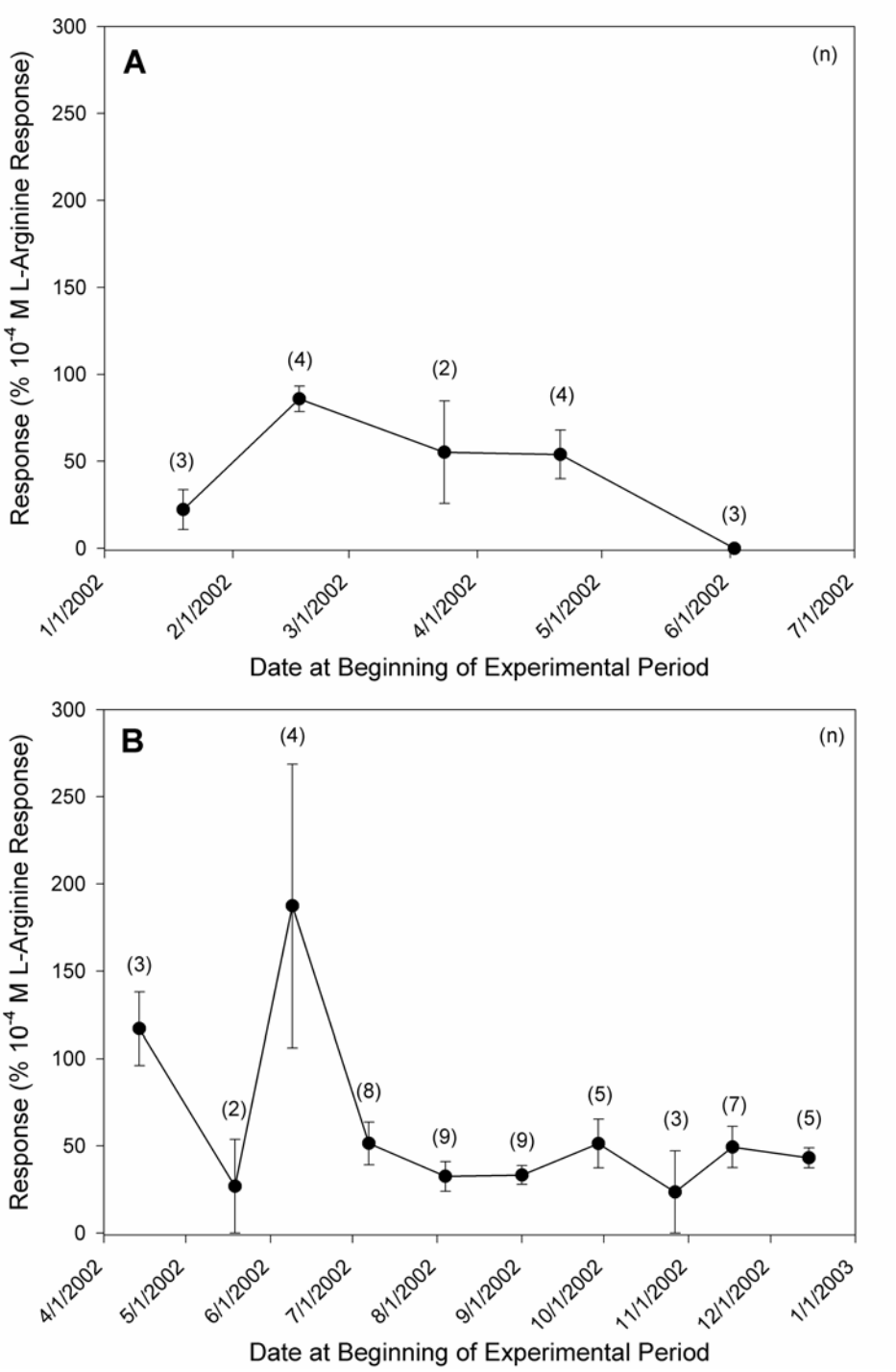

Figure 7. Olfactory responses of adult Pacific lampreys to 10^{-6} M petromyzonol sulfate (PS) in 2002. A) 2001 upstream migrants measured from January to June, and B) 2002 upstream migrants measured from June to December. Numbers above points are the sample sizes for each point. Error bars are ±1 standard error.

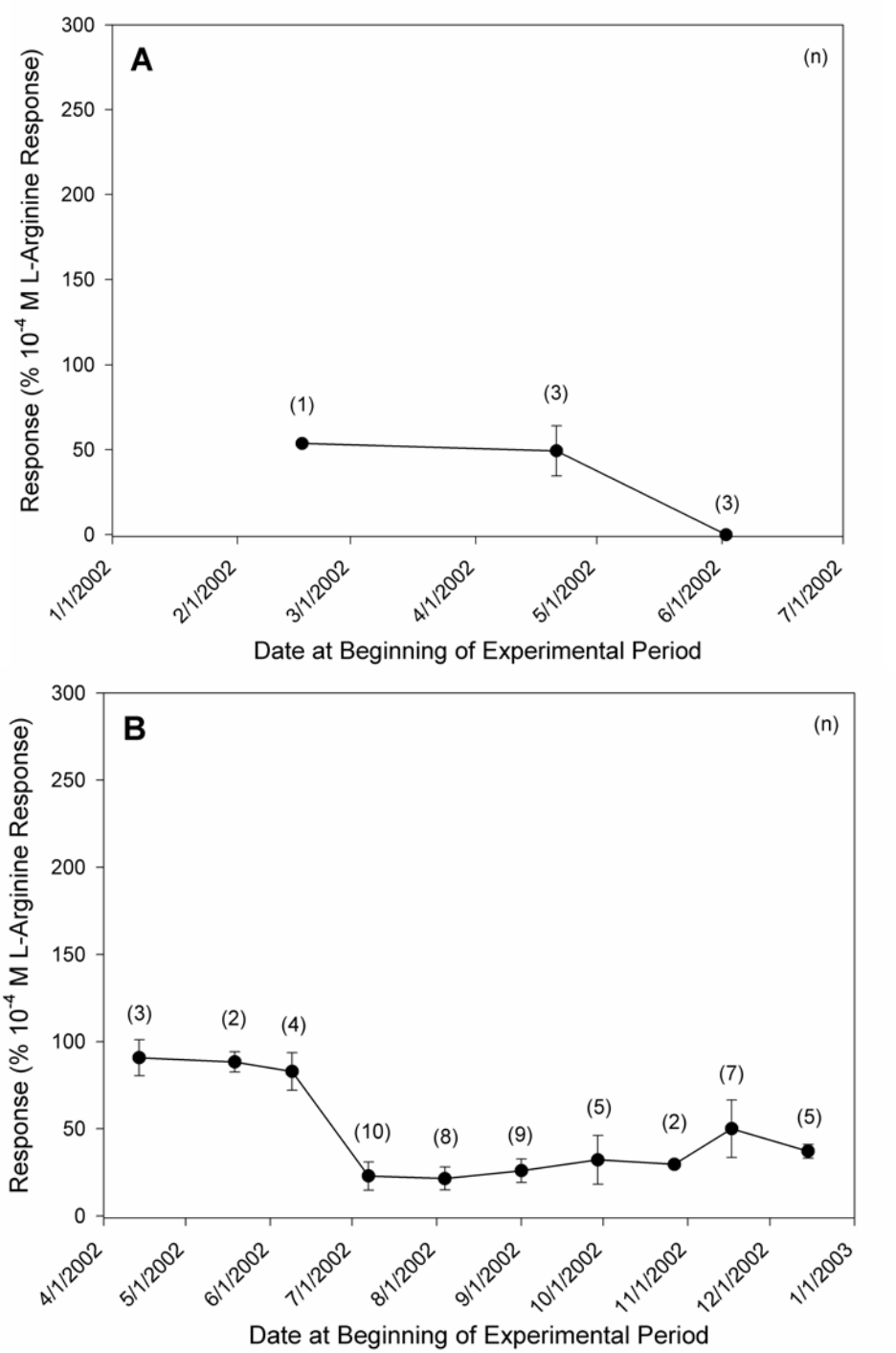

Figure 8. Olfactory responses of adult Pacific lampreys to 10^{-6} M 3-keto petromyzonol sulfate (3-keto PS) in 2002. A) 2001 upstream migrants measured from January to June, and B) 2002 upstream migrants measured from June to December. Numbers above points are the sample sizes for each point. Error bars are ±1 standard error.

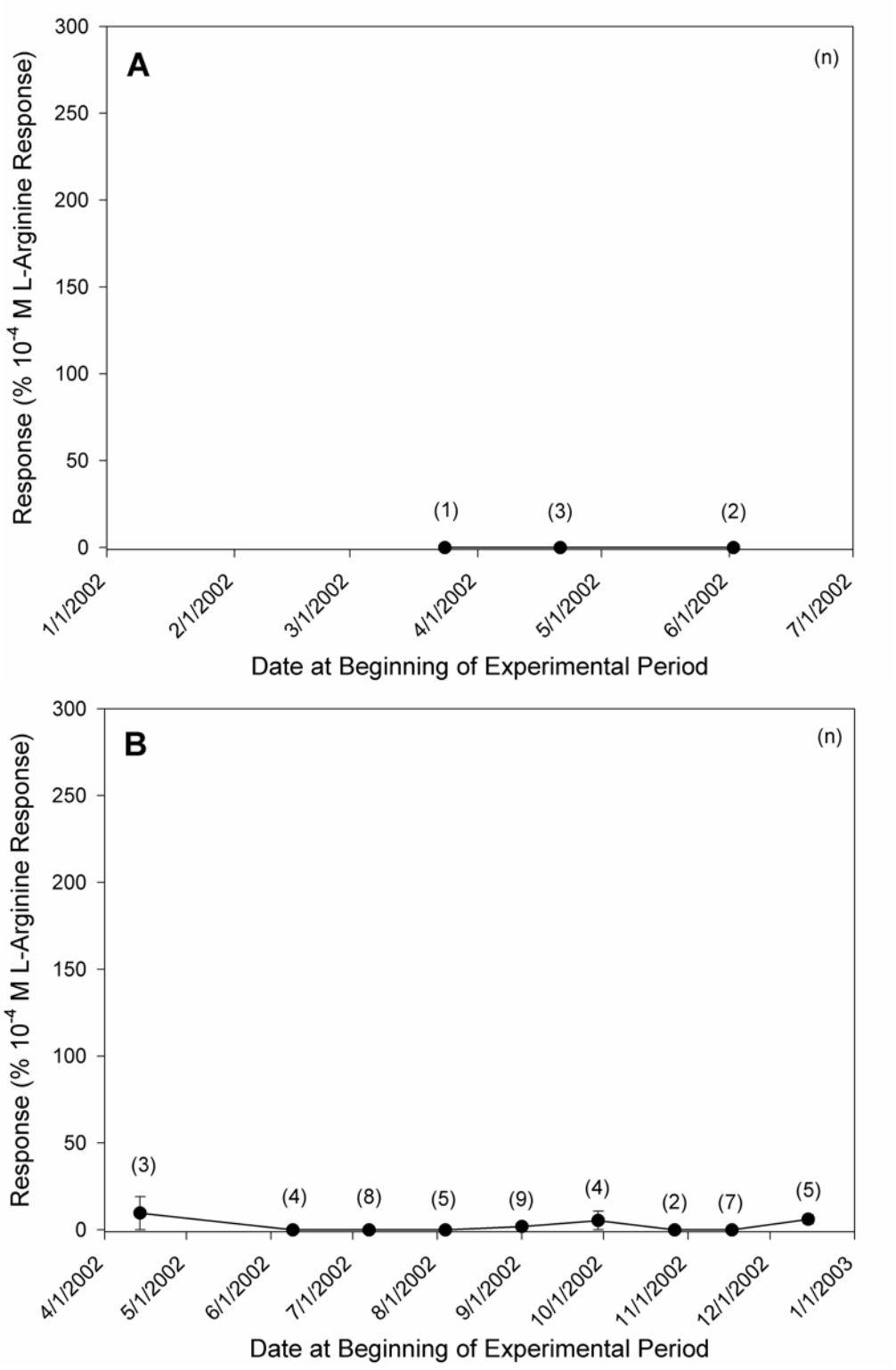

Figure 9. Olfactory responses of adult Pacific lampreys to 10^{-6} M allocholic acid (ACA) in 2002. A) 2001 upstream migrants measured from January to June, and B) 2002 upstream migrants measured from June to December. Numbers above points are the sample sizes for each point. Error bars are ±1 standard error.

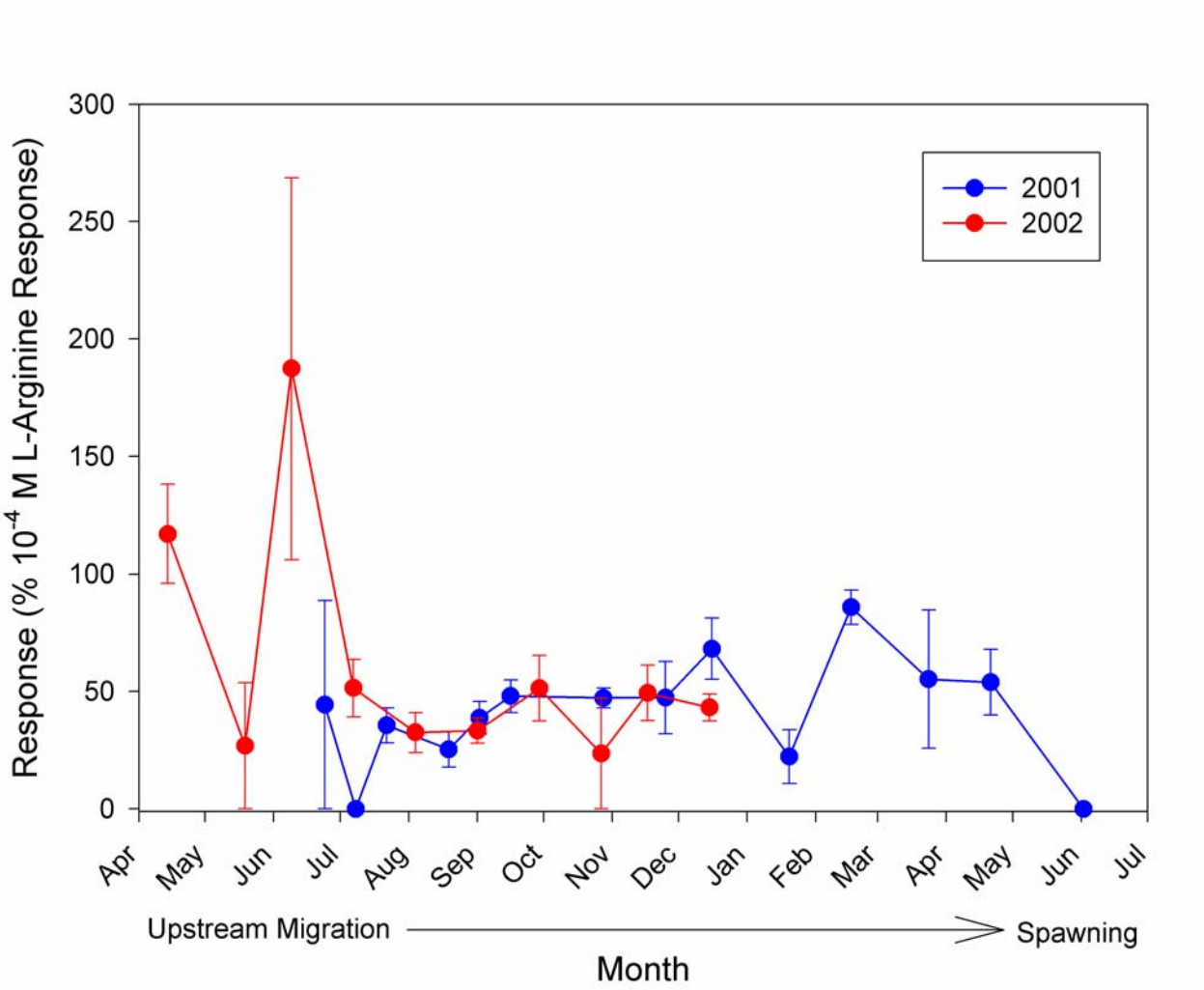

Figure 10. Olfactory responses of adult Pacific lampreys to 10^{-6} M petromyzonol sulfate (PS) from 2001 upstream migrants (blue) and 2002 upstream migrants (red). Error bars are ±1 standard error.

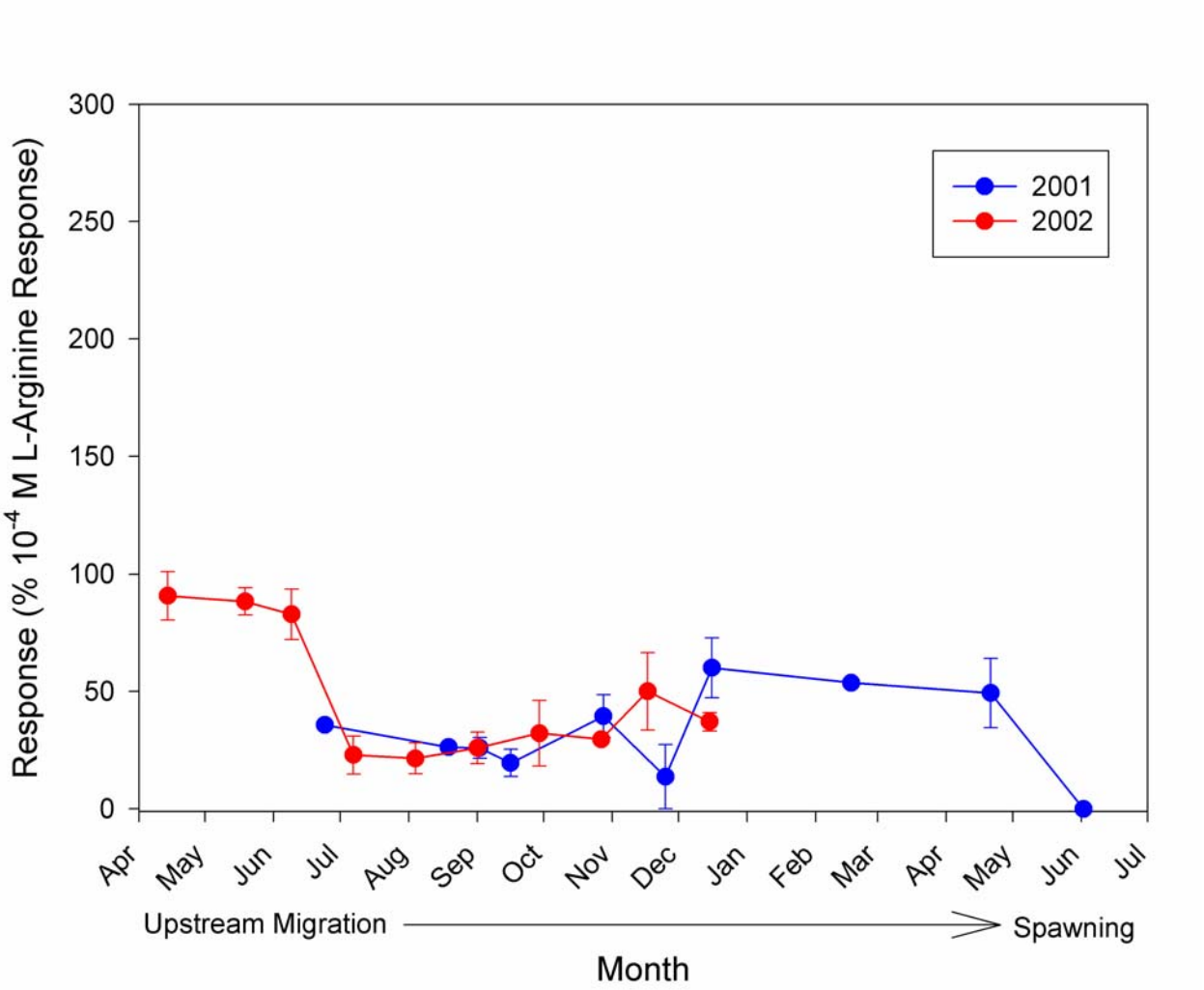

Figure 11. Olfactory responses of adult Pacific lampreys to 10^{-6} M 3-keto petromyzonol sulfate (3-keto PS) from 2001 upstream migrants (blue) and 2002 upstream migrants (red). Error bars are ±1 standard error.

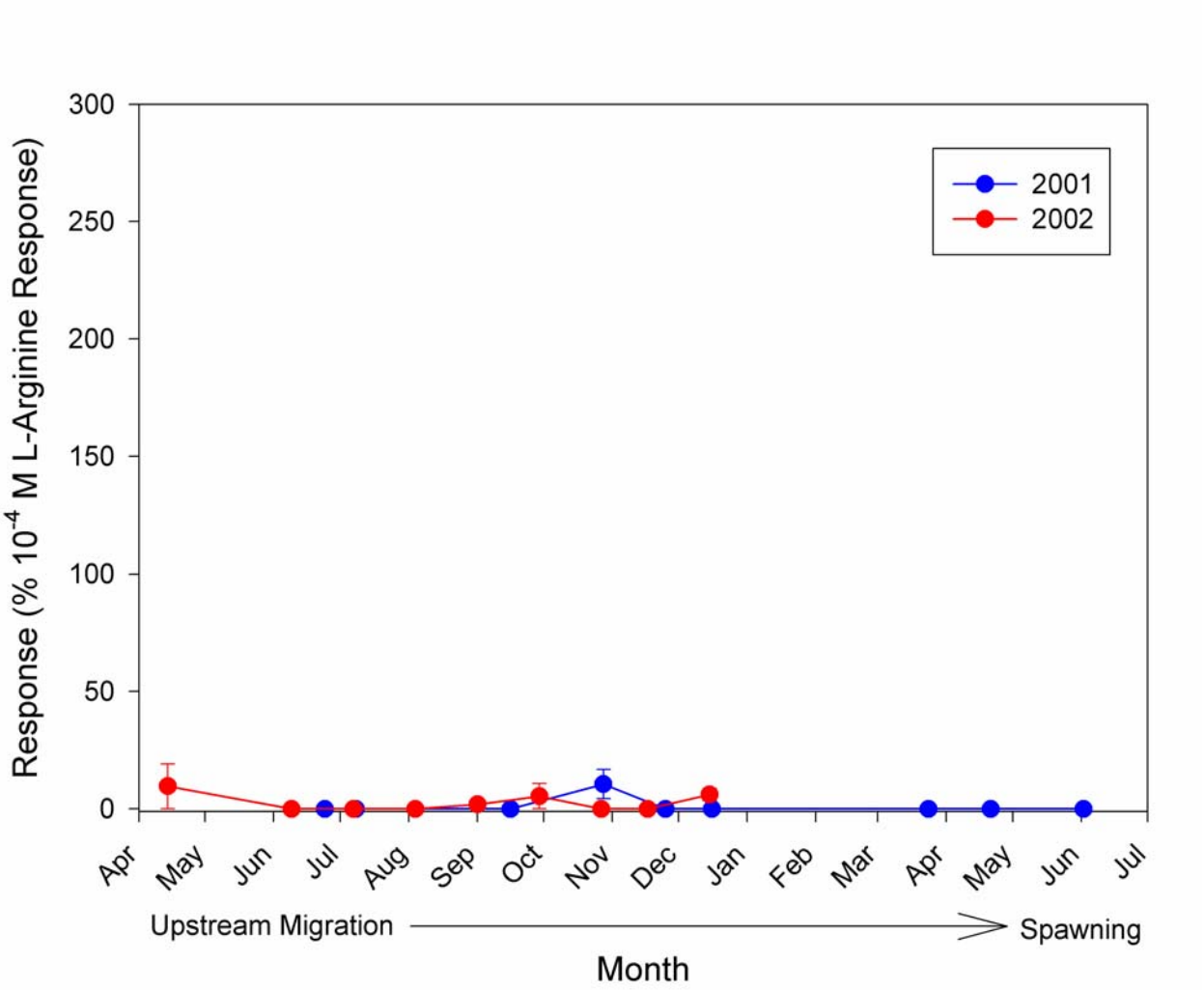

Figure 12. Olfactory responses of adult Pacific lampreys to 10^{-6} M allocholic acid (ACA) from 2001 upstream migrants (blue) and 2002 upstream migrants (red). Error bars are ±1 standard error.

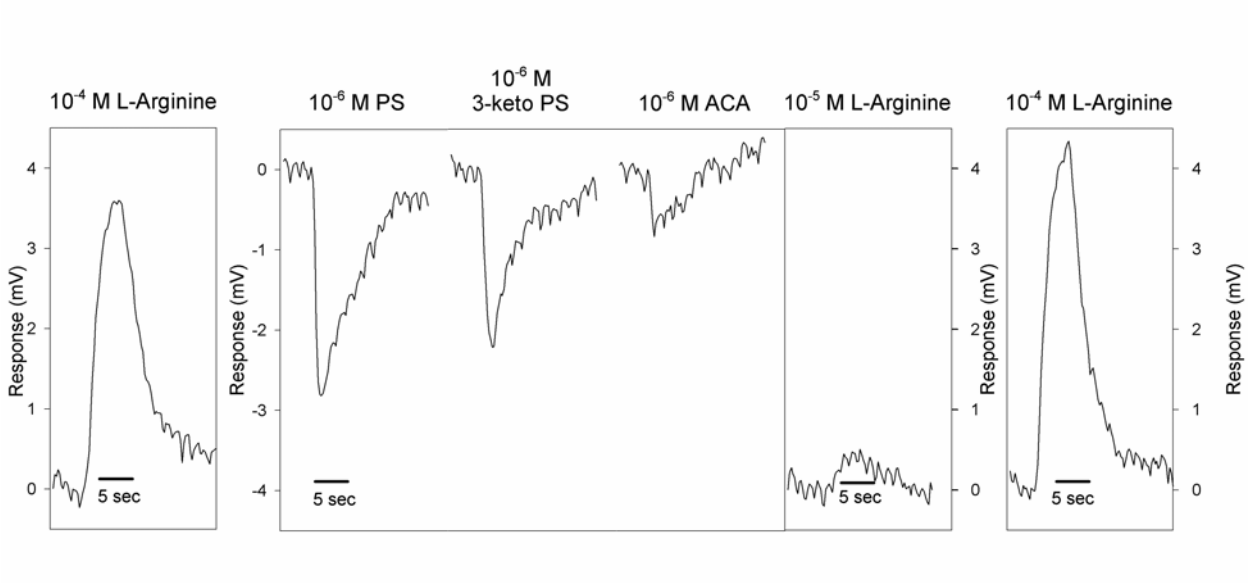

Figure 13. Electro-olfactogram (EOG) recordings from olfactory responses of adult sea lampreys to L-arginine, petromyzonol sulfate (PS), 3-keto petromyzonol sulfate (3-keto PS), and allocholic acid (ACA), in June 2002.

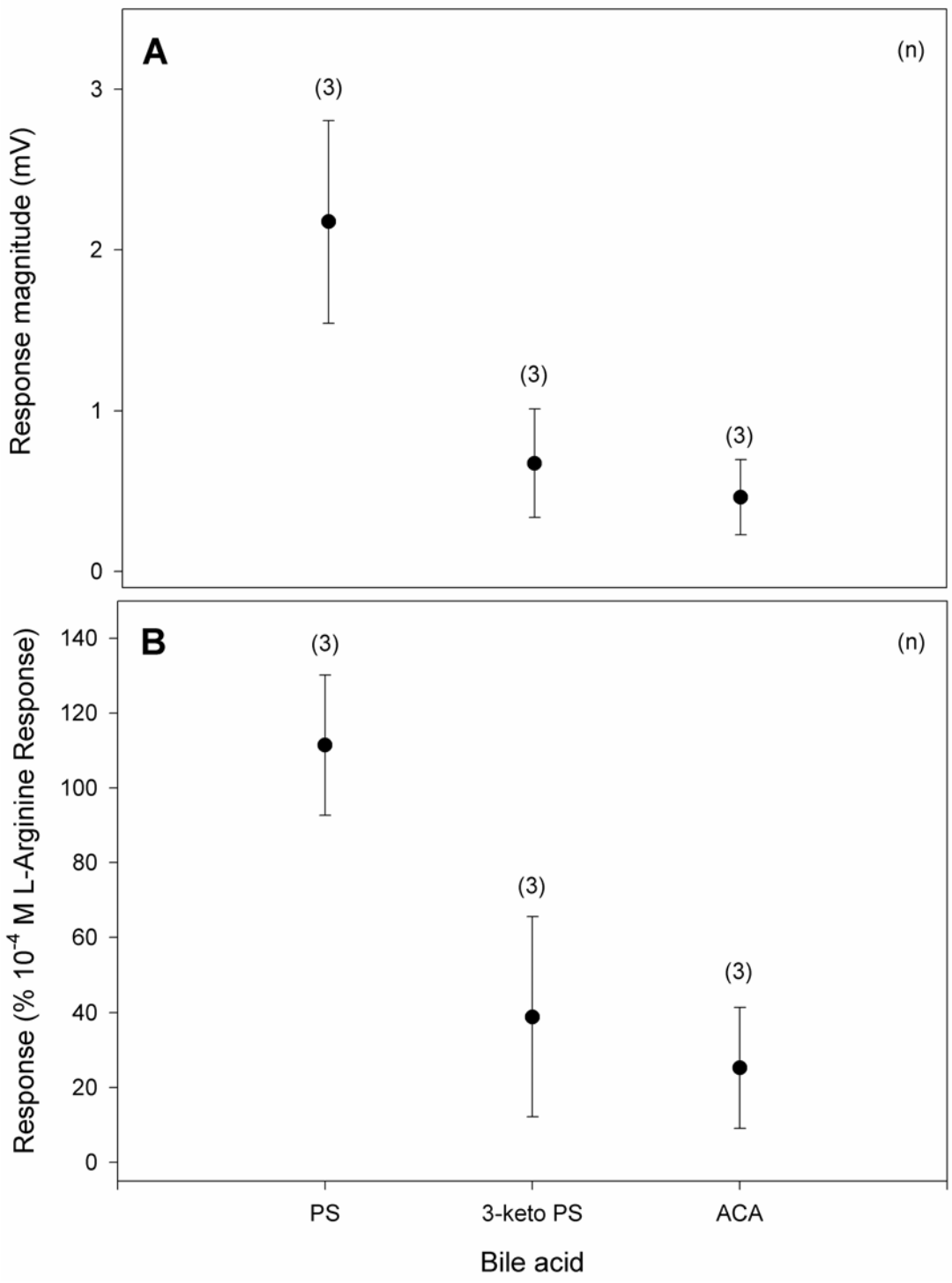

Figure 14. Olfactory responses of adult sea lampreys to 10^{-6} M bile acids in June 2002: petromyzonol sulfate (PS), 3-keto petromyzonol sulfate (3-keto PS), and allocholic acid (ACA). Olfactory responses expressed as A) magnitude of response in millivolts (mV), and B) percent 10^{-4} M L-arginine standard response. Numbers above points are the sample sizes for each point. Error bars are ±1 standard error.

CHAPTER THREE

HPLC and ELISA analyses of larval bile acids from Pacific and western brook lampreys

Sang-Seon Yun[1], Alexander P. Scott[2], Jennifer M. Bayer[3], James G. Seelye[3], David A. Close[4], and Weiming Li[1*]

[1] Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI 48824 USA

[2] Center for Environment, Fisheries and Aquaculture Science, Barrack Road, Weymouth, Dorset DT4 8UB, UK

[3] U.S. Geological Survey, Columbia River Research Laboratory, 5501a Cook-Underwood Rd., Cook, WA 98605 USA

[4] Department of Natural Resources, Tribal Fisheries Program, Confederated Tribes of the Umatilla Indian Reservation, Pendleton, OR 97801 USA

Introduction

The bile acids produced and released by sea lamprey (*Petromyzon marinus*) have been intensively studied for their pheromone function [1-2]. Larval sea lamprey produced only 5α-bile acids, opposed to 5β-bile acids found in most vertebrates [3-5]. Two of these bile acids, petromyzonol sulfate (PZS) and allocholic acid (ACA), are suggested to function as chemical cues to guide the migration of adult lamprey to spawning streams [3, 6-7]. Furthermore, at a later stage of their life history, adult males release an oxidized form of PZS and ACA (3 keto petromyzonol sulfate and 3keto allocholic acid) to attract conspecific females for spawning [8-9]. It is likely that these life stage specific bile acids mediate several channels of chemical communication between conspecifics [9].

Little is known about whether the 5α-bile acids found in the sea lamprey are produced by other lamprey species. To determine whether 5α-bile acids are unique to the sea lamprey, we analyzed bile acid production and release in two *Lampetra* species, Pacific lamprey (*Lampetra tridentata*) and western brook lamprey (*L. richardsoni*). Like the sea lamprey, the Pacific and western brook lampreys spawn in freshwater rivers, where larvae spend an extended period before undergoing metamorphosis to the adult form. Following completion of metamorphosis, lampreys exhibit one of two life history forms. Sea lamprey and Pacific lamprey are examples of migratory, parasitic species that move from streams to the ocean or large bodies of freshwater to feed (where they are parasitic), and then return to streams to reproduce [10]. In contrast, the western brook lamprey is a non-migratory, non-parasitic species that remains in natal streams until sexually mature, at which time they spawn and die [11-12]. Hence, an experimental examination of bile acids produced in these two *Lampetra* species and a comparison to those of sea lamprey will determine whether PZS and ACA, bile acids suggested to function as chemical cues for migration, are unique to *Petromyzon* species or to migratory lamprey species.

Further, to assess the feasibility for PZS to function as a chemical cue in *Lampetra* species, it is essential to estimate accurately its rate of production and release in these species. The current method for measuring PZS using HPLC [4] requires an extensive effort to concentrate a large amount of water conditioned with larvae [5]. In a study

determining the release rate of a male sea lamprey sexual pheromone [13], 3keto-PZS, an enzyme linked immunosorbent assay (ELISA) was found to enable rapid, sensitive, and specific measurements of the bile acid in a large number of samples. Based on this success, we proceeded to develop an ELISA specific for PZS. A major challenge in developing an ELISA for PZS is producing an antibody to discriminate between PZS and 3keto-PZS. The only difference between these compounds is that 3kPZS has a 3-keto group whereas PZS has a 3-hydroxyl group. This concern was addressed successfully by making 3kPZ-24-HS conjugated with bovine serum albumin (BSA) and produced an antiserum that specifically recognizes 3keto-PZS, with negligible cross reactivity with PZS [13]. We used the same strategy to develop the ELISA for PZS.

In the present study, we performed a series of experiments to determine which known bile acids are produced and excreted by larval Pacific, western brook, and sea lampreys. We also estimated the amount and individual variation of bile acids release by larvae of each species. The information offered in this study sheds light on whether 5α-bile acids mediate conspecific chemical communication in the *Lampetra* genus. Understanding of pheromone communication systems may facilitate rehabilitation of these species in the Columbia River Basin, USA where they pose ecological, economic, and cultural significance [14].

Material and Methods

Chemicals

Acetylcholinesterase (AchE), Ammonium carbonate, N, N'-dicyclohexylcarbodiimide, glutaraldehyde, N-hydroxysuccinimide, 3α hydroxysteroid dehydrogenase (3α HSD), lithocholic acid, β-nicotineamide adenine dinucleotide (β-NAD), trifluoroacetic acid (TFA), and trypsin were obtained from Sigma (St.Louis, MO, USA). Hyocholic acid was from Steraloids (Newport, RI, USA). Aminopropyl controlled glass beads were from CPG Inc. (Lincoln Park, NJ, USA), and an empty column (30 x 50 mm) was from Alltech (Deerfield, IL, USA).

Animals

Ammocoetes (Pacific and western brook lampreys) were from two sources: 1) wild larval lampreys were collected from Walla Walla River, WA, Middle Fork John Day River, OR, Hardy Creek, WA and Gibbons Creek, WA in July and August 2001, using a AbP-2 electrofisher and transported to the US Geological Survey Columbia River Research Laboratory (CRRL); 2) progeny from manual spawning of Pacific lampreys at CRRL in 1999. All ammocoetes were held in aquaria provided with a continuous inflow of water (ca. 1-2 L/min) from the Little White Salmon River, WA. Water used for this research was heated to simulate the natural temperature regime of tributaries to the Columbia River and aquaria were exposed to a simulated natural photoperiod produced by incandescent lights controlled by timers. Substrate, food, and aeration were also provided. Wild lampreys were identified by experienced personnel at the CRRL using a commonly accepted key [15].

Larval sea lamprey were collected from Elliot Creek, Cheboygan County, Michigan in April, 2002 by the staff of US Geological Survey Hammond Bay Biological Station (HBBS) using an AbP-2 Electrofisher. The animals were transported to the main laboratory of HBBS. The larval lampreys were held in a flow-through tank (100 L) with Lake Huron water (7°C to 20°C).

The average body weight of the larval lamprey used for tissue extraction was 0.17 g (Pacific lamprey, n=4), 1.32 g (western brook lamprey, n=5), and 1.60 g (sea lamprey, n=5). The average body weight of 75 larvae used for releasing rate estimation was 1.83 g

(Pacific lamprey), 1.88 g (western brook lamprey), and 0.63 g (sea lamprey), respectively.

Tissue extraction

Individual gall bladders and livers were dissected under a microscope and removed into tubes with 1 ml of 100% methanol. Bile acids were extracted as described [16]. Tissue samples were homogenized in 1 ml ethanol and sonicated for 30 min. The sonicated tissue samples were refluxed for 30 min and centrifuged. The supernatant was removed and reflux was repeated after adding 1 ml ethanol. Reflux of the tissue samples with a methanol:chloroform (1:1) mixture was repeated twice. The pooled supernatants were dried down under a nitrogen stream and redissolved in 1 ml methanol. The recovery rate of this method is known to be over 90% [4, 16-17].

Conditioned water collection and extraction

Larval lamprey-conditioned waters were collected from three groups of 25 larval lamprey separately for the three lamprey species. Each group was held in a 1-L glass beaker containing 500 ml of well water at 12 °C (Pacific and western brook lampreys) or lake water at 11.6 °C (sea lamprey) for 4 h under aeration. Conditioned water was removed and stored at –80°C until used. Ten ml of the conditioned water was passed through a Sep-Pak and eluted with 4 ml of methanol. The methanol elutes were concentrated and subjected to ELISA analysis (see below).

Preparation of enzyme column for HPLC

Immobilization of 3α HSD onto aminopropyl controlled glass beads was performed as described [18]. Briefly, 350 mg of glass beads were treated with 2.5% glutaraldehyde in 0.05 M phosphate buffer, pH 7.2, for at least 60 min under vacuum. After washing 6 times with phosphate buffer, the beads were incubated with 50 units of 3α HSD in 0.6 ml phosphate buffer for 3 h at room temperature. The beads were separated using a filter manifold and washed 3 times with phosphate buffer. The enzyme immobilized beads were packed into an empty column (5 x 30 mm) under vacuum.

HPLC analyses of bile acids

The HPLC system consisted of a Waters 600 delivery system (Milford, MA), a Beckman pump (Fullerton, CA), and a Waters 474 fluorescent detector. Bile acids were eluted on a C18 analytical column (Nova-Pak, 3.9 x 150mm, Waters) using a linear gradient of acetonitrile in 0.3 % ammonium carbonate, pH 8.2 at a flow rate of 0.5 ml/min [17]. The gradient started at 20% of acetonitrile and linearly increased to 50% in 40 min. The bile acids separated on the reverse-phase column were mixed with 0.1 M Tris buffer (pH 8.5) containing 38 μM β-NAD, 0.8 M dithiothreitol, and 1.7 mM EDTA [19] using a tee. After reaction with the 3α HSD enzyme column, the β-NADH generated by enzymic reactions was detected fluorometrically [20]. The quantity of bile acids was determined by measuring the peak area using Millennium software (Oracle, Redwood Shores, CA) in reference to that of bile acid standards. Five hundred ng of two internal standards, hyocholic acid (HA) and lithocholic acid (LCA), was loaded along with the samples.

HPLC fractionation was performed on a few gall bladder extracts using the same column and mobile phase except the enzyme column. Fractions from 20 to 60 min were collected using a fraction collector (WFC III, Waters).

Mass spectrometry analysis of HPLC fractions

Gall bladder extracts from Pacific and western brook lampreys were fractionated on a C18 column as described above. Fractions corresponding to the elution time of bile acids were collected, passed through a Sep-Pak (Waters) and eluted with 5 ml of methanol. The methanol elutes were concentrated under a nitrogen stream and subjected to Fast Atom Bombardment mass spectrometry (FABMS) analysis. Mass spectra were obtained using a JEOL HX-110 double-focusing FAB mass spectrometer (JEOL, Peabody, MA, USA) that operates in either the positive or negative ion mode. Ions were produced by bombardment with a beam of Xe atoms (6 keV). The accelerating voltage was 10 kV and the resolution was set at 3000. High resolution mass spectrometry was performed by peak matching with a resolution of 10,000.

Preparation of antibody

For the preparation of antigen, petromyzonol-hemisuccinate (PZ-24-HS) was custom synthesized by Toronto Research Chemicals (North York, ON, Canada). To conjugate it to BSA, 21 mg of PZ-24-HS was dissolved in 1.5 ml of dimethylformamide (DMF) in a 20-ml glass beaker. The beaker was placed in crushed ice within a polystyrene container that was placed on top of a magnetic stirrer. A small magnetic stirrer was added to the beaker. The ice was prevented from thawing by the occasional addition of small amounts of liquid nitrogen to the container. With constant stirring, 12 µl tri-butylamine and 10 µl isochloroformate were added to the beaker and the reaction was allowed to proceed for 40 min. In the meantime, 80 mg BSA was dissolved in 3 ml distilled water, diluted with 3 ml DMF plus 1 drop of 2N sodium hydroxide and chilled on ice. This mixture was added to the beaker and left to stir for another 3 h. After this time, the mixture, which was slightly opaque, was centrifuged for 10 min at 1000 x g. The clear supernatant was divided into 2.5 ml aliquots for desalting on PD-10 columns [21] using distilled water to elute the protein fraction. The eluates were combined, frozen and freeze-dried.

To produce antisera, 2 mg of the powdered conjugate was dissolved in 1 ml 0.9% saline and mixed with 1 ml Freund's complete adjuvant. One ml antigen-adjuvant mixture was injected into 2 rabbits (New Zealand White). The rabbits were boosted with the conjugate in Freund's incomplete adjuvant two weeks after the first injection. They were bled for the first time at four weeks after the first injection. The serum was collected by centrifugation of the blood at 2700 rpm for 15 min. The supernatant was removed, aliquoted, and stored at –80°C.

Preparation of enzyme label

The preparation of the enzyme label was based on the procedure described [20] with slight modifications. G4-acetylcholinesterase was generated by treating AChE (1 mg) in 500 µl 0.1 M sodium phosphate buffer, pH 7.0 with 25 µl trypsin solution at 25 µg/ml in the same buffer for 24 h at room temperature. This reaction mixture was loaded on to a PD-10 column and eluted with 3.5 ml of 0.1 M borate buffer, pH 8.5. Next, PZ-24-HS was activated by dissolving 200 µg in 38 µl of N-hydroxysuccinimide solution

(1mg/ml in DMF) and then adding 32 µl of N, N'-dicyclohexylcarbodiimide solution (2 mg/ml in DMF) and leaving it overnight in the dark. Thirty µl of this reaction mixture was reacted with 400 µl G4-AChE stock for 2 h in the dark. This was purified on a PD-10 column by eluting with 3.5 ml of 0.01 M Tris buffer, pH 7.4 containing 0.01 M $MgCl_2$, 1M NaCl and 0.15 mM NaN_3. The eluate was stored at –20°C in 20 µl aliquots.

Titration of antibody and enzyme label

The optimum dilutions of AChE label and antibody were determined by checkerboard titration [22]. The starting dilutions for the titration of the AChE label and the antiserum were 1:40 and 1: 1000, respectively.

Assay procedure

Plates were coated with polyclonal goat anti-rabbit IgG (Sigma; product code R2004) by adding 120 µl of antibody diluted in 0.05 M potassium phosphate buffer, pH 7.4 to each well and incubating overnight at 4°C. The plates were blocked by addition of 100 µl of 3% BSA in 0.1 M potassium phosphate buffered saline and storage at 4°C for at least 12 h.

After washing the plates three times with wash buffer (0.05 M potassium phosphate buffered saline, pH. 7.4, 0.05% Tween 20), 100 µl of assay buffer (0.1 M potassium phosphate buffered saline, pH. 7.4, 0.1% BSA, 1 mM EDTA, 0.15% sodium azide) was added to each well and serial dilutions of PZS were made in a range of 20 pg to 10 ng/well. Non-specific binding (NSB) and maximum binding (B_0) were measured in separate wells. Samples were diluted appropriately in assay buffer and 100 µl was added to wells in duplicate; 50 µl of the diluted enzyme label (1:1000) was added to all wells and 50 µl of primary antibody (1:200,000) was added to all but NSB wells. Plates were incubated for 2 h at room temperature in a humid chamber. Then, after rinsing three times with wash buffer, 200 µl Ellmans reagent (4.3 mg DTNB, and 4 mg acetyl thiocholine in 20 ml 0.02 M potassium phosphate buffer) was added to each well. The plates were sealed and incubated overnight at room temperature in a humid chamber. Color development was measured at 405 nm on a Bio-Rad Benchmark plate reader.

Assay validation

To test the cross-reactivity of the antibody to structurally related compounds, serial dilutions of ACA, PZ, 3kPZS, cholic acid (CA) and 3kACA were assayed alongside the PZS standard. Intra-assay variation was determined by assaying eight replicates of PZS standard in the same plate. Inter-assay variation was determined by assaying a sample with approximately 50% binding, six times in different plates. In addition, parallelism was determined by diluting gall bladder extracts from three species.

ELISA analysis of samples

Tissue extracts were first diluted 1,000-10,000 times in assay buffer and added to the wells along with the standard. For extracts of conditioned water, methanol elutes were dried down, reconstituted in assay buffer and assayed.

HPLC fractions collected were diluted 100 times in assay buffer and assayed as above.

Results

HPLC analyses of bile acids

The five bile acid standard resolved well by the 3α HSD enzyme HPLC column (Fig. 1). The peaks corresponding to each bile acid were observed at 22-24 min (HA), 29-30 min (ACA), 38-40 min (PZS), 47-48 min (LCA), 51-52 min (PZ). Calibration curves for the three bile acids, ACA, PZS and PZ, based on the intergrated areas of the peaks, were linear at the range of 50 ng – 2 μg (Fig. 2). The type and quantity of bile acids in the liver and gall bladder of three lamprey species varied. Petromyzonol sulfate was found to be a major bile acid component in all three species studied. Neither ACA nor PZ was detected from Pacific lamprey gall bladder extracts while two out of five gall bladders from western brook lampreys contained ACA at amounts that are 3-7% of PZS levels. In sea lamprey larvae, both ACA and PZS were detected and ACA amount was ca. 1% of that of PZS (Fig. 3). The total amount of PZS in the gall bladder and the liver of Pacific, western brook, and sea lampreys were normalized by the body mass. The lowest PZS level (per g body mass) in the gall bladder was found in sea lamprey with an average of 127.40 (±28.90, n=5) μg/g fish while the highest level was observed from western brook lamprey with an average of 276.96 (±78.28, n=5) μg/g fish. The PZS level in Pacific lamprey was 145.86(±3.11, n=4) μg/g fish (Fig. 4A). The PZS concentrations in the liver tissues were 10 – 100 times lower than those of the gall bladders (Fig. 4B).

Mass spectrometry analyses of PZS

HPLC fractions corresponding to PZS were analyzed by negative mode FAB-MS to observe major ionized peak at m/z 473.04 for Pacific lamprey and 473.4 for western brook lamprey, confirming the identity of the compound in the fractions as shown in Fig. 5. High resolution MS analyses of the fractions further confirmed that the molecule ionized at m/z 473.04 and 473.4 conforms to the PZS formula at 4.3 ppm level for Pacific lamprey and 2.4 ppm level for western brook lamprey.

Establishment of an ELISA for PZS

Hemisuccinated PZ was successfully used to immunize the rabbits. The antiserum L216 was titrated using an antigen-enzyme label (AchE). The optimum dilutions were

determined to be 1:1000 for the label and 1:200,000 for the antiserum. A standard curve was established in a working range of 20 pg/well to10 ng/well. On a weight per weight basis, the antiserum cross-reacted equally well with PZS, PZ and ACA while the cross reactions with all other compounds such as 3kPZS and 3kPZ were negligible (data not shown). Intra-assay variation from 8 replicates fell lower than 5% in the standard range and inter-assay variation was 12%. Further, close parallelism was observed between dilutions of the gall bladder extracts from three larval lamprey species and synthetic standard (Fig. 6).

ELISA measurement of PZS

ELISA analyses of the lamprey holding water revealed that the bile acid release rates varied dramatically among species. Western brook and sea lampreys larvae released 20 times more PZS than Pacific lamprey (Fig. 7). The release rates of PZS from individual larva were 1.48 (±0.51, n=3) ng/g fish/h for Pacific lamprey, 30.68 (±12.18, n=3) ng/g fish/h for western brook lamprey, and 36.77 (±13.81, n=3) ng/g fish/h for sea lamprey.

Tissue extracts from three lamprey species were analyzed with the assay and compared with the values obtained using 3α HSD enzyme column HPLC (Fig. 8). Overall, PZS concentrations obtained by ELISA were higher but a linear relationship between the two data sets was observed ($R^2 = 0.87$).

ELISA analysis of HPLC fractions

ELISA analysis of HPLC fractions collected directly from a C18 column revealed that the major compound eluted around 36-38 minutes is PZS-immunoreactive (Fig. 9).

Discussion

Results from our HPLC and mass spectrometry analyses clearly demonstrate that petromyzonol sulfate is the main bile acid in gall bladders and liver of larval Pacific and western brook lampreys. The concentration of PZS in gall bladders of western brook lamprey was twice as that found in Pacific and sea lampreys while the concentration of PZS in the liver of Pacific lamprey was the highest among the three species (Fig. 3 B). The identity of the compound in the fractions corresponding to PZS was confirmed by MS analyses of the fractions of gall bladder extracts. Fractions from both Pacific and western brook lamprey gall bladder extracts observed the ionized peaks at m/z 473 and was confirmed to be PZS by high resolution MS.

In contrast to the sea lamprey, the bile acid composition of Pacific and western brook lampreys lacks minor bile acids. No significant sign of petromyzonol has been observed in the HPLC chromatograph of the gall bladder extracts from Pacific and western brook lampreys, and only two out of five western brook lamprey samples analyzed contained ACA. In Pacific and western brook lampreys no ionized peaks were observed from fractions corresponding to ACA. These results contrast a previous report that ACA and PZ are present in the sea lamprey [5]. We recognize the possibility that this discrepancy is attributable to the slightly different feeding and storing conditions for the larvae and also the possibility that the total amount of extracts used in this study was not enough to make the two bile acids detectable. However, we believe that it is not attributable to the difference in HPLC methods since our detection limit for the analyses was 50 ng, more sensitive than the reported detection limit of 200 ng [5].

An ELISA for PZS was developed based on an reported approach [13] and used to measure the immunoreactive PZS in the gall bladder and liver extract and to estimate its release rate. The assay was validated by a series of experiments and found to have virtually no cross-reactivity with 3kPZS and 3kACA. The levels of intra- and inter-assay variation are within the acceptable range for typical ELISA and comparable to those of the ELISA for 3kPZS [13]. The specificity of this ELISA is further confirmed by the close parallelism of the gall bladder extracts the three species (Fig. 6). Due to the structural similarity between PZS and ACA or PZ, the antibody cross reacted with ACA and PZ but not other compounds tested. In a previous study [13], it was shown that the

free bile acids can be separated with diethyl ether at different pH. In the present study, no attempt was made to separate the free bile acid from the sulfated one, mainly because in Pacific lamprey there is no indication that the free bile acid, PZ, exists. However, in sea and western brook lamprey, the immunoactivity observed may represent both PZS and ACA.

Our ELISA analysis of holding water revealed that western brook lamprey larvae release 5α-bile acids at a rate comparable to that of sea lamprey larvae, but approximately 20 times higher than that of Pacific lamprey larvae (Fig. 7). The release rates measured by ELISA in the sea lamprey is comparable to that obtained with HPLC [5], which suggested that sea lamprey larvae can release sufficient PZS to achieve picomolar concentrations in lamprey streams of the Laurentian Great Lakes basin, a concentration within the olfactory sensitivity of adult sea lamprey [4, 6]. However, it is not possible to establish whether detectable levels of bile acids can be achieved in the streams which Pacific and western brook lampreys are found. The olfactory sensitivity of Pacific lamprey to PZS is the subject of ongoing research. Our unpublished data have shown that migrating adult Pacific lampreys are sensitive to PZS but showed few measurable responses to ACA. These preliminary results indicate a possible role for larval and adult lamprey bile acids as pheromone cues for Pacific lamprey.

The results of the ELISA were closely comparable to those obtained using the HPLC method. The reason why ELISA results were slightly higher is probably due to 'dilution error'. The ELISA is much more sensitive than the HPLC method, which means samples have to be diluted nearly 10,000 times in order to bring them to levels covered by the standard curve. The other possibility, that ELISA concentrations were higher due to interference by other cross-reacting compounds, was ruled unlikely as ELISA analysis of HPLC fractions confirmed that the major cross-reacting material was eluted at the same position as PZS (Fig. 9).

Since the Pacific lamprey has a life history similar to that of the sea lamprey, it seems plausible that Pacific lamprey rely on PZS for stream selection, as is the case in sea lamprey [4, 7]. Since their releasing rate for PZS is 20 times lower than that of sea lamprey, the Pacific lamprey would need to be more sensitive in their olfactory detection of this bile acid, or have a relatively higher density of larval population in order to use

PZS as a migratory cue. We are currently conducting a series of physiological and behavioral experiments to investigate these possibilities.

Although it is unlikely that PZS functions as a migratory cue for selecting spawning streams in the western brook lamprey, it cannot be ruled out that it may function as a within stream migratory cue as western brook lamprey have a slight prespawning migration back up their natal streams [12], and PZS may function to promote upstream movement in migratory stage animals [2]. Interestingly, the release rate of PZS for western brook lamprey is comparable to the sea lamprey. Bile acids are critical to digestion and absorption of lipids [23]. It is possible that machinery for producing the 5□-bile acid in western brook lamprey is a vestigial trait that was inherited from ancestral lamprey. However, it cannot be ruled out that this compound may act as chemical cues for other functions. In the sea lamprey, ammocoetes have mature receptor neurons [24] that are sensitive to biologically relevant odorants [25]. Olfactory cues are thought to mediate regulation of growth rate [26] and onset of metamorphosis. It is critical to further our understanding of the ecological relationships of these lampreys, including examination of olfactory sensitivity of non-migratory species.

In summary, two *Lampetra* lamprey species, Pacific and western brook lampreys, produce and release a bile acid, petromyzonol sulfate, in their larval stage. An ELISA for this assay has been developed and may be useful for studies of bile acid release in all lamprey species. Some possible functions of this bile compound beyond the digestive system require further examination.

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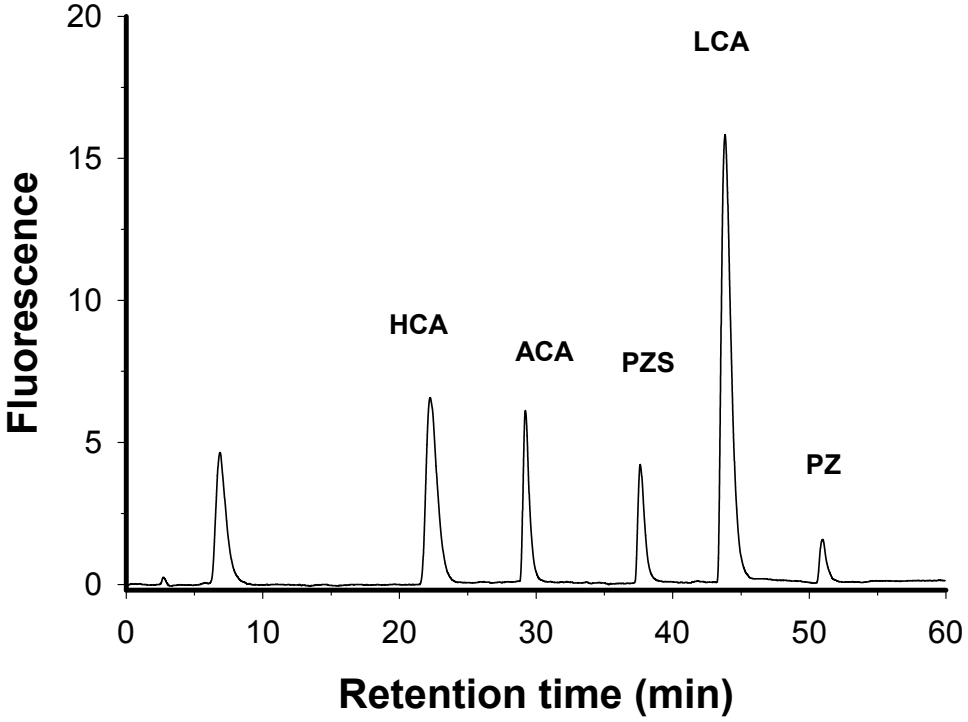


Figure 1. HPLC analyses of standard bile acids using a C18 and 3α HSD column. Fluorescence generated by NADH was measured at excitation 365 nm, emission 465 nm. Standards are hyocholic acid (HCA), allocholic acid (ACA), petromyzonol sulfate (PZS), lithocholic acid (LCA), and petromyzonol (PZ).

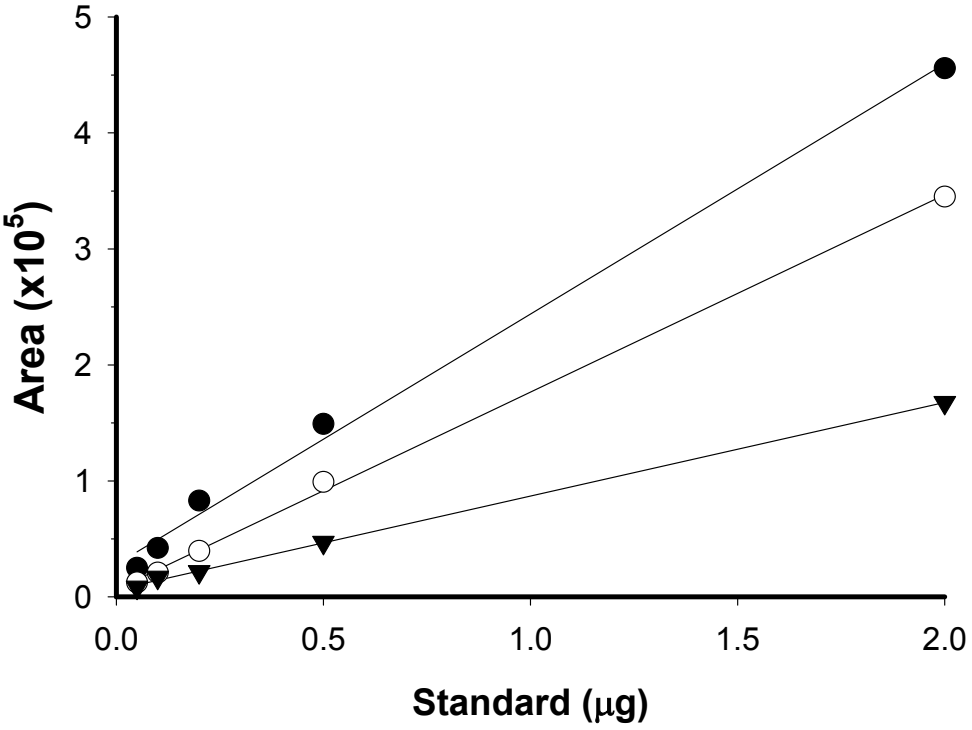


Figure 2. The calibration curves for three bile acids. The curves were obtained between 50 ng to 2 μg by integrating the fluorescent peaks for allocholic acid (●), petromyzonol sulfate (O) and petromyzonol (▼).

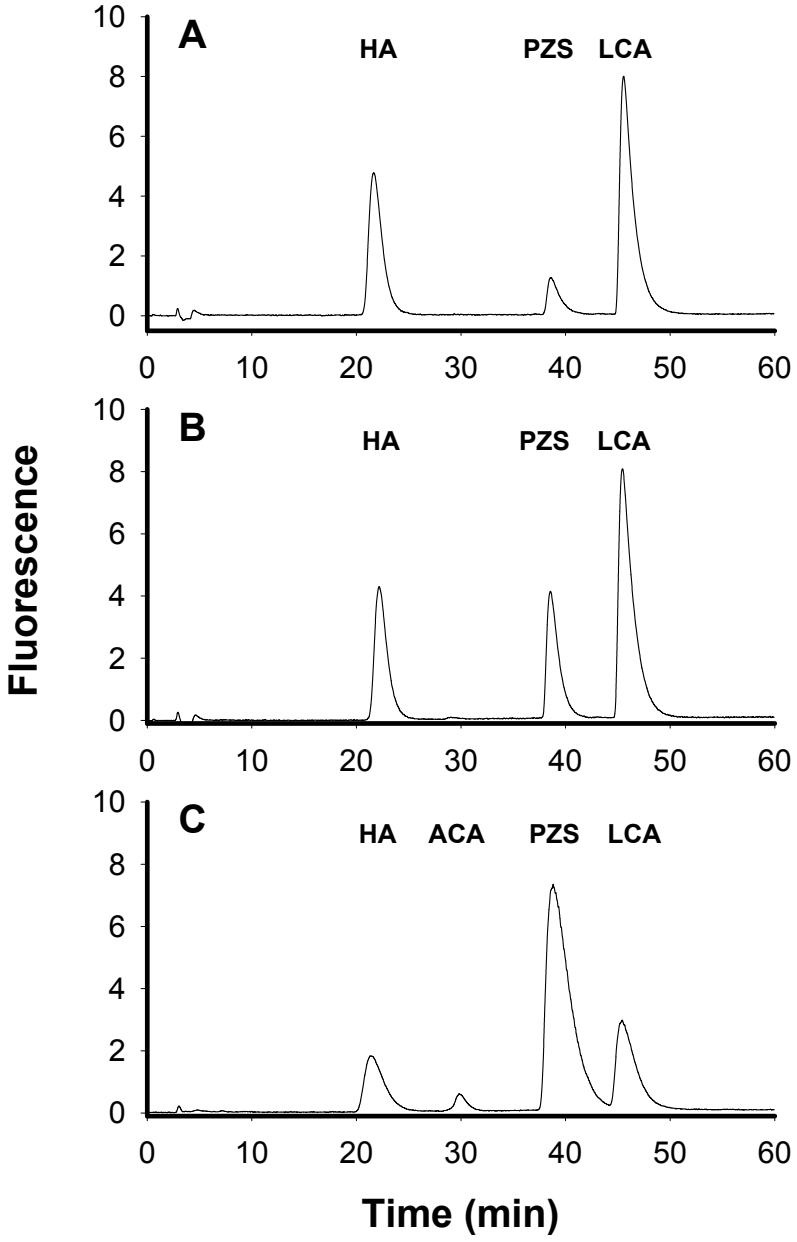


Figure 3. Representative chromatograms of gall bladder extracts from Pacific lamprey (A), Western brook lamprey (B) and sea lamprey (C).

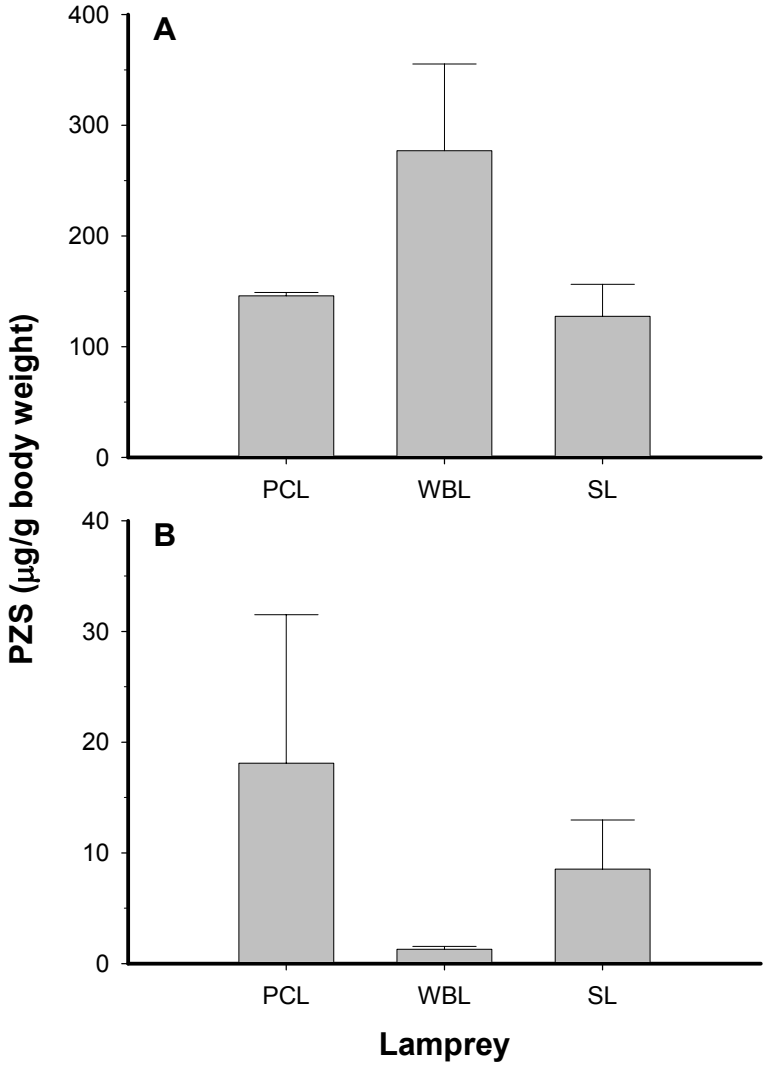


Figure 4. Tissue concentrations of PZS in larval gall bladders (A) and liver (B) determined by HPLC method Pacific lamprey (PCL), western brook lamprey (WBL) and sea lamprey (SL). Note the difference in scaling of Y-axis. Error bars represent standard error of means.

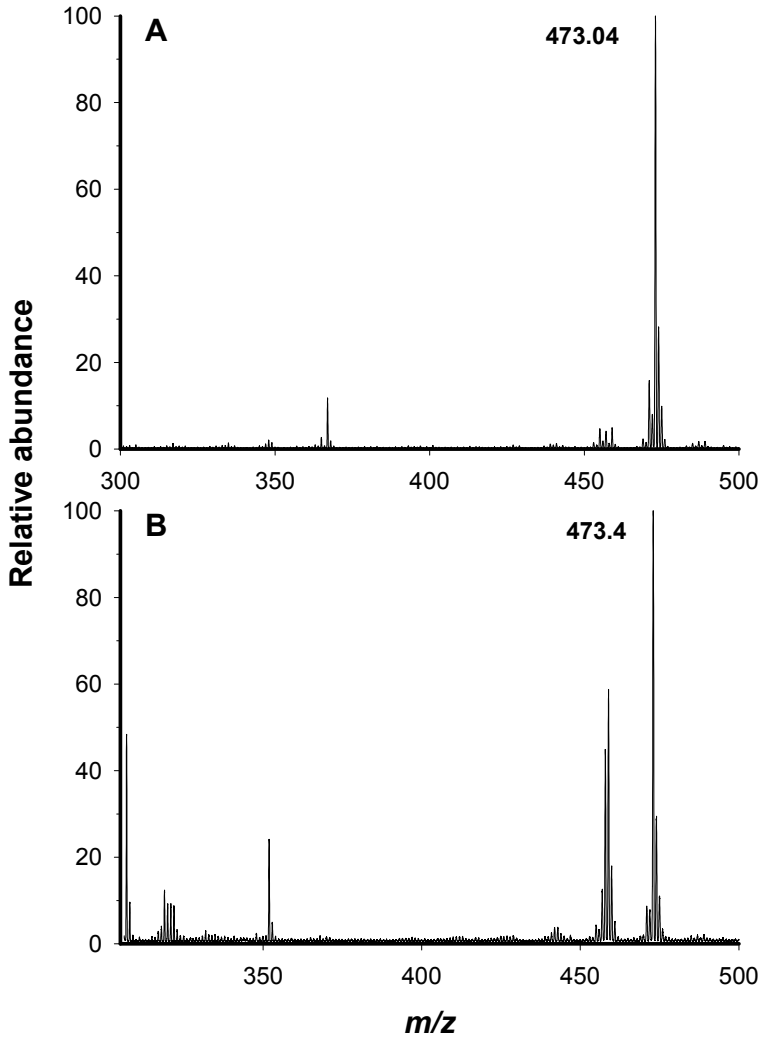


Figure 5. Fast atom bombardment mass spectrometry analyses (negative mode) of HPLC fractions from Pacific lamprey (A) and Western brook lamprey (B) gall bladder extracts. Note the base peaks for both species are at m/z 473.

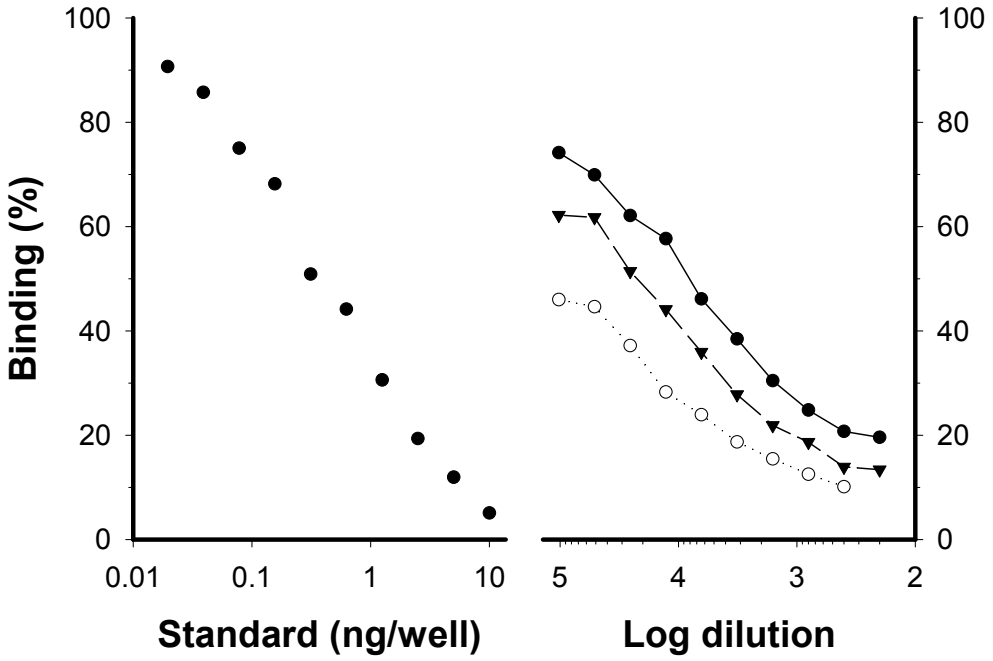


Figure 6. Parallelism of diluted gall bladder extracts from Pacific lamprey (●), Western brook lamprey (O) and sea lamprey (▼).

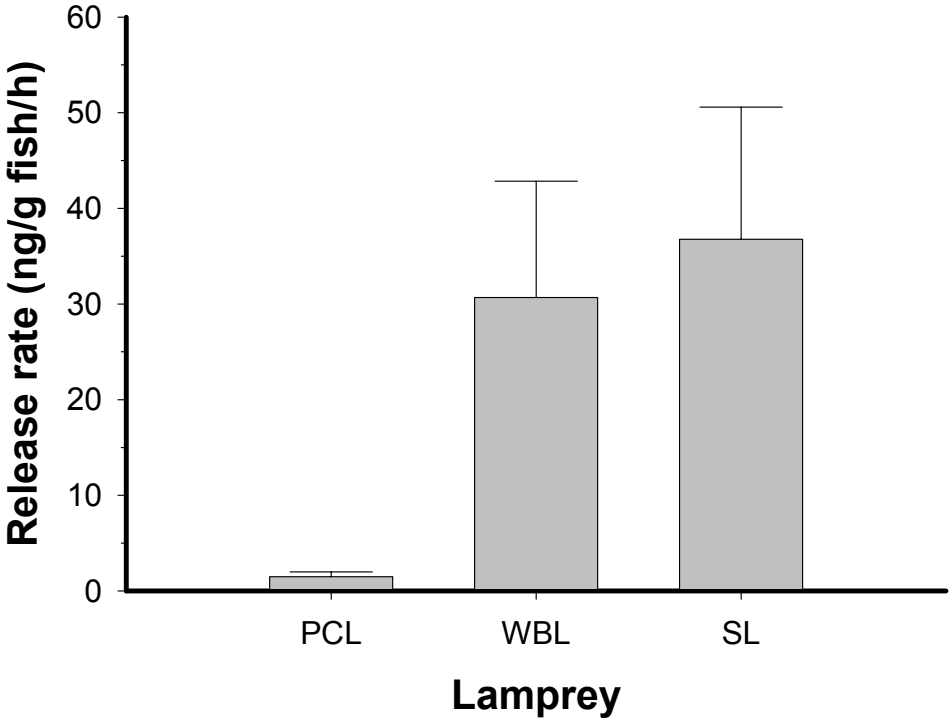


Figure 7. The release rates of petromyzonol sulfate determined by ELISA analyses of samples of water conditioned with larval Pacific lamprey (PCL), western brook lamprey (WBL) and sea lamprey (SL). Error bars represent standard error of means.

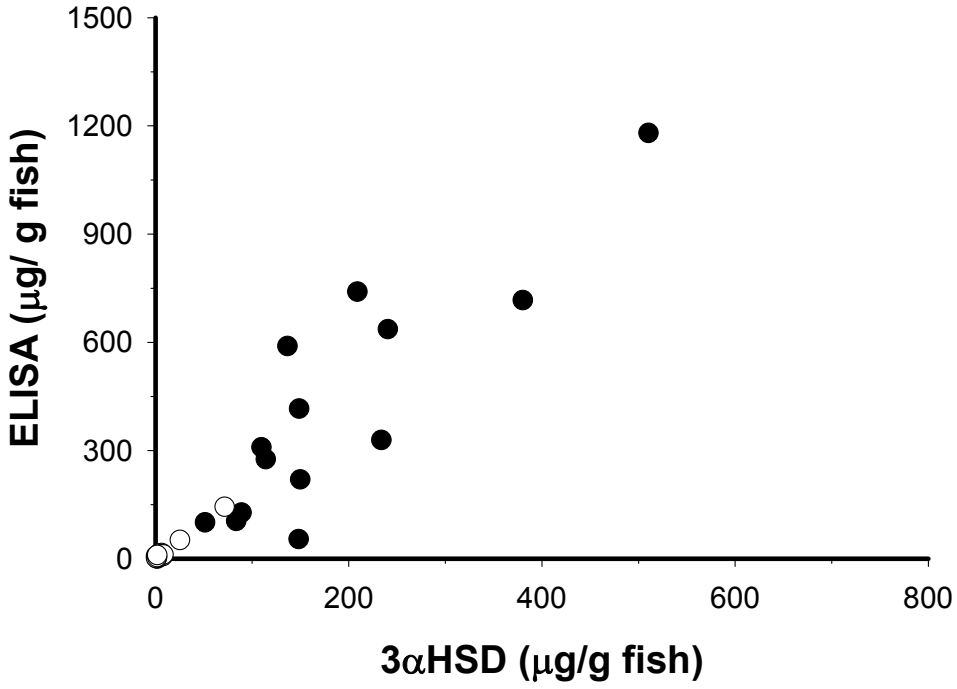


Figure 8. A comparison of petromyzonol sulfate concentrations obtained by two analytical methods, ELISA and 3α HSD/HPLC. Note that gall bladder samples (●) appeared at the higher concentration range while liver samples (O) at the lower range.

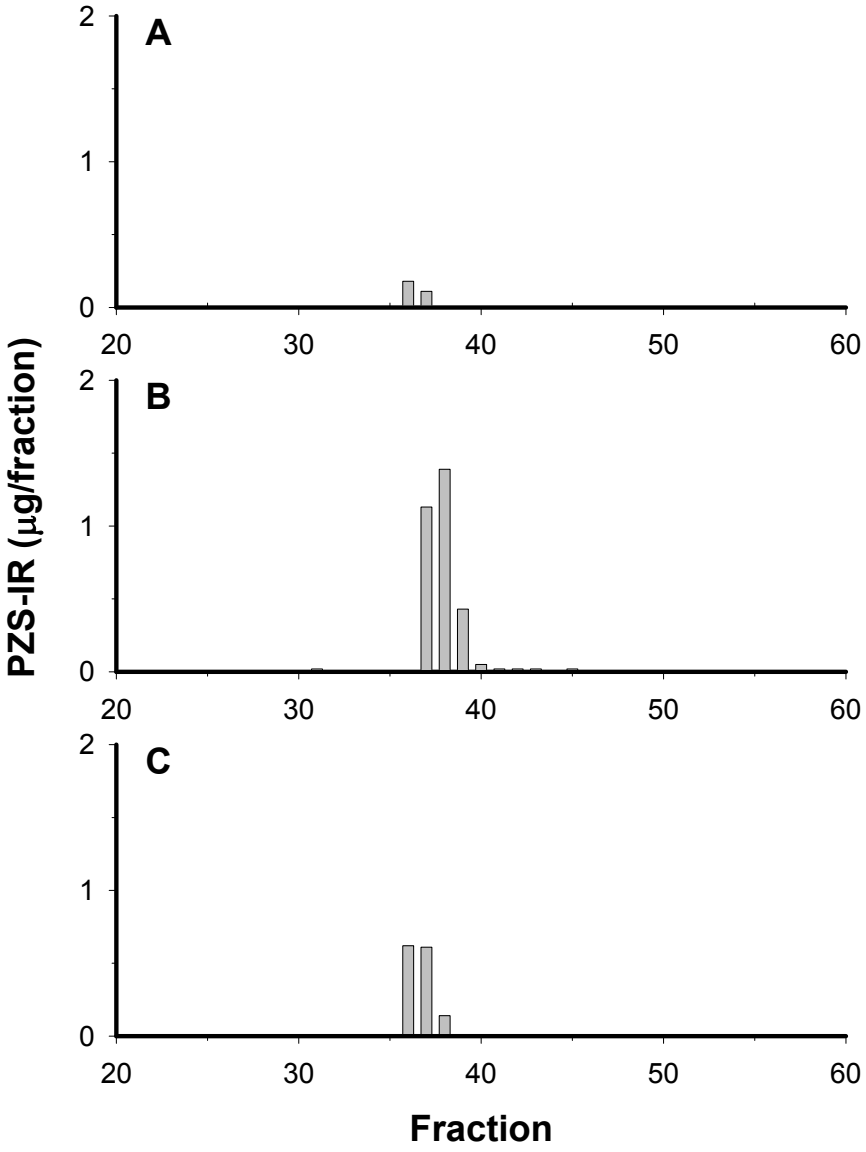


Figure 9. ELISA analysis of HPLC fractions. One gall bladder extract each from Pacific lamprey (A), western brook lamprey (B) and sea lamprey (C) was fractionated on a C18 column and assayed with the ELISA. Note most of PZS immunoreactivity was observed at fractions 36-38.

CHAPTER FOUR

Habitat suitability criteria for larval Pacific lamprey

Kimmo K. Aronsuu[1], Christian Torgersen[2], and David A. Close[1]

[1] Tribal Fisheries Program, Department of Natural Resources, Confederated Tribes of the Umatilla Indian Reservation, Pendleton, Oregon, U.S.A.

[2] Forest and Rangeland Ecosystems Science Center, U.S. Geological Survey, Corvallis, Oregon, U.S.A.

Introduction

The Physical Habitat Simulation System (PHABSIM) is part of a broad conceptual and analytical framework for addressing stream flow management issues called the Instream Flow Incremental Methodology (IFIM) (Stalnaker et al. 1995). IFIM provides a problem-solving outline for water resource issues in streams and rivers. IFIM and PHABSIM were developed as aids to instream flow decision making. Thus, the structure addresses the decision making environment as well as the techniques for quantifying incremental differences in instream habitat that result from proposed alternative instream flow

The relationship of physical habitat to fish (or any other aquatic organism) production assumes the production of benefits for fish is limited by the availability of physical habitat. Microhabitat is typically described by a combination of hydraulic and/or physical variables such as, depth, velocity, substrate, and cover at a spatial scale of near zero to a few meters. PHABSIM estimates changes in physical microhabitat as a function of flow, but does not directly address other elements of stream systems such as water quality and energy inputs.

Use of PHABSIM involves development and/or selection of habitat suitability criteria curves for use in the habitat models. In general, suitability curves have been classified according to the following categories (Bovee et al. 1998):

1) Expert opinion or literature curves. These are typically derived from a consensus of experts' accumulated knowledge of habitat use by a species' life stage(s) or by evaluating habitat use information found in the professional literature.
2) Habitat Utilization Curves. These are derived directly from observations of habitat use of the target life stage and species.
3) Habitat Preference Curves. These are derived from observation data on habitat use corrected for habitat availability.

PHABSIM is used in Columbia River basin to predict the effects of flow alternations on fish habitat availability (citations). The need to evaluate the effects of changes in flow regime on habitats used by larval lamprey is important due to the decline of Pacific lamprey in

the Columbia Basin. However, the lack of suitability curves has prevented fisheries management from including ammocoete habitat in the models. In 1999, Torgersen and Close (2002) collected data to predict the relative abundance of larvae among different scales in the Middle Fork John Day River. We used this data to calculate habitat preference curves for six of the sample level habitat variables which significantly ($p< 0.05$) explained the abundance of larval Pacific lamprey in bivariate logistic regression performed by Torgersen and Close (2002).

Material and methods

Data collection

Larval Pacific lamprey were collected in the upper 55 km of the Middle Fork John Day River, a fourth- to fifth-order stream in north-eastern Oregon, USA (Figure 1). The upper Middle Fork John Day River ranges in altitude from 1000 to 1300 m and flows through semi-arid rangelands in alluvial valleys and alluviated canyons vegetated on the upslopes with mixed conifer forest (*Pinus ponderosa* and *Abies grandis*). The basin has been influenced by a number of land-use practices, including mining, timber harvest, channelization, and grazing, which have nearly eliminated deciduous riparian vegetation (*Populus trichocarpa*, *Crataegus douglasii*, and *Alnus rubra*) in unconstrained alluvial valley reaches.

Detailed maps of stream habitat (channel unit type and dimensions) and channel gradient provided a high-resolution, spatially continuous context for selecting larval sampling sites in the Middle Fork John Day River study section. Longitudinal profiles of water depth and channel gradient derived from extensive habitat surveys (conducted by state and federal agencies for salmonid research) and 10-m digital elevation models (DEM) were rectified 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 (rkm 0). Thirty sites were distributed along the survey section and stratified based on longitudinal patterns in water depth and channel gradient. Sampling sites were located in the field with a hand-held global positioning system (GPS) to ±50 m.

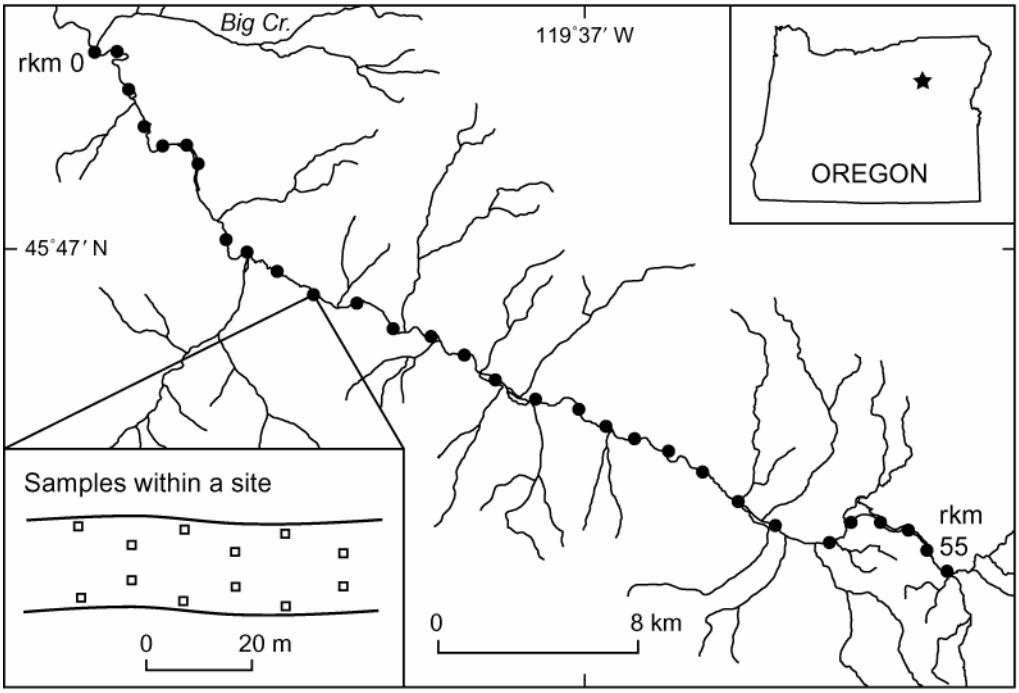


Figure 1. Study area and sampling design for the survey of larval Pacific lamprey in the upper 55 km of the Middle Fork John Day River, Oregon, USA. Solid circles indicate the locations of larval sampling sites. The direction of river flow is from right to left.

Larval sampling

Sampling was completed in one month (base flow in August 2000) to obtain synoptic data during a time when larval movement was limited. A nested sampling design was used (figure 1). Sampling locations (1 x 1 m quadrates, $n = 12$) 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 two 90-s passes with a backpack model AbP-2 larval lamprey electrofishing unit (Engineering Technical Services, University of Wisconsin, Madison, Wisconsin, USA). 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. After collection, larvae were anaesthetised in buffered MS-222 (tricaine methanesulfonate at 250 mg·L^{-1}), identified on the basis of caudal pigmentation patterns (Richards, Beamish and Beamish, 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 (Zippin 1958; White et al. 1982). Larval abundance was defined as the sum of larval densities per site. In addition, density estimates were calculated for two larval size classes: total length 30-69 mm and 70-109 mm.

Habitat description

Assessment of larval habitat was conducted at the sample or site level depending upon the nature of stream habitat variables. Only variables measured at the sample levels were used for creating habitat preference curves. Measurements of water velocity at 60% depth (Model 201D flowmeter, Marsh-McBirney, Inc.) and total water depth were taken once per sample. Dominant substrate and larval habitat type were estimated visually within each 1 x 1 m sampling quadrate. Substrate classes were organic debris, silt (<0.1 mm), sand (0.1-2 mm), small gravel (3-10 mm), large gravel (11-100 mm), cobble (101-300 mm), boulder (>300 mm) The median depth of overlying organic depris in the sampling quadrate was estimated based on 1 to 10 measurements with a meter stick. Channel unit type (pool/riffle) was classified based on channel morphology and surface water velocity (Bisson et al., 1982). Larval habitat type were estimated visually within each sampling quadrate. The following definitions were used to classify larval habitat: type I—a mixture of soft sediment particles including silt, clay, fine organic matter, and some sand; type II—similar to type I habitat but with a larger component of sand; type III—bedrock, hard clay, cobble, or coarse gravel substrates.

Torgersen and Close (2002) used bivariate logistic regression to describe the relationship between larval abundance and habitat variables at sample and site level. Six of the sample level habitat variables which statistically ($p< 0.05$) explained the abundance of larval Pacific lamprey was selected for further analysis. Selected variables were 1) dominant substrate type, 2) depth of overlying organic debris, 3) water velocity, 4) channel unit type (pool/riffle), 5) position (margin/middle), and 6) habitat type (I-III). Typically water depth is also used in physical habitat simulation; however, preference curves for water depth were not developed because of the non-significance in explaining larval abundance.

To Proportional use (P_i) values for every category of habitat variables were computed as:

$$P_i = \frac{n_i / N}{v_i / V_t},$$

where n_i is the estimated number of larvae in the *i*th category of habitat variable V, N is the total number of larvae estimated, v_i is the frequency of occurrence of the *i*th value of variable V, V_t is the total number of all measurements of variable V. Suitability index (Si) values were obtained by dividing all the Pi values by the maximum Pi value for each variable. This procedure results in a score range of 0-1 representing 'unsuitable' and 'optimal' habitat conditions respectively (Bovee 1986).

In addition to suitability index, the Jacobs' electivity index (D) was computed as:

$$D = \frac{U - A}{(U + A) - (2 \times U \times A)},$$

where $U = n_i / N$ and $A = v_i / V_t$. Jacobs' index ranges from -1 to +1; negative values suggest avoidance, and positive values suggest preference.

Results

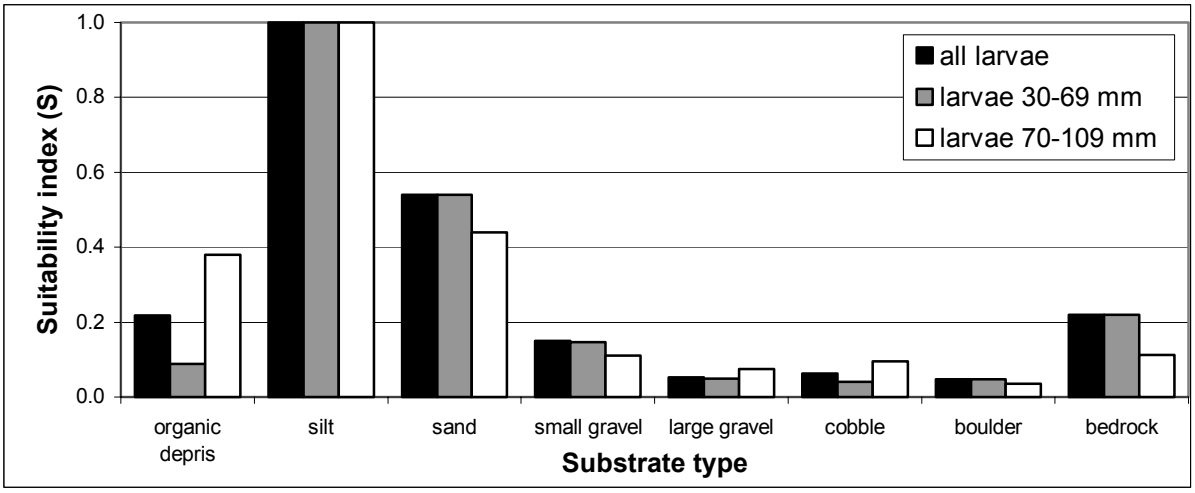
Larvae preferred areas where soft sediments were dominant (Figure 2, Table 1). The habitats where silt was the dominant substrate were most preferred followed by sand and then organic detritus. Larvae avoided coarse mineral sediments from small gravel to boulders. Even though the suitability of cobble substrate was low; the availability was so high that 31 % of larvae were estimated to select the cobble substrate. Cobble substrate was more suitable for large larvae (70-109). Consequently, 41 % of this size class of larvae was estimated to have selected habitat where cobble were the dominant substrate. Suitability of burrowing habitat increased when depth of the organic material increased (Figure 2, Table 1).

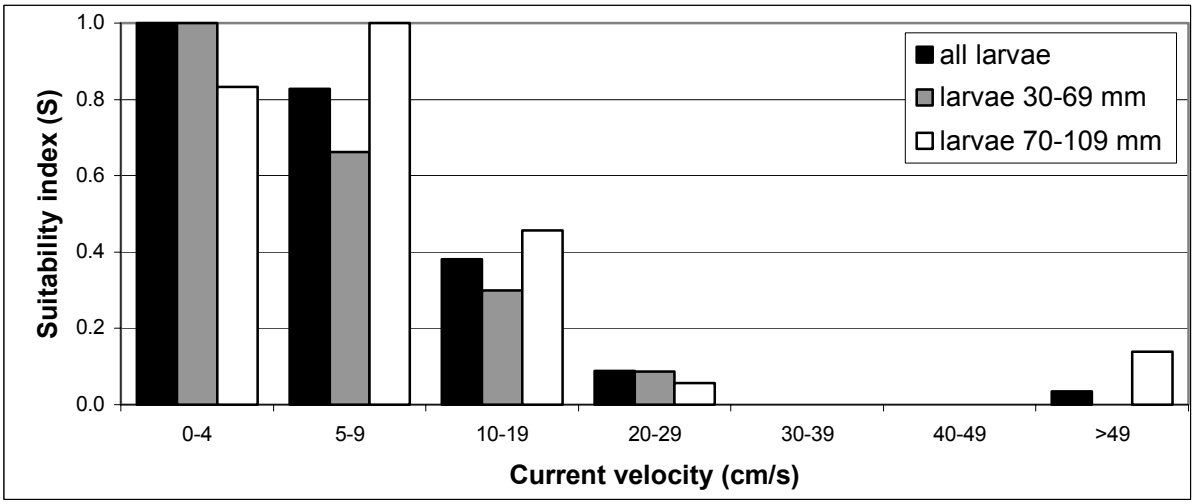
Larvae showed preference for low, under 0.1 m/s, current speed and strong avoidance for current speed higher than 0.19 m/s (Figure 2, Table 1).

Larvae preferred pool to riffle area and channel margins to mid channel (Table 1).

Habitat type 1 was strongly preferred, habitat type 2 preferred and habitat type 3 strongly avoided.

The indices showed some differences between size groups. The larger larvae didn't avoid cobble substrate and mid channel as strongly as the smaller larvae. Furthermore, the most





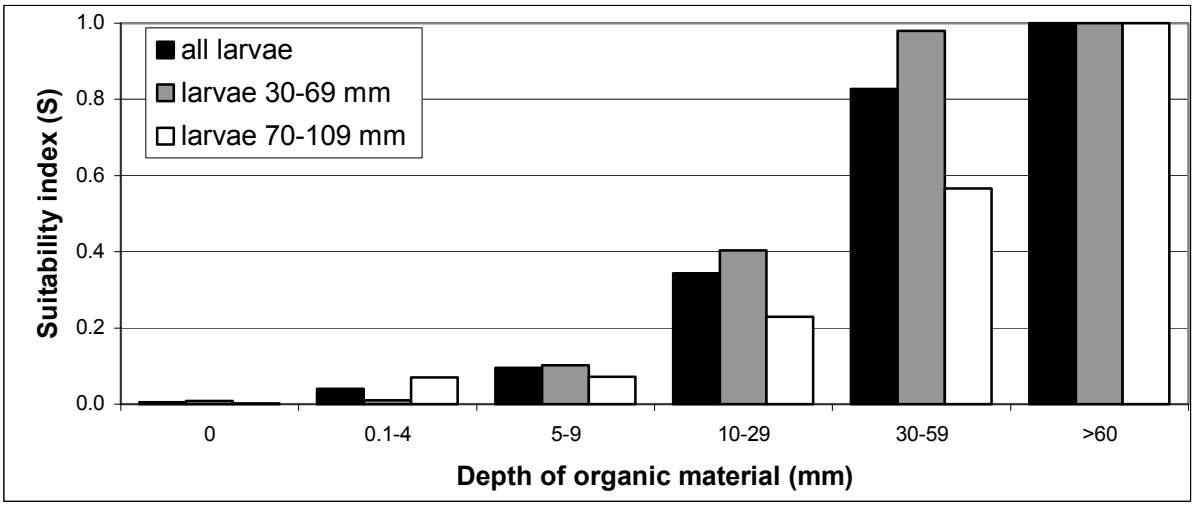


Figure 2. Suitability indices (S) of substrate types, current velocity and depth of organic material for all the larvae and two size groups.

Table 1. Suitability indices (S) and Jacobs' electivity indices (D) of substrate types, current velocity, depth of organic material, habitat type, channel type and channel location for all larvae and two size groups (total length 30-69 mm and 70-109. v_i = occurrence of variable, n_i = estimated number of larvae in the ith category, P_i = proportional use of ith category. The total estimated number of larvae (N) was 1609. The total number of larvae in the size group 30-69 mm was 1011 and in the size group 70-109 mm 581 individuals. The total number of sites (V_t) was 348.

substrate		all larvae				larvae 30-69 mm				larvae 70-109 mm			
type	v_i	n_i	P_i	S	D	n_i	P_i	S	D	n_i	P_i	S	D
organic depris	13	112	1.87	0.22	0.32	34	0.90	0.09	-0.05	62	2.85	0.38	0.51
silt	6	237	8.56	1.00	0.82	178	10.21	1.00	0.85	75	7.48	1.00	0.79
sand	17	364	4.63	0.54	0.70	272	5.50	0.54	0.75	93	3.29	0.44	0.58
small gravel	30	178	1.29	0.15	0.14	130	1.50	0.15	0.22	42	0.83	0.11	-0.10
large gravel	55	116	0.46	0.05	-0.41	79	0.50	0.05	-0.38	52	0.57	0.08	-0.31
cobble	200	496	0.54	0.06	-0.50	239	0.41	0.04	-0.63	238	0.71	0.10	-0.32
boulder	19	35	0.40	0.05	-0.44	27	0.49	0.05	-0.36	9	0.27	0.04	-0.59
bedrock	8	70	1.88	0.22	0.32	52	2.24	0.22	0.39	11	0.84	0.11	-0.09
current velocity		all larvae				larvae 30-69 mm				larvae 70-109 mm			
cm/s	v_i	n_i	P_i	S	D	n_i	P_i	S	D	n_i	P_i	S	D
0-4	71	782	2.38	1.00	0.57	548	2.66	1.00	0.64	232	1.96	0.83	0.44
5-9	42	383	1.97	0.83	0.39	214	1.76	0.66	0.32	165	2.35	1.00	0.49
10-19	87	365	0.91	0.38	-0.06	201	0.80	0.30	-0.15	156	1.07	0.46	0.05
20-29	71	70	0.21	0.09	-0.70	48	0.23	0.09	-0.68	16	0.14	0.06	-0.80
30-39	29	0	0.00	0.00	-1.00	0	0.00	0.00	-1.00	0	0.00	0.00	-1.00
40-49	25	0	0.00	0.00	-1.00	0	0.00	0.00	-1.00	0	0.00	0.00	-1.00
>49	23	9	0.08	0.04	-0.85	0	0.00	0.00	-1.00	13	0.33	0.14	-0.53
depth of org. mater.		all larvae				larvae 30-69 mm				larvae 70-109 mm			
cm	v_i	n_i	P_i	S	D	n_i	P_i	S	D	n_i	P_i	S	D
0	67	10	0.03	0.01	-0.95	9	0.05	0.01	-0.93	1	0.01	0.00	-0.99
0.1-4	77	81	0.22	0.04	-0.69	12	0.05	0.01	-0.92	67	0.51	0.07	-0.38
5-9	85	209	0.53	0.09	-0.37	128	0.51	0.10	-0.39	74	0.52	0.07	-0.38
10-29	95	847	1.91	0.34	0.49	569	2.04	0.40	0.54	264	1.65	0.23	0.37
30-59	13	280	4.60	0.83	0.69	189	4.94	0.98	0.71	89	4.07	0.57	0.64
>60	7	182	5.56	1.00	0.72	104	5.05	1.00	0.69	85	7.19	1.00	0.78
habitat		all larvae				larvae 30-69 mm				larvae 70-109 mm			
type	v_i	n_i	P_i	S	D	n_i	P_i	S	D	n_i	P_i	S	D
1	12	319	5.75	1.00	0.75	196	5.62	1.00	0.74	123	6.13	1.00	0.76
2	115	924	1.74	0.30	0.46	579	1.73	0.31	0.46	323	1.68	0.27	0.44
3	221	366	0.36	0.06	-0.71	236	0.37	0.07	-0.70	135	0.36	0.06	-0.70
channel		all larvae				larvae 30-69 mm				larvae 70-109 mm			
type	v_i	n_i	P_i	S	D	n_i	P_i	S	D	n_i	P_i	S	D
pool	244	1504	1.33	1.00	0.72	970	1.37	1.00	0.82	520	1.28	1.00	0.57
riffle	104	106	0.22	0.16	-0.72	41	0.14	0.10	-0.82	61	0.35	0.27	-0.57
channel		all larvae				larvae 30-69 mm				larvae 70-109 mm			
location	v_i	n_i	P_i	S	D	n_i	P_i	S	D	n_i	P_i	S	D
margin	174	1298	1.61	1.00	0.61	849	1.68	1.00	0.68	435	1.50	1.00	0.50
middle	174	311	0.39	0.24	-0.61	162	0.32	0.19	-0.68	146	0.50	0.34	-0.50

preferred current speed for larger larvae was higher than that of smaller larvae. Differences were not tested statistically.

Discussion

A lamprey larva burrows by inserting its head into the substrate with vigorous contractions of the tail (Hill and Potter 1970). In the burrow, the body is arched such that the anterior end is close to the surface. By secreting mucous from endostyle line, the larva forms a fragile tube at the entrance of the burrow. The substrate must be suitable to provide burrow construction and water exchange. While in the burrow larvae depend on unidirectional flow of water through their branchial chamber for the provision of food and exchange of respiratory gases and metabolic wastes (Hardisty and Potter 1971).

The habitat preferences we found for larval Pacific Lamprey were mainly consistent to results of earlier larval habitat studies (Malmqvist 1980, Potter et al. 1986, Young et al. 1990, Beamish and Jebbnik 1994, Kelso and Todd 1993, Beamish and Lowarz 1996, Almeida and Quintella 2002, Sugiyama and Goto 2002). Simplified, larvae prefer areas with soft sediment, which allows burrow construction and helps to maintain a vital water flux. In our study, two variables, dominant substrate type and depth of overlying organic sediment, were used to reflect the burrowing substrate. The content of organic material and the proportion of different sized mineral particles in burrowing habitat influence abundance of lampreys (Beamish and Jebbnik 1994, Beamish and Lowarz 1996). Our substrate classification included only three classes of soft sediments (Organic depris, silt and sand). Still, we think that for physical habitat simulation purposes, substrate classification combined with depth of soft sediments reflect the available burrowing habitat.

The larval distribution was closely associated with low water velocity. However, since substrate composition in the lotic environment is closely related with current velocity it is suggested that the ammocoete distribution is mostly a function of sediment particle size (see Young et al. 1990, Almeida and Quintella 2002). For example, current velocity may temporally be at the preferred range (<0.1 m/s) of larvae due to restricted flow. However, during normal flow sedimentation may not occur in these areas causing these areas to be unsuitable for larvae.

Three other variables, channel unit type (pool/riffle), position (margin/middle) and habitat type (I-III), are more or less combination of first three variables. The channel unit type

(pool/riffle) is closely related to current velocity and habitat type was mainly estimated based on substrate type and depth of soft sediment. The position variable (margin/middle) is more complex. Margin habitat is associated with low current velocity and deposition of fine substrate and shade, which in some studies correlated with larval densities (Potter et al. 1986).

Larval size has been shown to affect habitat selection in many studies (Kainua and Valtonen 1980, Beamish and Jebbnik 1994, Sugiyama and Goto 2002, Almeida and Quintella 2002) but there is also reverse results (Beamish and Lowarz 1996). Based on our data, there seemed to be some differences in habitat selection between size-groups in habitat selection. We speculate that larger larvae are able to utilize habitat at higher velocities and mid channel area because of better swimming capacity. It is noteworthy that the upper limit of total length for larger size group was 109 mm. Still, larvae are approximately 150 mm before metamorphosis. Our data did not include enough large larvae to create suitability indices for larva over 109 mm.

When creating suitability criteria it is assumed that all habitat is equally available to larvae. The data we used for creating preference curves were collected throughout a 55 km section and therefore it was not the best one for suitability criteria use. Torgersen and Close (2002) showed that in addition to habitat variables longitudinal position in the stream section explained additional variation in the larval abundance in the study area. For example, availability of suitable spawning habitats and number of spawning adults could lead to differences in larval abundance between sites or longitudinal positions (see Morman et al. 1980, Almeida and Quintella 2002). The data for creating suitability curves would be better if collected in a more restricted area to avoid the above mentioned problem. Another weakness of the data was that it included only six sites where the most preferred substrate (silt) was the dominant substrate, consequently, the suitability index of all substrate types were dependant on only few samples. Regardless of how the data are collected, suitability curves will demonstrate some specificity to the stream in which they were developed. With limited resource availability and the high cost associated with development of stream-specific suitability curves, use of habitat suitability curves from other streams is common. Thus, checking for the appropriateness of the transfer is important. The investigator must apply professional knowledge and judgment to evaluate if the source curves are meaningful for the current application.

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