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PACIFIC LAMPREY RESEARCH AND RESTORATION PROJECT

Annual Report 2001

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PACIFIC LAMPREY RESEARCH AND RESTORATION PROJECT

ANNUAL REPORT 2001

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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 1999). 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 2001.

Chapter I

A total of 249 adult lampreys were collected at the John Day Dam fish ladder in the winter of 2000-2001. 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 early May. Nest and egg viability surveys were conducted to determine reproductive success 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. 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 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 spawned successfully in the mainstem Umatilla River and Meacham Creek. In total 49 viable nests were located during the surveys (06/01-07/01). All nests were above river kilometer 117.7 in the mainstem Umatilla River. 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. The mean survival rate of eggs at stages 12-14 was 86.2 % (range 57.2-100 %) in 13 nests. Based on our data, we suggest that

environmental conditions in the uppermost reaches of the Umatilla River and Meacham Creek are good for spawning and successful embryological development.

Larval densities in index sites above river kilometer 100 in the mainstem Umatilla River were much higher than previous years. The mean density above river kilometer 100 in the mainstem Umatilla and Meacham Creek (sites 20-34) was 12.6 $ind.m^{-2}$. Larval length distributions have shown that the largest portion of larvae was year class 2000 and most likely the offspring of adults outplanted in 2000. Year class 2000 larvae had not yet dispersed into the lower part of the river. Below river kilometer 100 the mean density of larvae in index sites (1-19) was only 0.1 $ind.m^{-2}$. The growth rate of larvae has been high. The overall median length of larvae found above rkm 100 was 62 mm. The median length of larvae in the sites was negatively correlated with river kilometer.

The total number of captured outmigrating metamorphosed and larval lampreys was 1,988 and 755 individuals. The number of outmigrating metamorphosed lampreys was estimated in the tens of thousands of individuals during the migration season 2000-2001. The catch of larvae and metamorphosed lampreys were positively correlated with flow. The mean length of captured lampreys was higher than observed in other rivers reflecting size selectivity of the traps or a different metamorphosing size in the Umatilla River compared to other rivers.

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.

Chapter II

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 from June through December 2001, and compares these data 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 June 2001 (the time of capture during upstream migration) through June 2002 (the time of natural spawning). Olfactory responses of juvenile steelhead (*Oncorhynchus mykiss*) to selected L-amino acids were periodically measured, confirming that the EOG apparatus was properly functioning. Between June and December 2001, 95 adult Pacific lampreys and 10 juvenile steelhead were tested on the EOG apparatus. The olfactory system of early 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. These preliminary results from 2001 indicate a possible role for larval and adult lamprey bile acids as pheromonal cues for Pacific lampreys.

CHAPTER ONE

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

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INTRODUCTION

The Pacific lamprey (*Lampetra tridentata*) is an 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. 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 in press). Adult Pacific lamprey have 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 2001.

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."

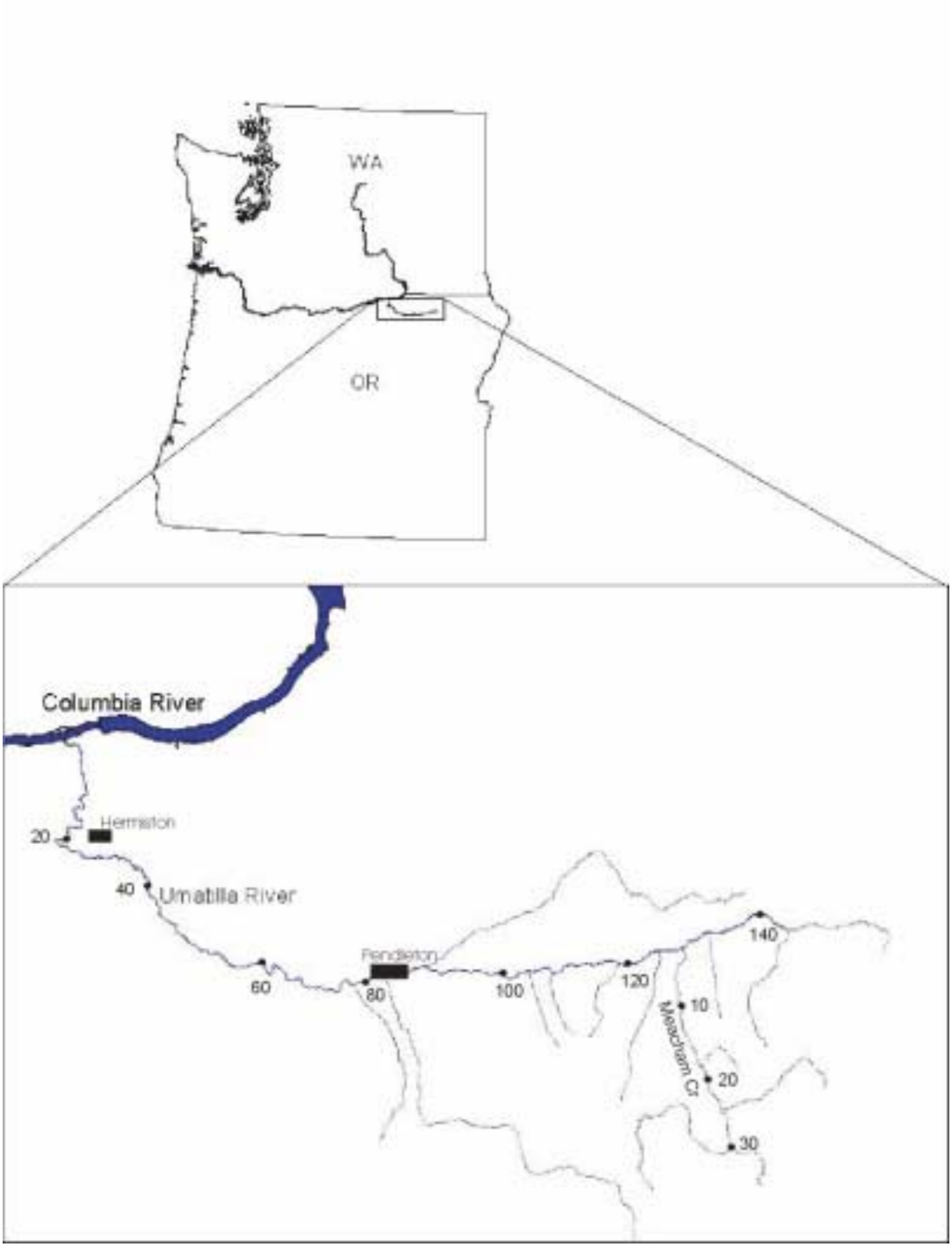

Figure 1. The Umatilla River basin in Northeast Oregon. Numbers in the map represent river kilometers.

Flow and water temperature during the study period

During the study period from October 2000 to September 2001, the daily mean discharges in the Umatilla River at river kilometers (rkm) 131, 90 and 4.5 were 4.5, 9.7 and 10.5 m^3s^{-1}, respectively. Discharge was highest during April and early May (Figure 2). In the middle and lower part of river, discharge was higher than 40 m^3s^{-1} and approximately 25 m^3s^{-1} above Meacham Creek confluence (rkm 131). In the two uppermost stations, flow decreased by early May. In the lowermost station (near the river mouth) discharge dropped quickly in early July and varied between 0.08 and 0.51 m^3s^{-1} (mean 0.21 m^3s^{-1}) from July 7th through August 16th (Figure 2).

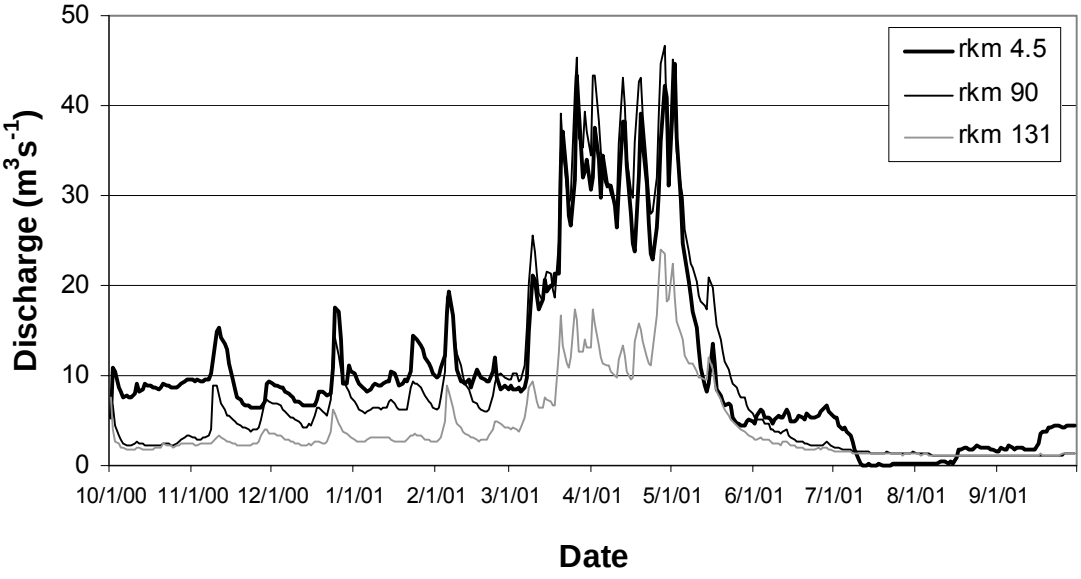

Figure 2. Daily mean discharge in the Umatilla River at river kilometers 131, 90 and 4.5 from October, 2000 to September, 2001.

Temperature is variable in the Umatilla River (Figure 3). The mean water temperatures during the summer season (06/01/01-09/15/01) collected by stream thermographs at rkm 140.0, 131.5, 117.3, 108.9 and 81.7, were 13.9, 15.7, 17.9, 19.2, and 21.4 $^{\circ}$C, respectively. Mean temperatures during same period in Meacham Creek at rkm 20.9, 8.4 and 3.3 were 15.3, 16.9, and 17.7 $^{\circ}$C, respectively.

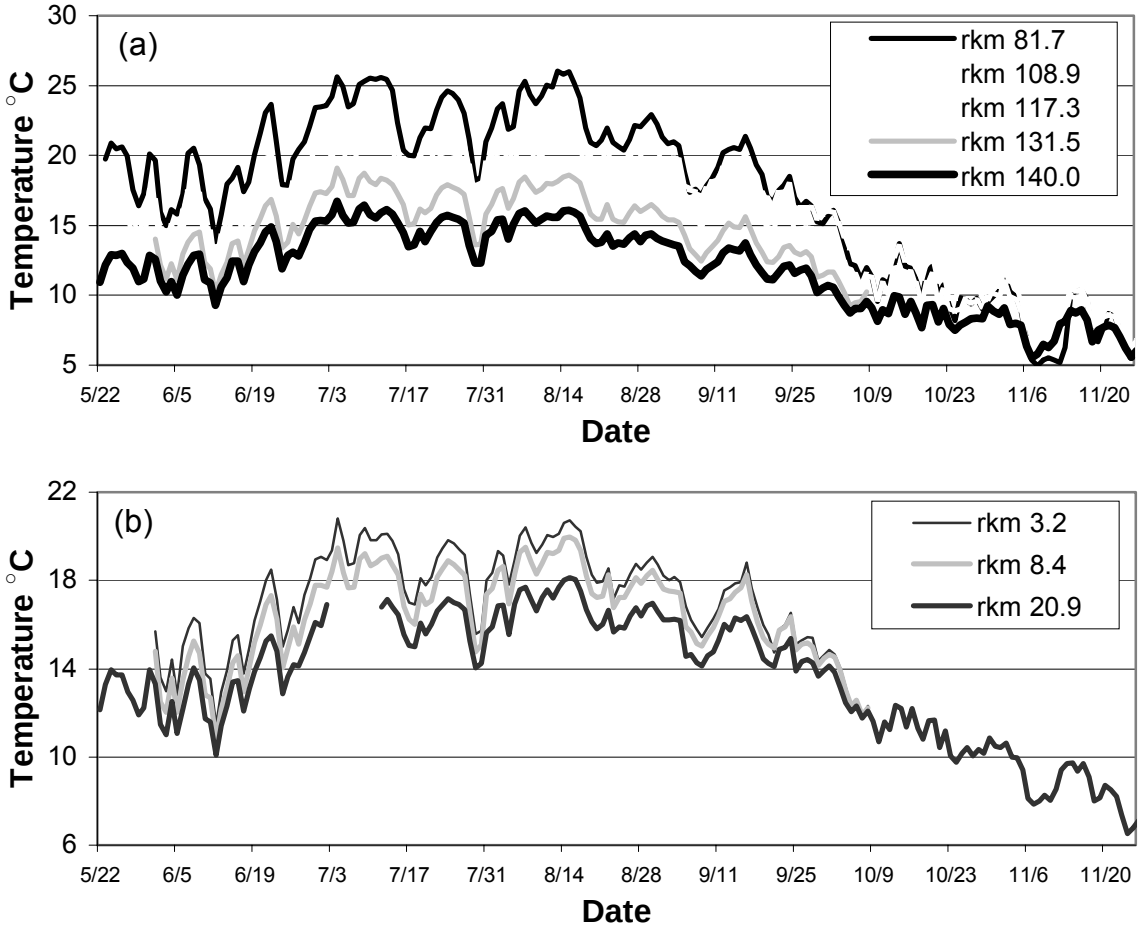

Figure 3. Daily mean temperatures at five locations in the Umatilla River (a) and three locations in Meacham Creek (b) from 05/22/01 to 11/25/01.

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 249 adult lampreys were collected during the winter dewatering and maintenance at the John Day Dam fish ladder on December 12th, 2000 and January 5th, 2001. 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 of all individuals was measured. The mean length of the adults was 634 mm (range 542-734 mm). Fish were maintained in a raceway located at the Three Mile Falls facility. The mean temperature was 8.2°C in the raceways during holding period and varied between 3.3 and 15.9°C. Lampreys were checked weekly then daily for ripeness. Before adults were release into the Umatilla River they were checked for disease by ODFW fish pathology in La Grande, Oregon.

Releasing

When lampreys were almost ready to spawn, they were transferred (05/02/01) in aerated tanks to releasing sites. Eighty two and 81 adults were released into two locations in the Umatilla River at river kilometers 118.4 and 139.9, respectively. In addition, 81 adults were released into Meacham Creek (rkm 17.5), a major tributary of the Umatilla River. The daily mean temperature in the raceway on the day before releasing was 10.4°C. On the releasing day the daily mean flow at rkm 131 was 22.5 m^3s^{-1} (Figure 2).

NEST AND EGG VIABILITY SURVEYS

Objective

Nest surveys were conducted for the first time in 2000 to assess the distribution and the number of nests after outplanting adult lamprey. In 2001, the main goal of the surveys was to find viable nests and collect eggs for the survival study, which was conducted to determine reproductive success of adults and embryological development. In addition, viable nests were located and recorded.

Methods

The mainstem Umatilla River (rkm 90 to 140) and Meacham Creek (0 to 19) were surveyed to locate lamprey nests. Nest surveys were conducted from early June through early July. 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 was located surveyors recorded approximate location

with a hand held GPS unit and later mapped with Arcview (GIS version) in the office. The whole area was surveyed once to locate spawning areas, then resurveyed to find additional nests.

To study egg viability, 16 of the nests found were sampled for eggs. A probe sample of 10-20 eggs was taken to determine stage of egg development. Eggs were gently dislodged with a weed picker and were captured in a plankton kick net placed below the nest. The probe sample was used to predict when stage 12 was reached (Piavis 1961). Once eggs reached stage 12 (06/11/01-07/10/01) a sample of 200 eggs were sampled from each nest. Eggs were fixed in 10 % formalin, then stored in carosafe. Later in the laboratory, the number of viable and unviable eggs was counted using a dissecting microscope. Only nest samples with 200 or more eggs at stages 12-14 were used to estimate percentage of viable eggs. Eggs were classified as unviable if covered with fungus or deformed.

Results

During the surveys, a total of 49 viable nests were found in the mainstem Umatilla River and Meacham Creek. Nineteen of the located nests were in the mainstem Umatilla River and 30 in Meacham Creek. In the Umatilla River, five of the detected redds were 700 m below the lower releasing site (rkm 117.7) and the rest of the nests between the releasing sites (rkm 118.4-139.9) (Table 1). In Meacham Creek, 27 of 30 located nests were situated no more than one kilometer away from release site (Table 1). Eighteen of 27 redds were above the releasing site, two at the releasing site, and 10 below the releasing site. In addition three viable nests were found in Meacham Creek at rkm 8.3.

In both the Umatilla River and Meacham Creek most of the new viable nests were detected during the first two weeks of June (Table 1). Mean daily temperatures at rkm 117.3 and 140 in the mainstem Umatilla River was 12.7-16.5°C and 9.3-12.9°C, respectively. In Meacham Creek at the Duncan thermograph (rkm 20.9), the daily mean temperature varied between 10.1 and 13.3°C from 06/01 to 06/14.

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							
date	rkm 117.7	rkm 123.5	rkm 128.7	rkm 133.7	rkm 134.6	rkm 135.1	rkm 138.1-138.5	total
6/3/01	0	0	0	0	0	0	1	1
6/6/01	5	0	0	0	0	0	0	5
6/12/01	0	0	0	0	0	0	1	1
6/13/01	0	0	0	2	1	2	0	5
6/14/01	0	3	0	0	0	0	0	3
6/27/01	0	0	2	0	0	0	0	2
7/3/01	0	0	0	0	0	0	2	2
total	5	3	2	2	1	2	4	19

(b)	Number of nests					
date	rkm 8.1-8.5	rkm 16.6-17.0	rkm 17.1-17.5	rkm 17.6-18.0	rkm 18.1-18.5	total
6/4/01	1	0	0	4	0	5
6/5/01	2	0	0	0	0	2
6/11/01	0	1	2	0	0	3
6/13/01	0	0	0	8	3	11
6/14/01	0	0	2	0	0	2
6/25/01	0	3	1	0	0	4
6/27/01	0	0	0	1	2	3
total	3	4	5	13	5	30

We found 13 of the 16 nests containing enough eggs for the study. The proportion of viable eggs in those nests varied between 57.8 % and 100.0 % and was on an average 86.2 % (Figure 4). In seven of 13 nests the proportion of viable eggs was over 99 %. Seventy five percent of the unviable eggs were covered by fungus and 25 % were deformed.

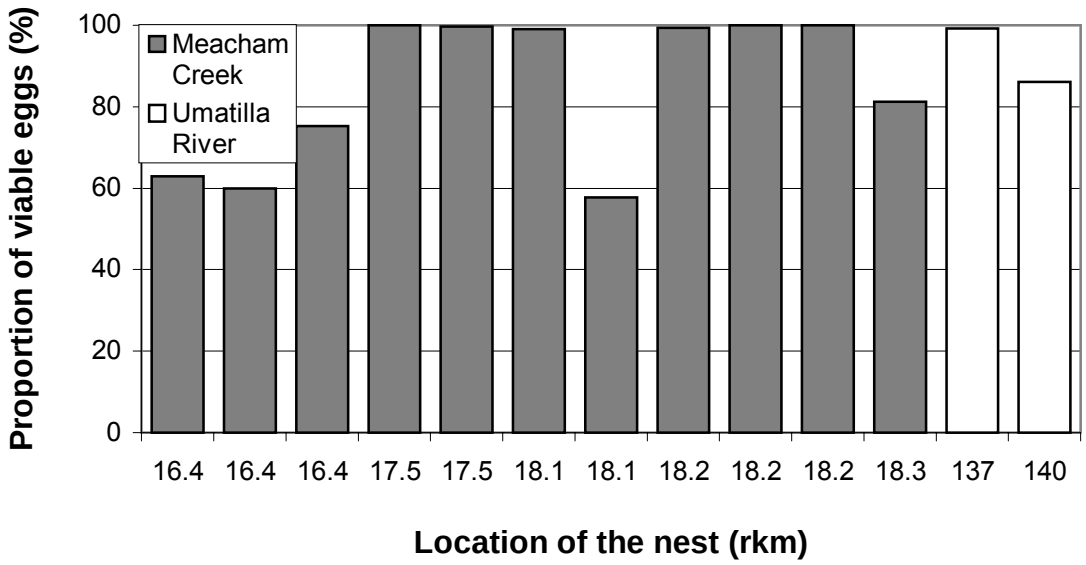

Figure 4. The proportion of viable eggs at stages 12-14 in 11 nests in Meacham Creek and two nests in the mainstem Umatilla River.

Conclusions

On the basis of these results, we are not able to say exactly what portion of the released females spawned. Surveys were mainly conducted to find nests for the egg viability study, thus surveys were concentrated mainly in areas where most of the nests were located and it is likely that all of nests were not detected. In addition, communal and superimposed spawning, which is quite usual for Pacific lamprey (Pletcher 1963 and Kan 1975), made it difficult to estimate how many females spawned in the 49 viable nests. However, nest surveys and egg viability of the nests have shown that a majority of the outplanted females spawned successfully in the uppermost part of river and it also seems that outplantings, started in 2000, have also increased the number of ammocoetes in the uppermost part of the river (page 17).

All lamprey nests were located above rkm 117 in the upper reaches of the river. This was expected since the lampreys were transplanted above rkm 118 and Pacific lamprey has a natural behavior of upstream migration before spawning (Bayer et al. 2001). Furthermore, spawning in the upper reaches may be a natural behavior; it allows for maximum use of suitable larval habitat assuming young ammocoetes gradually migrate downstream (Potter 1980).

In Meacham Creek, a majority of the adults spawned less than one kilometer from the releasing site. In contrast, lampreys in the mainstem spread more widely and possibly migrated into Meacham Creek. From our observations, lampreys do not need to migrate away from

Meacham Creek's releasing site**,** but in the mainstem they are migrating much more to find appropriate spawning sites. 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). More information is needed to find out why lampreys select one but reject other spawning areas and the best release locations.

Water temperature is an important triggering factor for lamprey spawning (Applegate 1950, Manion and McClain 1971). The observations of spawning temperature in the Umatilla River and Meacham Creek were consistent with earlier observations. Pacific lamprey along the coast of Oregon has been observed spawning in May with temperatures between 10 and 15 $^{\circ}$C (Kan 1975). Inland populations can spawn through the end of July (Kan 1975, Farlinger and Beamish 1984). Most of the nests in 2001 were located in the beginning of June when the daily mean temperature was between 9-16 $^{\circ}$C.

Spawning habitat, water quality, and temperatures seem to be good enough for successful spawning and embryological development in the uppermost reaches of the Umatilla River and Meacham Creek based on egg viabilities. Manion and Hanson (1980) reported the fertilization and survival rate of eggs deposited in the nests is high and may approach 90 % for sea lamprey (*Petromyzon marinus*). The survival rate for Pacific lamprey eggs in the Umatilla River was similar to sea lamprey in the Great Lakes.

Outplanted Pacific lampreys in the Umatilla River potentially produce large quantities of larvae. Relative fecundity of Pacific lamprey is approximately 500 eggs/g body weight (Kan 1975). Based on assumptions that 1) half of outplanted lampreys were females, 2) all females spawned, and 3) 5 % of eggs remained in body cavity like in sea lamprey (Appelgate 1950**),** and 4) according to the weight data from lampreys we outplanted in 2001, the female lampreys released approximately 23.5 millions eggs. Only 14 % of the sea lampreys eggs remain in the nest (Manion & Hanson 1980). If the proportion is the same for Pacific lamprey, and if we account for egg viability, approximately 2.8 million prolarvae hatched in the nests during the summer of 2001. While this estimate includes a lot of assumptions, it still gives us an idea of how much larvae a few hundred outplanted lampreys are able to produce in the uppermost part of the Umatilla River and Meacham Creek when environmental conditions are good.

LARVAL ABUNDANCE AND DENSITIES

Objective

The approach has been to follow the development of larval densities and length distribution in the index sites selected in 1998 by conducting a larval lamprey electrofishing survey. In 1998-2000 we studied larval densities before and after outplanting adult lampreys. In 2001, larvae were detected for the first time in the upper drainage after outplantings.

Material and Methods

In 1998, a total of 42 sites were selected in the Umatilla River for following 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. All the selected sites were habitat type 1 (i.e. silty substrate characteristics) where larvae are typically most abundant. From some index sites 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 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 was 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). In addition, 691 ammocoetes were weighted ($\pm$ 0.01 g). After recovery, larvae were returned to the river. The electrofishing survey was conducted from 08/03/01 through 09/05/01.

A population estimate was conducted within each plot. The Two Catch method was used to estimate abundance in area sampled.

Mathematics: $N_o=C_1^2/(C_1-C_2)$

$P=(C_1-C_2)/C_1$

$q=1-p$

Var $N=C_1^2C_2^2(C_1+C_2)/C_1-C_2)^{\wedge 4}$

$(SD)(Z_\alpha)$=confidence interval

$bias=q(1+q)/p^3$

Where: N_o = population to be estimated

C_1= catch at first fishing

C_2= 2nd catch

P= probability that any one fish is caught

Variances of sites were summed and confidence intervals (95%) for the estimate of total number larvae caught were calculated using the square root of the sum of variances (CI = 1.96 x square root of the variance).

Results

A total of 1,178 ammocoetes were collected, and the estimate of the total amount of larvae (± 95% confidence interval) for the sampled area was 1,433 ± 112 individuals. The estimated mean density for the sampled area was 5.6 ± 0.4 ind.m^{-2}. Ammocoetes were detected in all index sites in Meacham Creek (sites 32-34) and all but one index sites above river kilometer 102.2 in the mainstem Umatilla River (sites 20-31), but below that ammocoetes were found only in site 3 (Figure 5). The mean density estimate for sites 20-34 was 12.6 ± 1.0 ind.m^{-2}. The highest density was detected at site 27 with 34.7 ± 6.4 ind.m^{-2}.

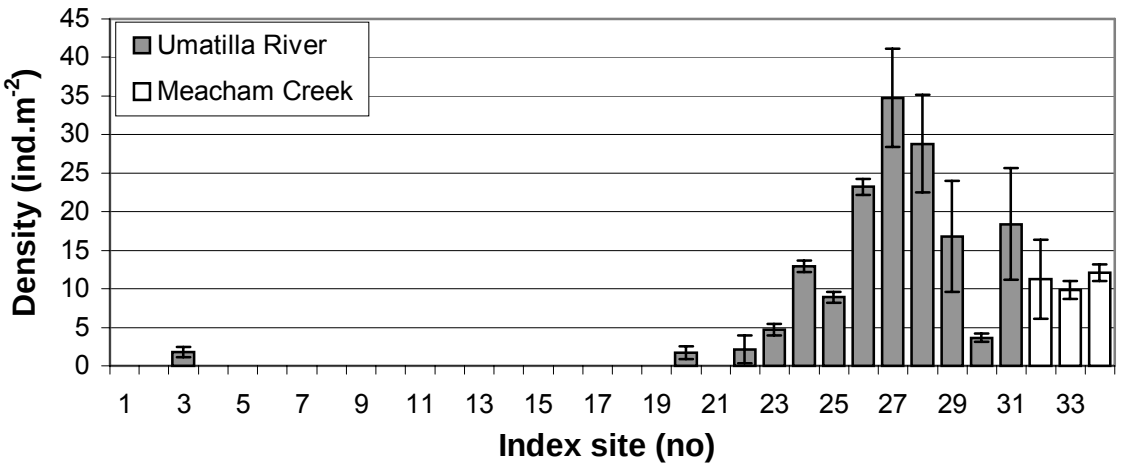

Figure 5. Estimated mean densities (± 95 % confidence interval) for index sites in the Umatilla River and Meacham Creek in 2001.

The median length of larvae captured in the Umatilla River was 63 mm. The median length of larvae caught from sites 22-31 varied between 47 and 76 mm (Figure 6). Median length of larvae in sites were negatively correlated with river km (Spearman rank correlation rs = -0.823, n = 10, $P < 0.01$). In site 20, the median length of ammocoetes was only 33 mm, which suggests that larvae were mainly year class 2001. In site 3, larvae were much longer (median length 111 mm) than in the other sites. In Meacham Creek, the median length of the larvae in the lowermost site (32) was 70.5 mm and in the two uppermost sites (33 and 34) 44.0 mm and 48.0 mm, respectively.

In the sites where the numbers of captured larvae were high, length distribution was unimodal reflecting one major year class (Figure 6). However, in a few sites the length distribution wasn't clearly unimodal even when the number of larvae was quite high. In site 29 there was small separate peak of larger larvae and in site 32 a separate peak of smaller larvae.

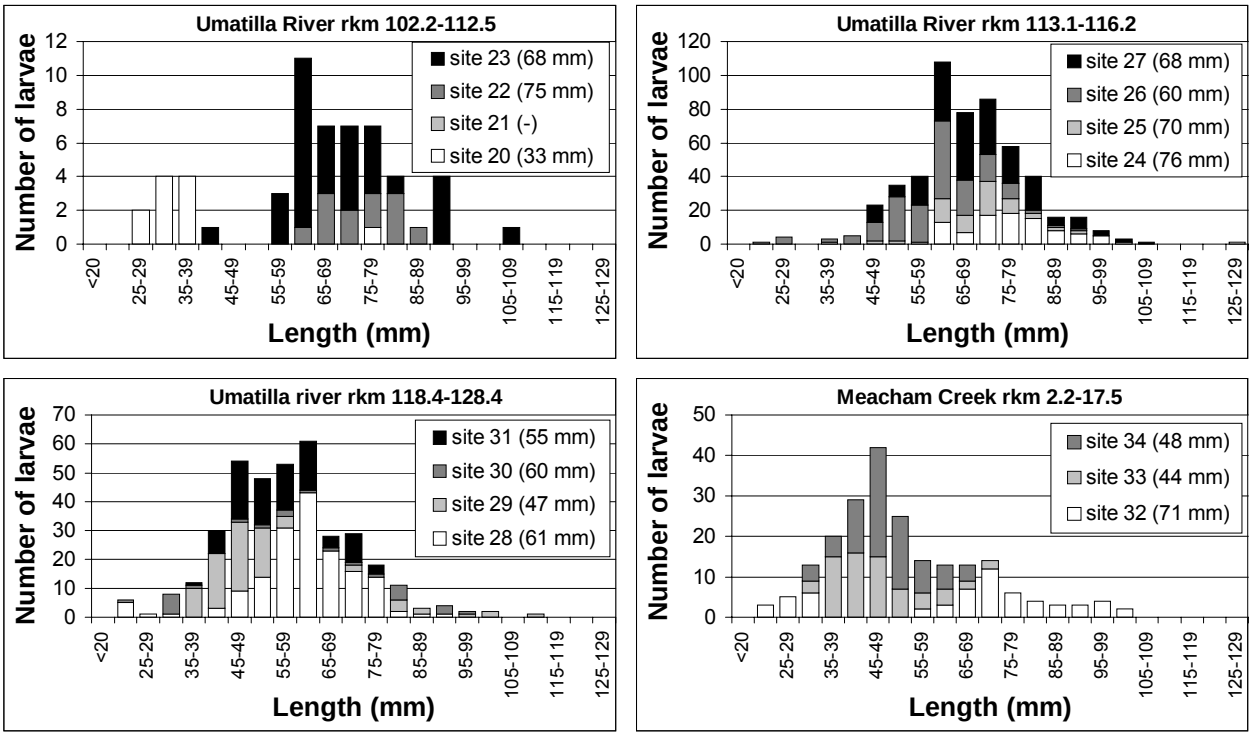

Figure 6. Length distributions and median lengths (in parentheses) for index sites 20-34 in the Umatilla River and Meacham Creek in 2001.

Conclusions

Larval surveys have shown that outplanting mature lampreys (starting in the spring of 2000) have been successful. In earlier larval surveys from 1998-2000, no larvae were found in the index sites near the detected spawning areas of outplanted adults (sites 20-31) (Close 2000, Moser and Close in press, Close et al. 2002), therefore it is probable that almost all larvae found in sites 19-34 were year class 2000. In addition, unimodal length distributions are showing that there was only one major year class.

Lamprey larvae move downstream during their life cycle. It is believed that this downstream movement disperses larval lamprey throughout the watershed (Potter 1980). 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 class 2000 haven't yet migrated to the lower portion of the river and natural reproduction has been very low during the last few years. Before outplanting adults in the upper part of the river, there was an empty niche for the year class 2000 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). After a few more years we will be able to determine how effectively 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 in press). In surveys conducted from 1998-2001 no larvae have been found between river kilometers 36.8 and 102.2 (Close 2000; Moser and Close in press; Close et al. 2002). If larval densities do not increase through time in the middle and lower sections of the river, we should investigate why.

Ammocoetes of year class 2000 grew very fast during the first year. Even though length distributions showed that there were few specimens other than year class 2000 we think that in the sites 22-28 the median lengths (60-76 mm) are reflecting the growth of first year of the year class 2000. In the Willamette and Umpqua River systems in Oregon, the approximate median length for 1+ ammocoetes was only 40 mm. In lampricide treated rivers the growth of sea lamprey larvae was found to be high after treatments because of reduced population densities (Torblaa and Westman 1980). We speculate that high growth rates observed in the Umatilla River are also due to lack of competition. The growth rate will probably decrease through time as we continue adult outplantings.

Water temperature is an obvious reason why median length increased when river kilometer decreased. In many studies it has been observed that larval growth is faster in creeks with warm water temperatures compared to creeks with cooler water temperatures (Manion and Smith 1978; Potter 1980; Holmes 1990; Young et al. 1990). Even though distance between sites 22-31 is only is only 19.3 km (rkm 109.1-128.4) temperature conditions are quite different between sites due to elevation (Figure 3). In the lower river, lampreys spawn earlier, eggs hatch

faster, and the growing season is longer than in the uppermost part of the river. It's also probable that temperatures between river kilometers 110-120 in the Umatilla River are closer to optimum for growth of larvae than in the uppermost part of the river. The optimum temperature for growth of Pacific lamprey larvae is unknown, but for larval sea lamprey it is approximately 21 $^{\circ}$C (Holmes and Lin 1994).

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 10/03/00-03/08/01 using a 1.5 m diameter rotary-screw trap (RST). 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, a majority of lampreys was returned to the river.

Mark-recapture studies were conducted to calculate trapping efficiency of the rotary screw trap and to estimate the total number of outmigrants during trapping. In total, 343 metamorphosed and 411 larval lampreys were fin clipped then released about 400 m up from the trap to the channel on the right side of the river for recapture (Table 2). The number of fin clipped recaptured lampreys were counted and a pooled trapping efficiency estimate for all tagged fish was determined as the ratio of the total fish recaptured to total fish released during the collection period. Variance for estimation was estimated using bootstrap method (Efron and Tibshirani 1986, Thedinga *et al.* 1994) with 1,000 iterations. Confidence intervals (95%) for the

abundance estimate were calculated using the square root of the Bootstrap variance estimate (CI = 1.96 x square root of the variance).

From March 8, 2001 to September 7, 2001, 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, however, trapping efficiency estimates of the FCF was not conducted.

The correlation between discharge and the number of caught larvae and metamorphosed lampreys were tested using Spearman rank correlation test. A nonparametric test was used because the assumption of normal distribution was not fulfilled. The variable reflecting the flow during catching night was the average of the mean flow of the day before and day after the catching night. The time period analyzed was between days when the first and last lampreys were caught (12/22/00-5/1/01). Analyses were conducted separately for RST- and FCF-catch.

Results

During the study period, 1,988 metamorphosed lampreys were collected. 1,822 individuals were caught with RST and 161 individuals at the FCF at the West Extension Canal. The first individual was caught on 12/22/00 and last one on the 5/1/01. Catch was highly variable through time (Figure 7). On 124 of the 150 trapping days, no metamorphosed lampreys were caught and 1,642 individuals i.e. 83 % of the total catch was captured in two days (6^{th} and 7^{th} of February). The daily catch of metamorphosed lampreys from the RST and FCF was positively correlated with flow (Spearman rank correlations, $P < 0.01$).

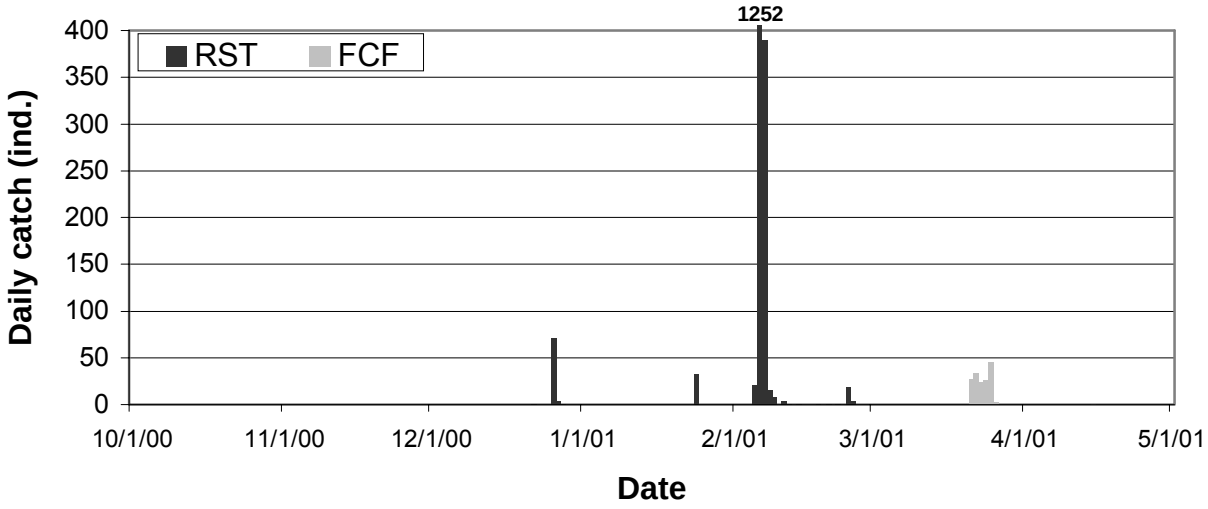

Figure 7. Daily catch of metamorphosed lampreys during the collection period 10/3/00-5/1/01.

In total 22 fin clipped metamorphosed lampreys were recaptured (Table 2) resulting in a pooled trapping efficiency estimate of 0.064. Based on the trapping efficiency estimate, 28,547 ± 13,925 (95 % CI) metamorphosed lampreys migrated out of the Umatilla River from 10/03/2000 - 3/8/2001.

Table 2. The number of fin clipped and recaptured larval and metamorphosed lampreys.

	Number of metamorphosed lampreys		Number of larval lampreys	
Date	released	recaptured	released	recaptured
12/27/00	37	0	108	0
12/31/00	0	1	0	0
1/25/01	28	0	47	0
1/26/01	0	0	0	1
2/6/01	21	0	44	0
2/7/01	75	6	48	0
2/8/01	130	2	95	0
2/9/01	15	5	0	0
2/11/01	0	7	0	0
2/12/01	11	0	1	0
2/16/01	2	0	0	0
2/21/01	0	1	0	0
2/25/01	20	0	47	1
2/27/01	4	0	21	0
2/28/01	0	0	0	1
Total	343	22	411	3

The mean length of metamorphosed lampreys was 153 mm and ranged between 124 and 186 mm (Figure 8). The length distributions of metamorphosed lampreys caught by rotary screw trap and fish collection facility were almost identical.

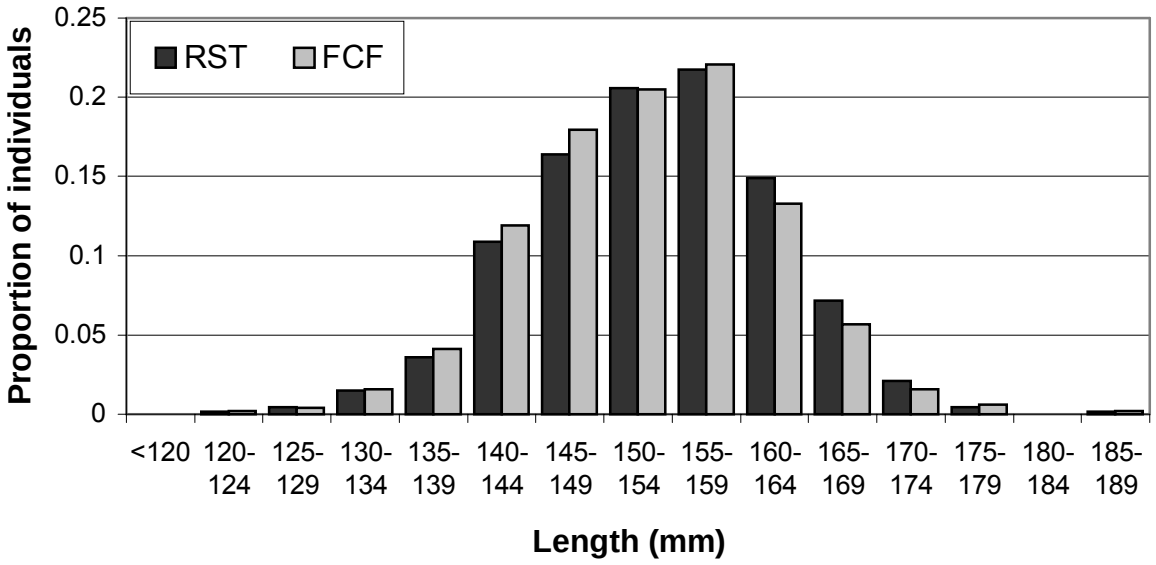

Figure 8. Length distributions of metamorphosed lampreys collected by rotary screw trap (RST) and fish collection facility (FCF) in the Umatilla River.

In total, 755 larval lampreys were caught during the study period (Figure 9). Also daily numbers of captured larvae were positively correlated with flow (Spearman rank correlations, $P < 0.01$). Only three fin clipped larvae were recaptured (Table 2) resulting in a pooled trapping efficiency estimate of 0.007. The mean length of captured larvae was 158 mm and ranged from 61 mm to 188 mm. Ninety nine percent of captured larvae were over 129 mm (Figure 10).

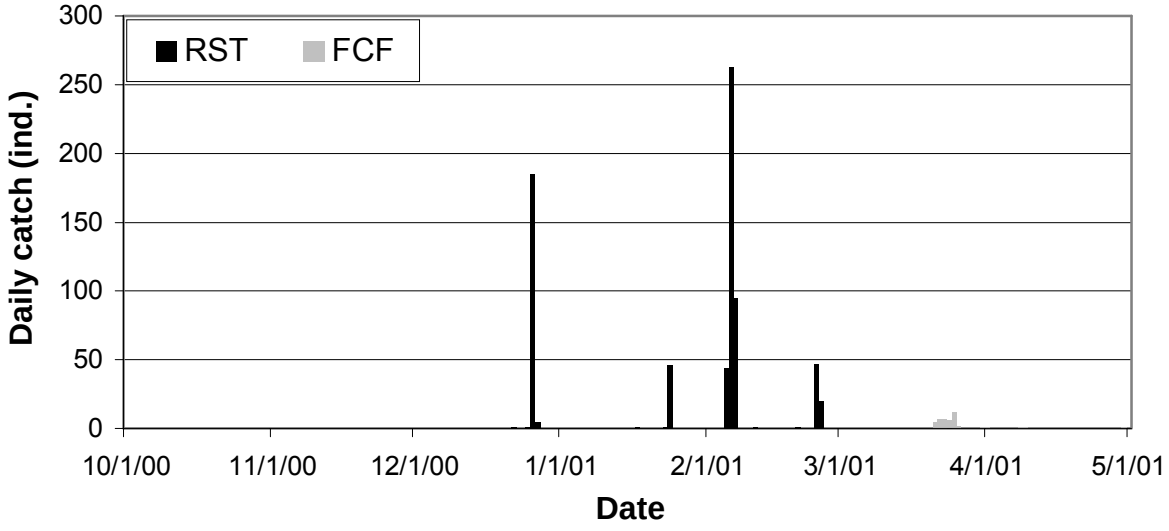

Figure 9. Daily catch of larval lampreys during the collection period 10/3/00-5/1/01.

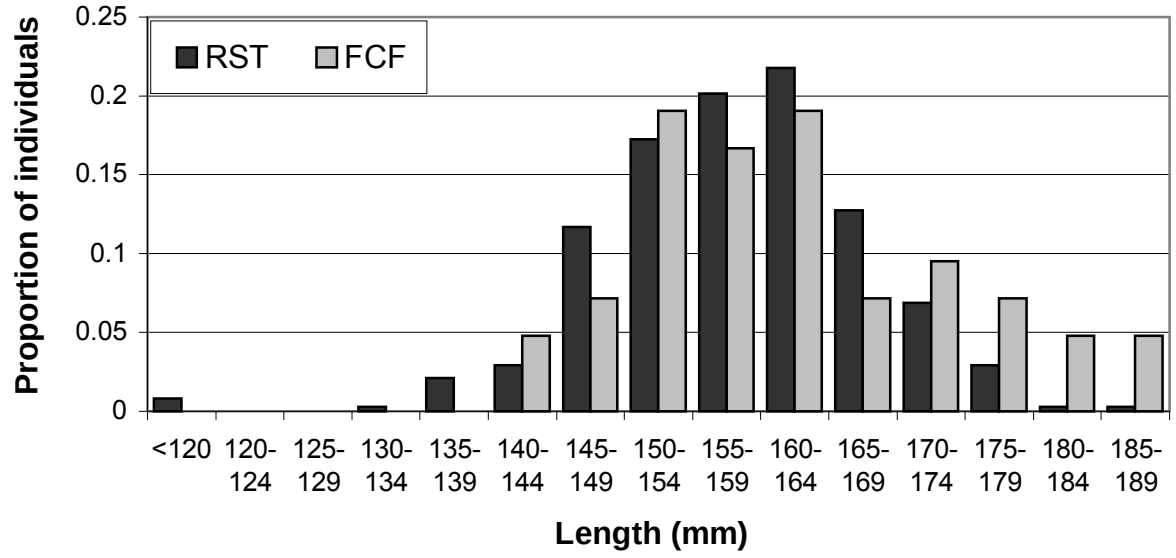

Figure 10. Length distributions of larval lampreys collected by rotary screw trap and fish collection facility in the Umatilla River.

Conclusions

Pacific lamprey begins metamorphosis in the summer and completes metamorphosis by the fall. During the winter and spring, metamorphosed lampreys are migrating to the ocean. In earlier studies there has been variation in seasonal timing of outmigration of Pacific lamprey (Richards 1980, Beamish and Levings 1991, van de Wetering 1998). During the collection

period 2000-2001, metamorphosed lampreys in the Umatilla River were captured between late December and May and migratory activity was closely associated with increasing flows as has been shown in other studies (Beamish and Levings 1991; van de Wetering 1998; Close et al. 2002).

During 2000-2001, the number of captured outmigrating lampreys was much higher than in 1997-2000. Catch of larval and metamorphosed lampreys were 3 times and 20 times higher than the average catch in 1997-2000 (Knapp et al. 2000; Ehlers et al. 2001; Close et al. 2002). We suggest that a warm spring temperature was one the of the reasons for the high numbers of captured metamorphosed lampreys, but it is likely that higher trapping efficiency and total outmigrant production of the river affected the high number of outmigrants in the 2000-2001 trapping season.

Spring time (June) river temperatures influence the number of metamorphosed lampreys. More animals will metamorphose when spring temperatures are warm compared to cool temperatures (Youson et al. 1993; Holmes et al. 1994; Holmes and Youson 1994). In June 2000 the daily mean water temperature in the lower reach of the Umatilla River was over one Celsius degree higher than in 1999 or 2001, which may partially explain the high number of metamorphosed lampreys. In 1997-2000 the ratio of larval and metamorphosed lampreys have been approximately 3:1 (Knapp et al. 2000; Ehlers et al. 2001; Close et al. 2002). In 2000-2001, the ratio of catch was opposite (1:3), which lends support to the idea that a bigger than usual portion of large larvae have metamorphosed during the summer of 2000. Still, the number of outmigrating larval lampreys was higher than earlier reflecting better trapping efficiency or higher numbers of outmigrants than earlier, probably both.

The efficiency estimate of the rotary screw trap for metamorphosed lampreys in the trapping season 2000-2001 was eight times higher than the trapping season 1999-2000 (Close et al. 2002). Lower peak flows during trapping season 2000-2001 explain inpart why trapping efficiency and the number of captured outmigrants was higher than 1999-2000. Even though the rotation speed of the screw-rotary trap increases with increasing current, the proportion of river flow sampled is lower during the high flows than in the moderate or low flows (Knapp et al. 1997). In addition, the amount of debris is lower during low and moderate flows and does not hamper trapping compared to high flows.

Flows alone may not explain eight fold differences between trapping efficiency estimates. In 2000 lampreys were released approximately 3 km above the trap just below the Three Mile Falls Dam, while in 2001, the releasing site was the channel just above the trap. It is probable that in 2001 released lampreys didn't mix in the whole water column but remained in the channel leading to high trap efficiency estimates i.e. too low population estimates.

The trapping efficiency of FCF for metamorphosed lampreys is unknown, but for salmonid-smolts it is approximately 10 times higher than the efficiency of the RST (Knapp *et al.* 1998). When the FCF-catch of metamorphosed lampreys was only 8 % of the total catch and size selectivity of FCF is similar to RST, we speculate that over 95 % of the metamorphosed lampreys migrated during the RST-trapping.

The mean length of captured metamorphosed lampreys in the Umatilla River (153 mm) was much higher than observed in British Columbia rivers and Oregon coastal streams (Beamish 1980, Beamish and Levings 1991, van de Wetering 1998). Either lampreys metamorphose at a larger size in the Umatilla River or the traps used are size selective, which may lead to overestimation of mean length and underestimation of the number of outmigrating lampreys. Length distribution of captured larval lamprey shows that fish under 130 mm are not captured. Even though mainly large larvae are migrating out of river, usually smaller ones are migrating too. In the Fraser River, approximately 9 % of outmigrating larvae were under 100 mm (Beamish and Levings 1991) while in the Umatilla River, the proportion of larvae under 130 mm was 0.7 %.

Due to the factors mentioned above, we suggest that the total amount of outmigrating metamorphosed lampreys in 2000-2001 was higher than the calculated population estimate. Even though larval densities in the index sites have been at very low levels, still the Umatilla River produced several tens of thousands of metamorphosed lampreys in the summer of 2000. To get a more accurate estimate of the total number of outmigrants, studies should be aimed at size selectivity, and trapping efficiency of the RST and FCF. Outmigration is correlated mainly to rising or high flows (Beamish and Lewings 1991, Close 2002, this study) and it occurs mostly at night (Beamish and Levings 1991, van de Wetering 1998). During high flows, the RST should be emptied and cleaned every two-three hours especially at night to prevent debris clogging the trap and effecting trapping efficiency.

Because of the burrowing habit of larval lampreys it is difficult to get reliable trapping efficiencies. It's almost impossible to estimate the proportion of larvae that will continue downstream migration after tagging and releasing. However, high numbers of captured larvae compared to the number of metamorphosed lampreys shows that a remarkable proportion of larvae hatched in the Umatilla River leave the river and metamorphosis may take place in the mainstem Columbia River.

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 2001, the number of upmigrating adult lampreys to the Umatilla River was not determined, because the fish trapping facility at Three Mile Falls Dam was not functional due to damages caused by high flows with debris.

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 in press). Number of daily counts of Pacific lamprey at the John Day dam 5/1/01-10/31/01 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.

Results

In 2001 a total of 4,005 lampreys were counted at the John Day Dam. The peak migration occurred in July and August which accounted for 72 % of all lampreys passing the dam (Figure 11). Approximately 50 % of lampreys were counted from 07/11-08/15, when discharge in the Umatilla River was very low (range 0.08 - 0.51 m^3 s^{-1}).

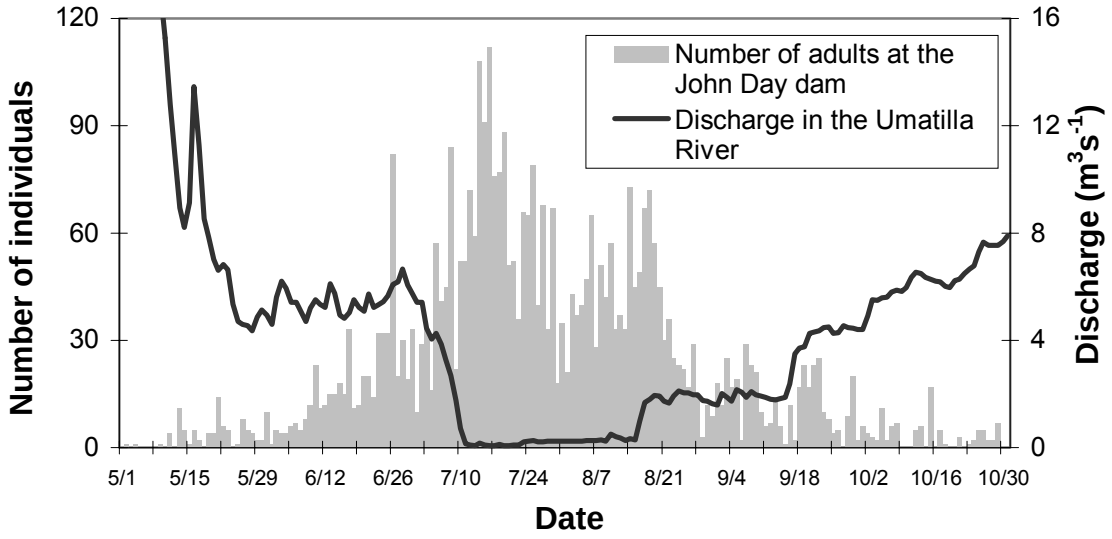

Figure 11. Daily counts of lampreys at the John Day Dam and discharge at rkm 4.5 in the Umatilla River.

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 quite 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. However, almost 50 % of lampreys passed the Umatilla River, when discharge in the Umatilla River is higher than it has been historically (Close 1999). Because the number of migrating adult lampreys in the Columbia River is currently very 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.

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CHAPTER TWO

Olfactory Sensitivity of Pacific Lampreys to Petromyzonal Sulfate

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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, collaborating with the Columbia River Inter-Tribal Fish Commission (CRITFC) to examine trends in lamprey passage at hydroelectric facilities in the CRB, and working with researchers at Oregon State University (OSU), University of Minnesota (UMN), 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 (Close 1999).

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 PS (Table 1), excreted through the gills of spermiating male sea lampreys (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. A preliminary study by Sorensen and Close (2000) indicated that larval Pacific lampreys produce PS and that the olfactory organ of upstream migrating adults responds to stimulation by PS when measured by EOG. Based on these results, CTUIR decided to expand the investigation of migratory pheromones in Pacific lampreys and contracted the USGS to build and test an EOG apparatus, which was completed in 2000 (Seelye and Bayer 2001). In 2001, we began to test the olfactory sensitivity of Pacific lampreys to PS, ACA, and 3-keto PS. 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). The extended residence of adult Pacific lampreys in fresh water during upstream migration through spawning suggests that they may exhibit a longer period of sensitivity to larval and adult bile acids than sea lampreys. Thus, we decided to use EOG to track the olfactory sensitivity of adult Pacific lampreys from their capture in the CRB in June

through spawning a year later. This report examines data collected from June through December 2001, and compares these data to studies of sea lamprey olfaction, which is a well-understood model of pheromones as migratory and spawning cues in lampreys.

METHODS

Study Animals

In June 2001, upstream migrating adult Pacific lampreys were collected from fish ladders at Willamette Falls on the Willamette River in Oregon and Bonneville Dam on the Columbia River in Washington. 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 river water from the Little White Salmon River. Water temperature was 15°C from June through October 2001, and near ambient Columbia River values, as measured at Bonneville Dam, during November and December 2001 (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), respectively. 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 from the same sand-filtered, river water source 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 (tip diameter = 347.5 μm ± 28.0 μm (S.D.), 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 (Table 2) and a bile acid, taurolithocholic acid 3-sulfate (TLCS, Table 1). An odorant delivery device similar to one used by Li et al. (1995) was used to minimize temperature and flow differences between blank perfusion water and test solutions, and to provide 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 only used for one experiment. Lampreys that showed no response to the standard were not further tested 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 employed to allow the olfactory receptors to recover. Exposures to the L-arginine standard were performed every 30 minutes to 1 hour. A methanol blank was periodically employed 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, and sex was determined by examination of the gonad.

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), 3-keto PS (Dr. Weiming Li, MSU), and TLCS (Sigma-Aldrich, St. Louis, MO) 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 juvenile steelhead

Olfactory responses of juvenile steelhead (*Oncorhynchus mykiss*) to selected L-amino acids were periodically measured in order to confirm that the EOG apparatus was functioning properly. 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 juvenile steelhead enabled us to confirm that our equipment was producing data comparable to previous studies. Steelhead were prepared for EOG recording using the same methods described above for Pacific lampreys with minor changes to accommodate differences in physiology and morphology between the species. Juvenile steelhead were anesthetized and immobilized with intramuscular injections of metomidate hydrochloride (3 mg/kg body weight) and gallamine triethiodide (15 mg/kg body weight), respectively. The skin above the left olfactory organ was removed, exposing the olfactory lamellae. 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 one lamella. Placement of the recording electrode was optimized by changing the position of the electrode until a standard 10^{-4} M L-serine solution produced an acceptable response and baseline noise was minimized. L-serine was chosen as a standard because of its olfactory potency in other teleost species (Silver 1982, Bjerselius and Olsen 1993, Sorensen et al. 1995). Stock solutions of L-serine, L-leucine, L-histidine, L-glutamine, and L-arginine (10^{-2} M, Sigma-Aldrich, St. Louis, MO) were prepared in deionized water and stored at 4°C for a maximum of two weeks. 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. At the conclusion of each experiment, length and weight of juvenile steelhead were measured.

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

Olfactory responses of captive adult Pacific lampreys were measured at intervals from June 2001 (the time of capture during upstream migration) through June 2002 (the time of natural spawning). From June 2001 through September 2001, we attempted to record EOG responses from lampreys for five consecutive days each week, grouping data from two consecutive weeks for analysis. From October 2001 through June 2002, we attempted to record EOG responses from lampreys for five consecutive days every fourth week. Due to technical difficulties, we sometimes deviated from this schedule.

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

Between June 24 and December 31, 2001, 95 adult Pacific lampreys were tested on the EOG apparatus, with 81 adults tested for olfactory sensitivity to larval and adult lamprey bile acids (Table 1), and 14 adults screened for olfactory sensitivity to 20 L-amino acids (Table 2). Each test group showed similar morphometric statistics of length, weight, and girths (Table 3). Sex ratios among test groups varied, with an overall distribution of 45% females and 55% males (Table 4). The rate of successful EOG recordings varied among test groups, with 57 lampreys (60%) overall producing valid EOG recordings (Table 4). The rate of successful EOG recordings varied by sex as well, with 51% of females and 67% of males producing acceptable recordings (Table 4).

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, and ACA, as well as the recordings from exposures to the L-arginine standard immediately before and after the test odorants. 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). Based on this result, we screened 19 other L-amino acids (Table 2) as well as another bile acid, TLCS (Table 1), as possible standards. None of these compounds proved to be a suitable substitute for L-arginine (data not shown).

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. This relation held true whether expressed in response magnitudes of millivolts or as percentages of the L-arginine standard (Figures 3 and 4). The lower threshold of detectable olfactory responses varied between 10^{-9} M and 10^{-8} M for PS and between 10^{-8} 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 responses of adult Pacific lampreys (Figure 5). When tested at concentrations less than 10^{-9} M, bile acids listed in Table 1 produced no measurable EOG responses (data not shown). Thus, testing of these compounds below concentrations of 10^{-9} M was discontinued shortly after this study began. Increasing concentrations of L-arginine exposures produced a logarithmic dose-response curve (Figure 6), with a lower detection threshold of 10^{-4} M. Olfactory responses of adult Pacific lampreys to L-arginine were most consistent and reliable at this concentration.

Olfactory responses of adult Pacific lampreys to larval and adult bile acids varied over time. Olfactory responsiveness to 10^{-6} M PS varied between means of 0 to 2.83 mV (0% to 68% L-arginine standard) from June through December 2001 (Figure 7). Responses to 3-keto PS varied between means of 0.32 to 1.84 mV (14% to 60% L-arginine standard) from June through December 2001 (Figure 8). Responses to PS were generally greater than responses to 3-keto PS. When compared by sex, adult Pacific lamprey responses to 10^{-6} M PS and 3-keto PS were similar from June to December 2001 (Tables 5 and 6). Exposure to ACA produced no measurable olfactory responses during this same interval of time, except for two out of four individuals in October 2001 (Figure 9). Mean responses to 10^{-4} M L-arginine ranged from 1.07 to 5.80 mV and were centered at a median of 2.97 mV (Figure 10). This range of mean responses to 10^{-4} M L-arginine held constant from June through December 2001 (Figure 11).

EOG recordings from juvenile steelhead

Positive potential EOG recordings from adult Pacific lampreys when exposed to L-arginine and the lack of a suitably potent alternative L-amino acid for EOG measurements in this agnathan species led us to briefly explore olfactory responses of steelhead, a teleost species, to L-amino acids. In July and October 2001, 6 and 4 juvenile steelhead, respectively, were tested

for olfactory sensitivity to selected L-amino acids on the EOG apparatus (Table 7). Lengths and weights for both test groups were similar (Table 8). The rate of successful EOG recordings was greater for steelhead tested in October 2001 than those tested in July 2001 (Table 8).

EOG recordings from juvenile steelhead to selected L-amino acids confirmed that the EOG apparatus was properly functioning. Figure 12 shows EOG recordings from juvenile steelhead exposed to L-serine, L-leucine, L-glutamine, and L-arginine in July 2001. Juvenile steelhead responded to L-serine, L-leucine, and L-glutamine with classic sharp negative potential peaks that gradually returned to baseline (Figure 12 A, B, and C). However, L-arginine exposure elicited atypical positive potential EOGs in two trials; one of these data sets was rejected in the data screening process as uncontrolled (see *Data Analysis* above, data not shown) and the other was at a high concentration (10^{-3} M) in a dose-response curve that showed little concentration dependency (Figure 12 D). Subsequent exposures of juvenile steelhead to 10^{-4} M L-arginine in October 2001 elicited repeatable classic negative potential peaks (Figure 13). L-histidine exposure elicited typical negative potential EOGs in one trial with steelhead, but these data were rejected as uncontrolled (data not shown).

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, in the early 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 the sea lamprey. 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, W. Li and S. Yun, Michigan State University, personal communication). To date there are no published results of sea lamprey EOG responses to 3-keto PS. However, personal communications with Dr. Li of MSU indicate that responses to this bile acid in Pacific lampreys are also less than those recorded in sea lampreys. Additionally, at the early migratory phase of the adult life stage of Pacific lampreys, there does not appear to be a sexual dimorphism in responses to 3-keto PS as reported for spawning phase sea lampreys (Li et al. 2002). However, results from the spawning phase EOG trials of Pacific lampreys have not yet been analyzed.

Adult Pacific lampreys show an atypical response to L-arginine. The lack of response to 10^{-5} M L-arginine by adult Pacific lampreys and their positive potential responses to higher concentrations (10^{-4} and 10^{-3} M) of this L-amino acid are unlike results seen in studies of sea lampreys (Li et al. 1995, Li and Sorensen 1997). However, in 2002 we have recorded positive potential EOGs from sea lampreys on our EOG apparatus (data not shown) and have hypothesized that these results may reflect an unknown interaction between our water supply and the olfactory system of agnathan species. Positive potential EOGs were seen in two trials with juvenile steelhead (*Oncorhynchus mykiss*), but the data were questionable due to poor control responses and a lack of dose dependence. Subsequent trials have confirmed negative potential responses to L-arginine and other L-amino acids in steelhead. Despite the novelty of Pacific lamprey responses to L-arginine, we have concluded that it is still a viable standard for use in this study 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, respectively. 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 the current EOG experiments are 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 through December 2001 and continued to be measured through the winter and spring of 2002 (data not shown). 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.

To summarize findings from 2001, 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 to a reduced sensitivity to larval and adult lamprey bile acids, the limits of detectability for these compounds 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. These preliminary results from 2001 indicate that, similar to sea lampreys, larval and adult lamprey bile acids have the potential to

act as pheromones for migration and spawning of adult Pacific lampreys. Further data collection is needed, and thus, EOG studies of adult Pacific lamprey olfaction will continue through 2002 and into 2003. In 2002, we have begun to design and test experimental systems for behavioral studies of Pacific lamprey olfaction to begin in 2003.

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TABLES

Table 1. Bile acids tested by electro-olfactogram (EOG) in 2001 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
Taurolithocholic acid 3-sulfate (TLCS)	3α-Hydroxy-5β-cholan-24-oic-acid N-(2-sulfoethyl)-amide 3-sulfate

Table 2. Survey of L-amino acids tested by electro-olfactogram (EOG) in 2001 for olfactory responsiveness of adult Pacific lampreys.

Compound	*Abbreviation*	*Concentration*	*Number of trials*
L-Alanine	Ala	10^{-4} M	2
L-Arginine	Arg	10^{-9} to 10^{-3} M	6
L-Asparagine	Asn	10^{-4} M	3
L-Aspartic Acid	Asp	10^{-4} M	4
L-Cysteine	Cys	10^{-4} M	3
L-Glutamic Acid	Glu	10^{-4} M	3
L-Glutamine	Gln	10^{-4} M	3
L-Glycine	Gly	10^{-4} M	3
L-Histidine	His	10^{-4} M	4
trans-4-Hydroxy-L-Proline	trans-4-Hydroxy-L-Pro	10^{-4} M	3
L-Isoleucine	Ile	10^{-4} M	2
L-Leucine	Leu	10^{-4} M	3
L-Lysine	Lys	10^{-9} to 10^{-4} M	5
L-Methionine	Met	10^{-4} M	3
L-Phenylalanine	Phe	10^{-4} M	3
L-Proline	Pro	10^{-4} M	3
L-Serine	Ser	10^{-4} M	3
L-Threonine	Thr	10^{-4} M	3
L-Tryptophan	Trp	10^{-4} M	3
L-Valine	Val	10^{-4} M	3

Table 3. Morphometric data from adult Pacific lampreys tested by electro-olfactogram (EOG) in 2001. Data arranged by test interval.

Dates	n	Length ± S.D. (mm)	Weight ± S.D. (g)	Anterior girth ± S.D. (mm)	Mid girth ± S.D. (mm)	Posterior girth ± S.D. (mm)
Bile acid testing:						
6/24 to 7/7/2001	7	642.9 ± 56.8	382.99 ± 100.56	107.3 ± 11.3	103.6 ± 10.8	83.4 ± 6.9
7/8 to 7/21/2001	14	664.4 ± 53.8	444.39 ± 98.74	116.9 ± 9.7	112.1 ± 8.5	89.4 ± 6.4
7/22 to 8/4/2001	7	669.7 ± 58.3	459.84 ± 121.55	117.3 ± 14.5	113.1 ± 13.5	91.7 ± 9.6
8/19 to 9/1/2001	10	656.1 ± 26.9	450.52 ± 56.22	117.8 ± 8.2	112.5 ± 6.6	89.4 ± 5.5
9/2 to 9/15/2001	10	667.0 ± 33.4	471.78 ± 72.88	118.0 ± 6.9	113.0 ± 7.4	90.2 ± 5.9
9/16 to 9/29/2001	17	665.5 ± 32.9	472.31 ± 69.03	118.1 ± 7.1	114.2 ± 7.2	91.2 ± 5.3
10/28 to 11/3/2001	5	648.0 ± 29.4	408.92 ± 60.53	109.0 ± 6.6	106.2 ± 7.2	85.6 ± 5.7
11/25 to 12/1/2001	4	625.5 ± 19.4	366.75 ± 52.91	103.5 ± 6.6	100.5 ± 5.4	82.8 ± 5.5
12/16 to 12/22/2001	7	655.9 ± 40.4	393.09 ± 57.03	104.7 ± 6.6	103.7 ± 7.0	84.7 ± 5.3
Sub-total:	81					
L-amino acid screening:						
7/31 to 8/16/2001	14	660.1 ± 42.6	476.17 ± 62.56	120.8 ± 6.4	114.4 ± 6.7	91.5 ± 3.2
Total:	95					

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

Dates	n	Successful EOG recording (% n)	Female (% n)	Successful EOG Female (% female)	Male (% n)	Successful EOG Male (% male)
Bile acid testing:						
6/24 to 7/7/2001	7	3 (43%)	4 (57%)	1 (25%)	3 (43%)	2 (67%)
7/8 to 7/21/2001	14	3 (21%)	7 (50%)	0 (0%)	7 (50%)	3 (43%)
7/22 to 8/4/2001	7	4 (57%)	2 (29%)	2 (100%)	5 (71%)	2 (40%)
8/19 to 9/1/2001	10	8 (80%)	2 (20%)	2 (100%)	8 (80%)	6 (75%)
9/2 to 9/15/2001	10	5 (50%)	5 (50%)	2 (40%)	5 (50%)	3 (60%)
9/16 to 9/29/2001	17	14 (82%)	11 (65%)	9 (82%)	6 (35%)	5 (83%)
10/28 to 11/3/2001	5	5 (100%)	2 (40%)	2 (100%)	3 (60%)	3 (100%)
11/25 to 12/1/2001	4	4 (100%)	0 (0%)	0 (--)	4 (100%)	4 (100%)
12/16 to 12/22/2001	7	5 (71%)	4 (57%)	2 (50%)	3 (43%)	3 (100%)
Sub-total:	81	51 (63%)	37 (46%)	20 (54%)	44 (54%)	31 (70%)
L-amino acid screening:						
7/31 to 8/16/2001	14	6 (43%)	6 (43%)	2 (33%)	8 (57%)	4 (50%)
Total:	95	57 (60%)	43 (45%)	22 (51%)	52 (55%)	35 (67%)

Table 5. Olfactory responses of adult Pacific lampreys to petromyzonol sulfate (PS) and 3-keto petromyzonol sulfate (3-keto PS) by sex from June to December 2001. Response magnitudes are in millivolts (mV).

	10^{-6} M PS				**10^{-6} M 3-keto PS**			
Sex	**n**	**Mean Response ± S.D. (mV)**	**Min.**	**Max.**	**n**	**Mean Response ± S.D. (mV)**	**Min.**	**Max.**
Female	17	1.76 ± 0.95	0.72	3.77	10	0.85 ± 0.75	0	2.40
Male	20	1.27 ± 0.87	0	3.16	14	0.86 ± 0.53	0	2.02
All	37	1.50 ± 0.94	0	3.77	24	0.85 ± 0.61	0	2.40

Table 6. Olfactory responses of adult Pacific lampreys to petromyzonol sulfate (PS) and 3-keto petromyzonol sulfate (3-keto PS) by sex from June to December 2001. Responses expressed as percent 10^{-4} M L-arginine standard response (% L-arg std).

	10^{-6} M PS				**10^{-6} M 3-keto PS**			
Sex	**n**	**Mean Response ± S.D. (% L-arg std)**	**Min.**	**Max.**	**n**	**Mean Response ± S.D. (% L-arg std)**	**Min.**	**Max.**
Female	17	51.9 ± 22.8	24.2	95.1	10	27.4 ± 27.6	0	82.2
Male	20	37.9 ± 26.1	0	98.9	14	30.5 ± 15.9	0	59.9
All	37	44.3 ± 25.3	0	98.9	24	29.2 ± 21.0	0	82.2

Table 7. L-amino acids tested by electro-olfactogram (EOG) in 2001 for olfactory responsiveness of juvenile steelhead.

Compound	*Abbreviation*	*Concentration*	*Number of trials*
L-Arginine Standard	Arg	10^{-4} M	4
L-Arginine	Arg	10^{-7} to 10^{-3} M	3
L-Glutamine Standard	Gln	10^{-4} M	4
L-Glutamine	Gln	10^{-7} to 10^{-3} M	2
L-Histidine	His	10^{-7} to 10^{-3} M	1
L-Leucine	Leu	10^{-7} to 10^{-3} M	1
L-Serine Standard	Ser	10^{-4} M	4
L-Serine	Ser	10^{-7} to 10^{-3} M	2

Table 8. Morphometric data and electro-olfactogram (EOG) recording success rates from juvenile steelhead tested by EOG in 2001. Data arranged by test interval.

Dates	**n**	**Length ± S.D. (mm)**	**Weight ± S.D. (g)**	**Successful EOG recording (% n)**
July 2001	6	211.3 ± 13.5	94.93 ± 24.18	2 (33%)
October 2001	4	228.8 ± 9.4	120.50 ± 24.31	3 (75%)
Total:	10			5 (50%)

FIGURES

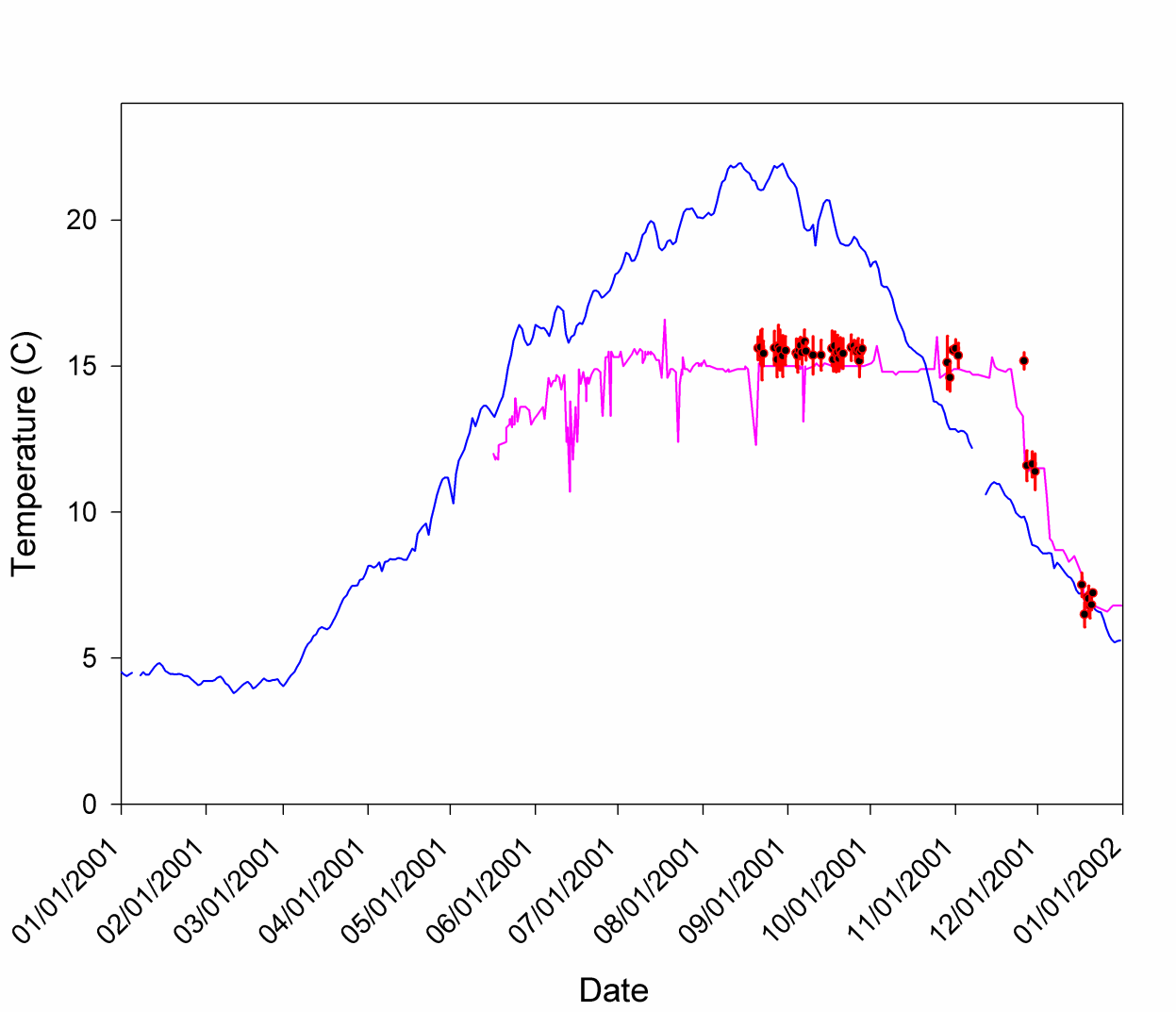

Figure 1. 2001 daily temperatures of the Columbia River at Bonneville Dam (RKM 234, blue line), lamprey holding tank at the Columbia River Research Lab (pink line), and electro-olfactogram (EOG) apparatus (black dot, red error bars). Columbia River temperature data are from DART (http://www.cqs.washington.edu/dart/dart.html). Holding tank temperature data are from daily single point readings. EOG temperature data are mean ± 2 S.D. of temperature during each experiment (monitoring by data logger begun 8/21/2001).

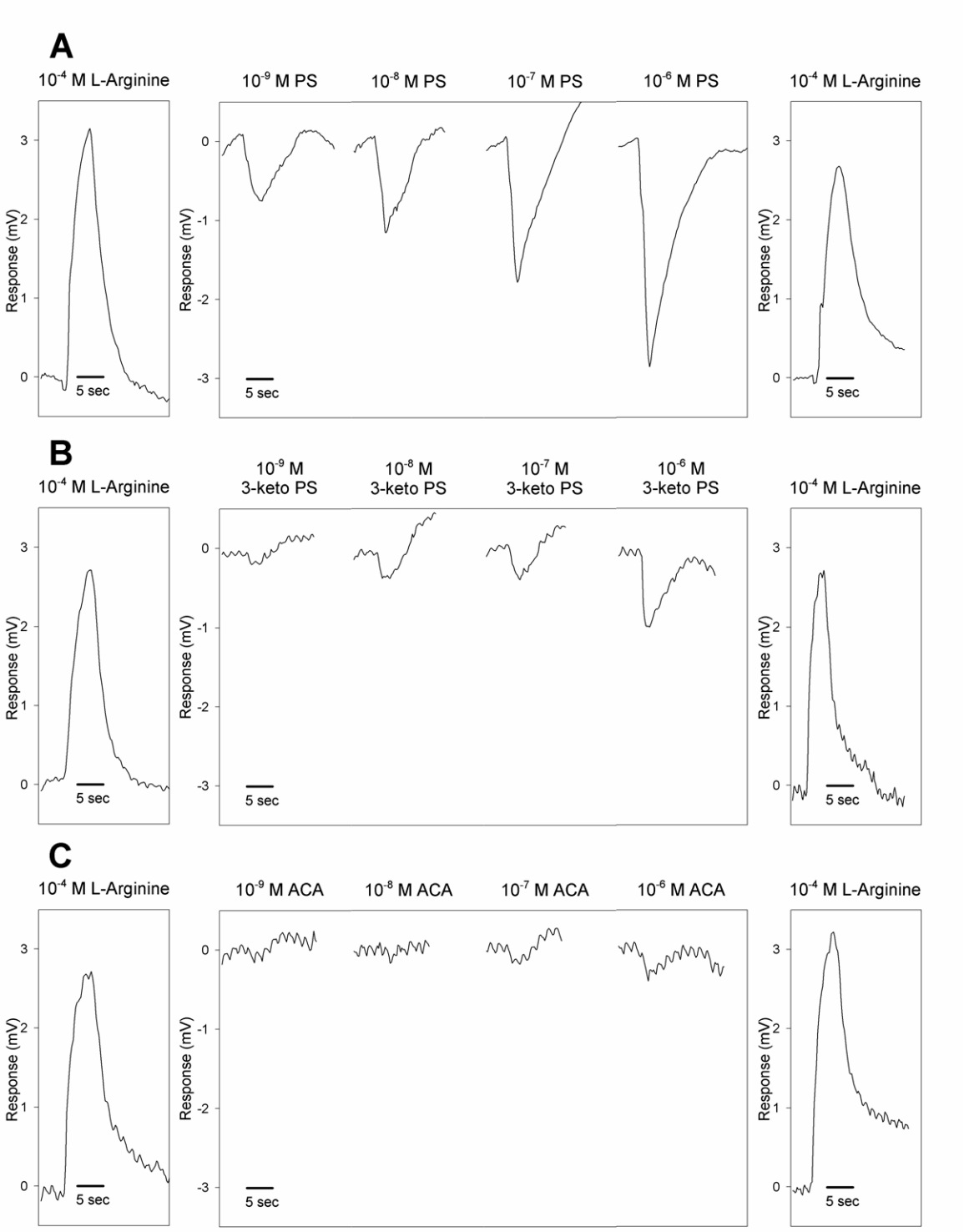

Figure 2. Electro-olfactogram (EOG) recordings from olfactory responses of adult Pacific lampreys to bile acids and 10^{-4} M L-arginine standard in 2001. A) petromyzonol sulfate (PS) in September 2001, B) 3-keto petromyzonol sulfate (3-keto PS) in October 2001, and C) allocholic acid (ACA) in October 2001.

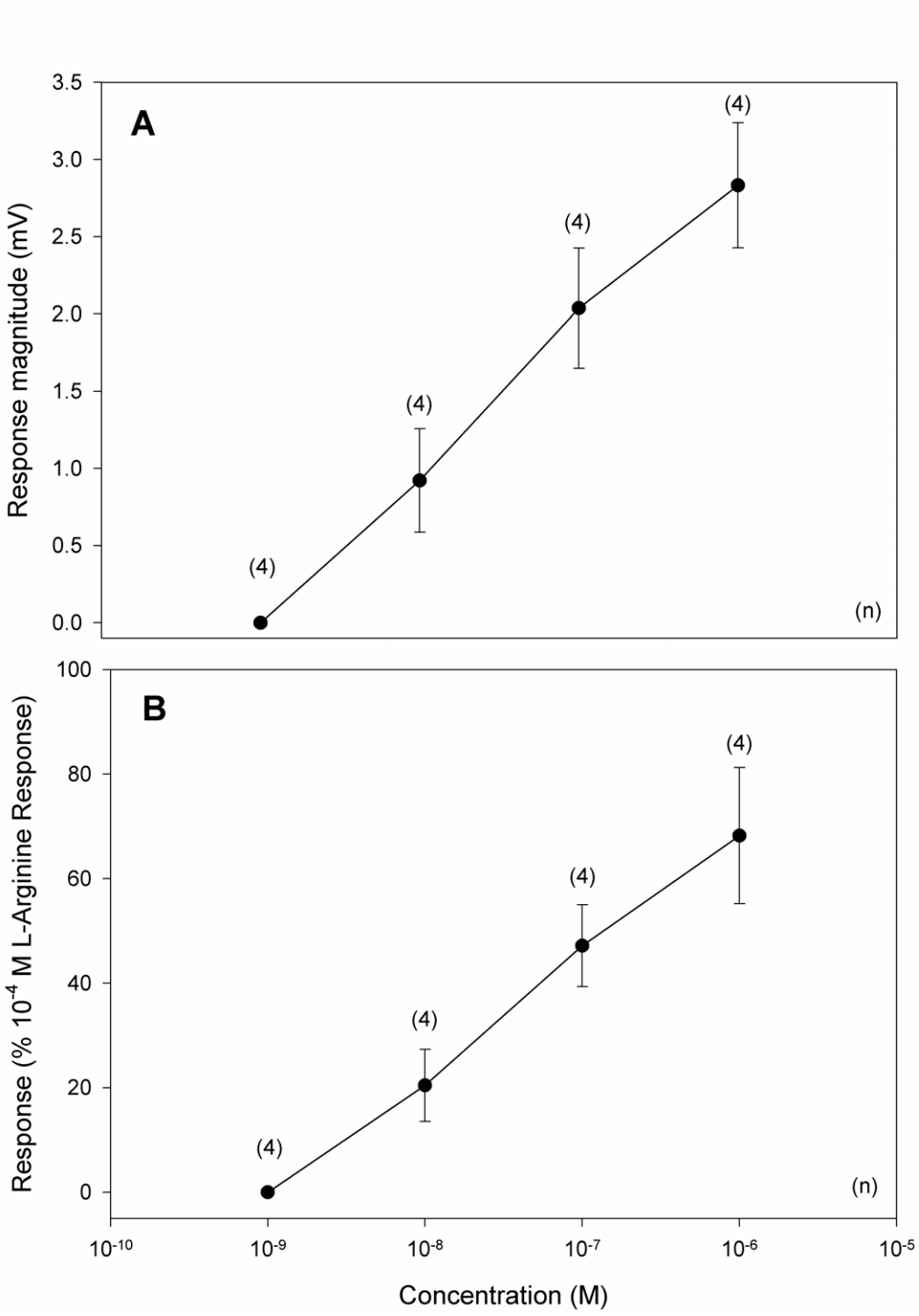

Figure 3. Representative graph of relation between the molar concentration (M) of petromyzonol sulfate (PS) and olfactory responses of adult Pacific lampreys in December 2001. A) Response magnitudes in millivolts (mV), and B) Responses expressed as percent 10^{-4} M L-arginine standard response. Numbers above points are the sample sizes for each point. Error bars are ±1 S.E.

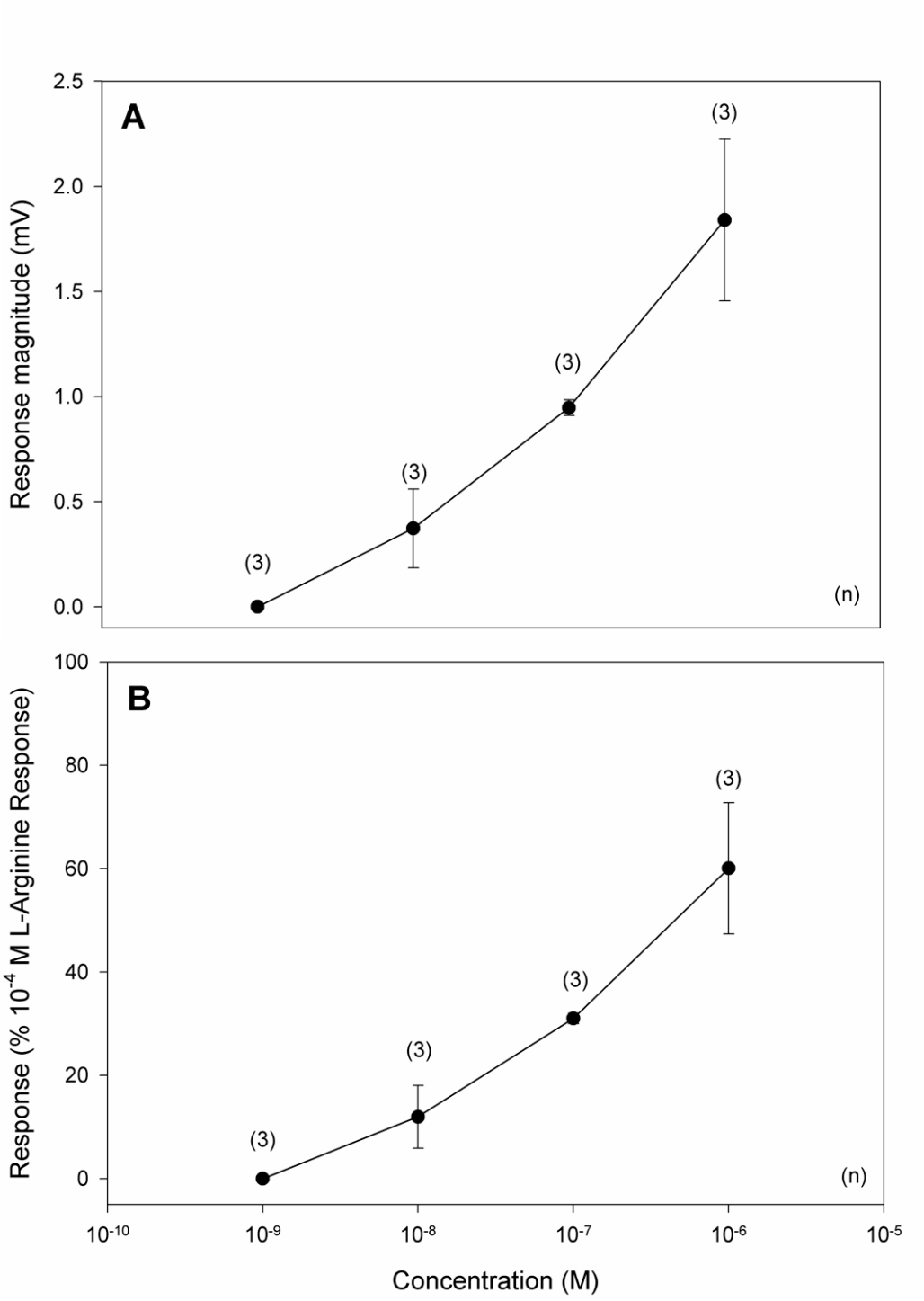

Figure 4. Representative graph of relation between the molar concentration (M) of 3-keto petromyzonol sulfate (3-keto PS) and olfactory responses of adult Pacific lampreys in December 2001. A) Response magnitudes in millivolts (mV), and B) Responses expressed as percent 10^{-4} M L-arginine standard response. Numbers above points are the sample sizes for each point. Error bars are ±1 S.E.

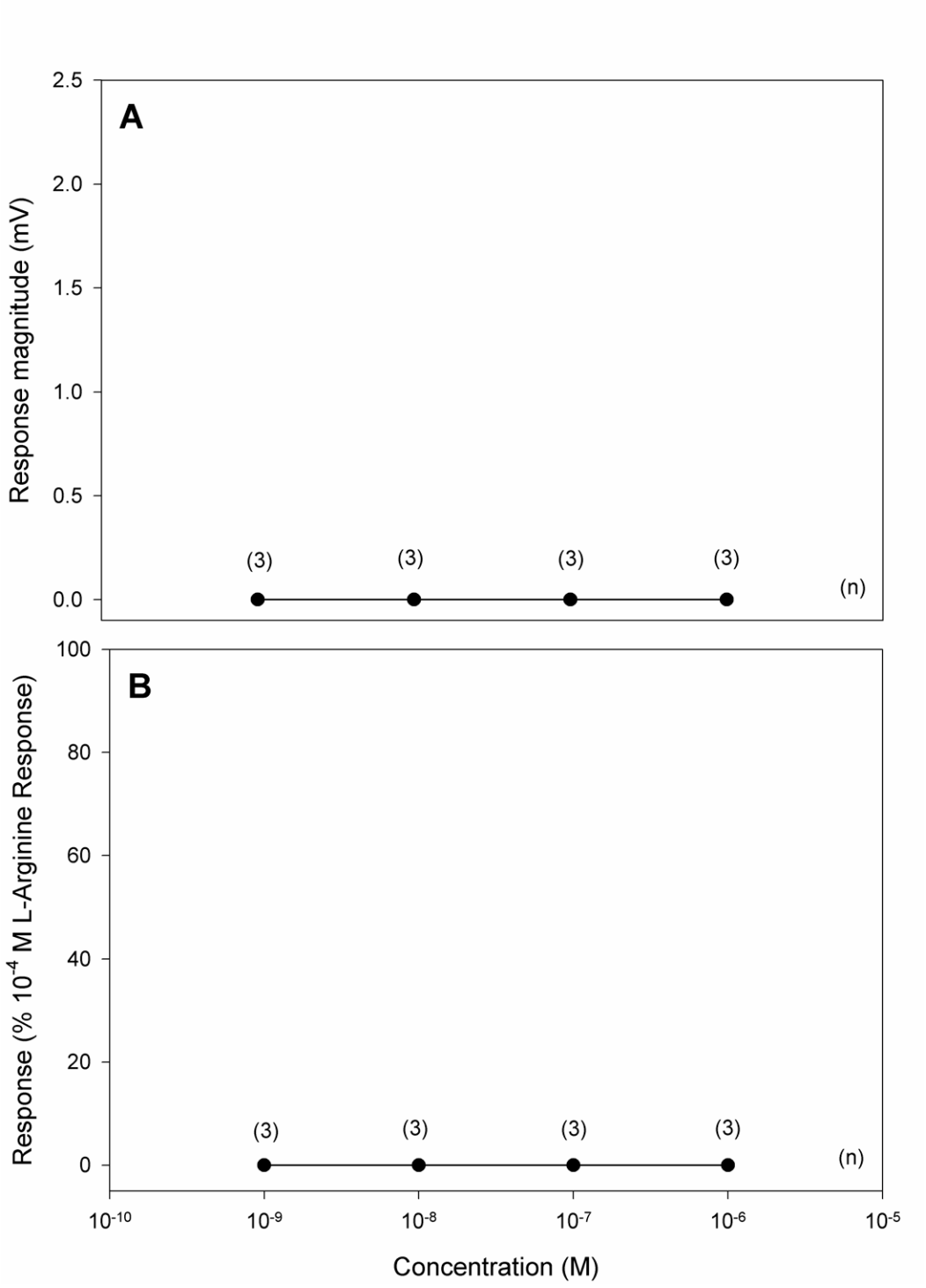

Figure 5. Representative graph of relation between the molar concentration (M) of allocholic acid (ACA) and olfactory responses of adult Pacific lampreys in December 2001. A) Response magnitudes in millivolts (mV), and B) Responses expressed as percent 10^{-4} M L-arginine standard response. Numbers above points are the sample sizes for each point. Error bars are ±1 S.E.

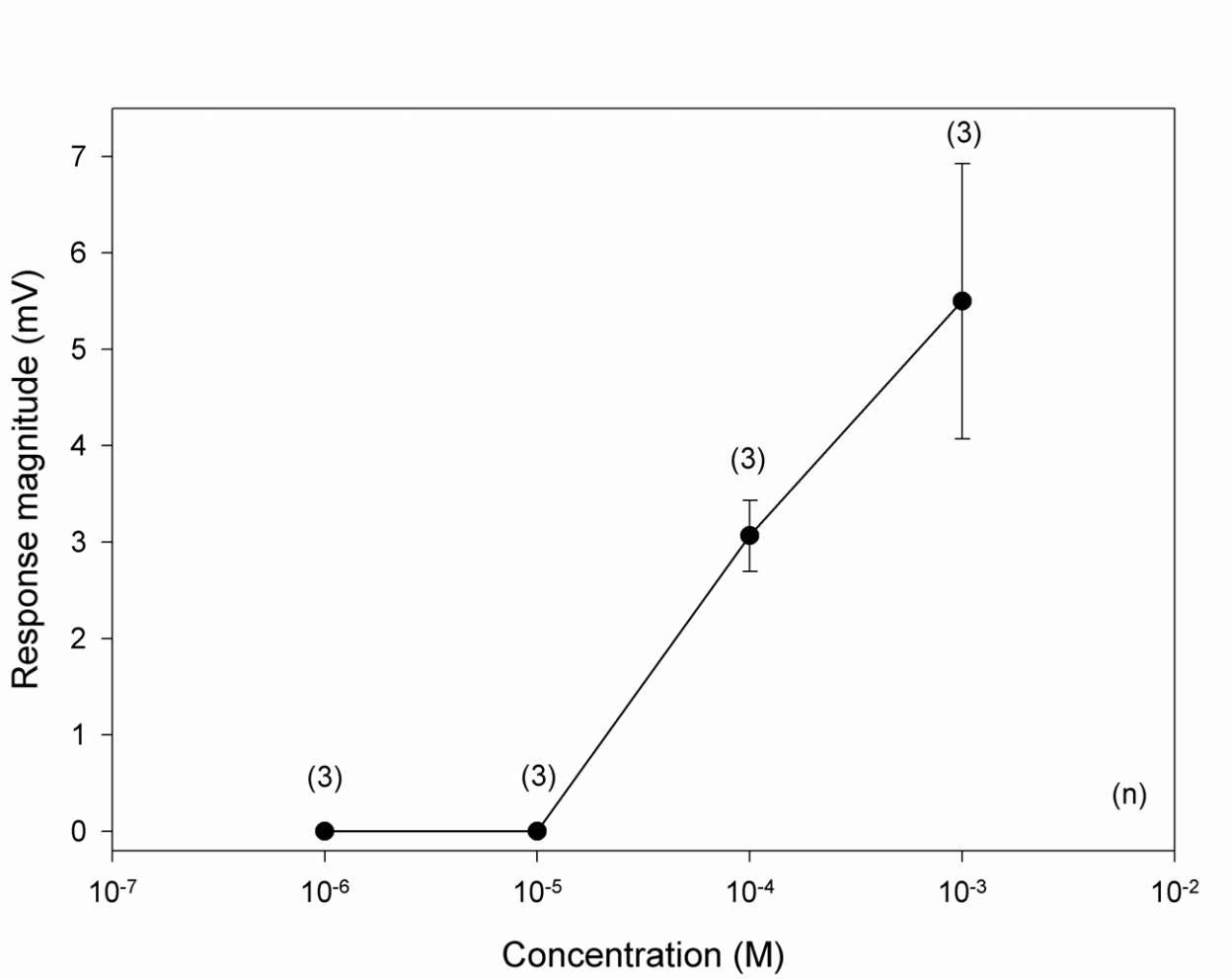

Figure 6. Representative graph of relation between the molar concentration (M) of L-arginine and olfactory responses of adult Pacific lampreys in September 2001. Response magnitudes in millivolts (mV). Numbers above points are the sample sizes for each point. Error bars are ±1 S.E.

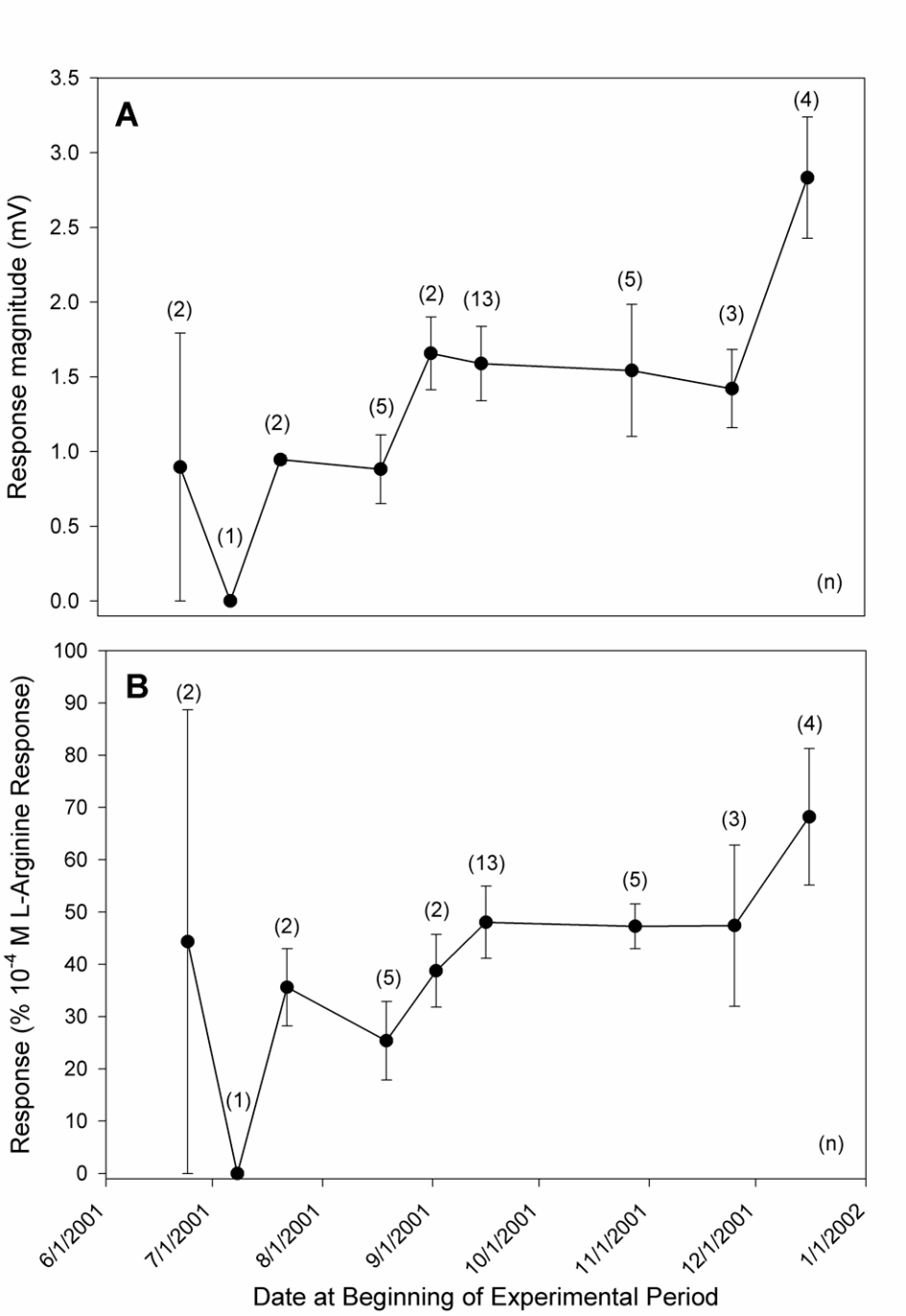

Figure 7. Olfactory responses of adult Pacific lampreys to 10^{-6} M petromyzonol sulfate (PS) from June to December 2001. A) Response magnitudes in millivolts (mV), and B) Responses expressed as percent 10^{-4} M L-arginine standard response. Numbers above points are the sample sizes for each point. Error bars are ±1 S.E.

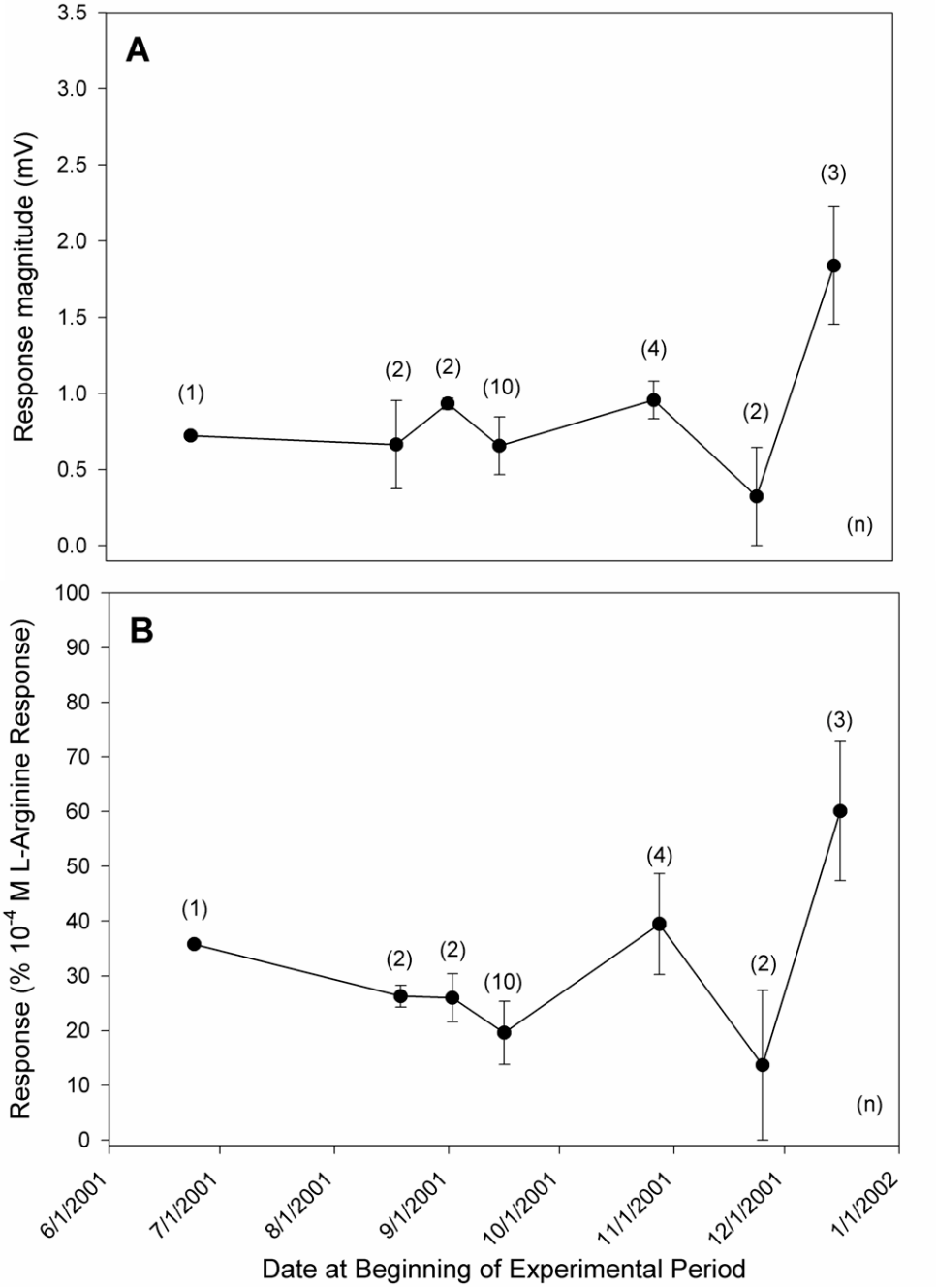

Figure 8. Olfactory responses of adult Pacific lampreys to 10^{-6} M 3-keto petromyzonol sulfate (3-keto PS) from June to December 2001. A) Response magnitudes in millivolts (mV), and B) Responses expressed as percent 10^{-4} M L-arginine standard response. Numbers above points are the sample sizes for each point. Error bars are ±1 S.E.

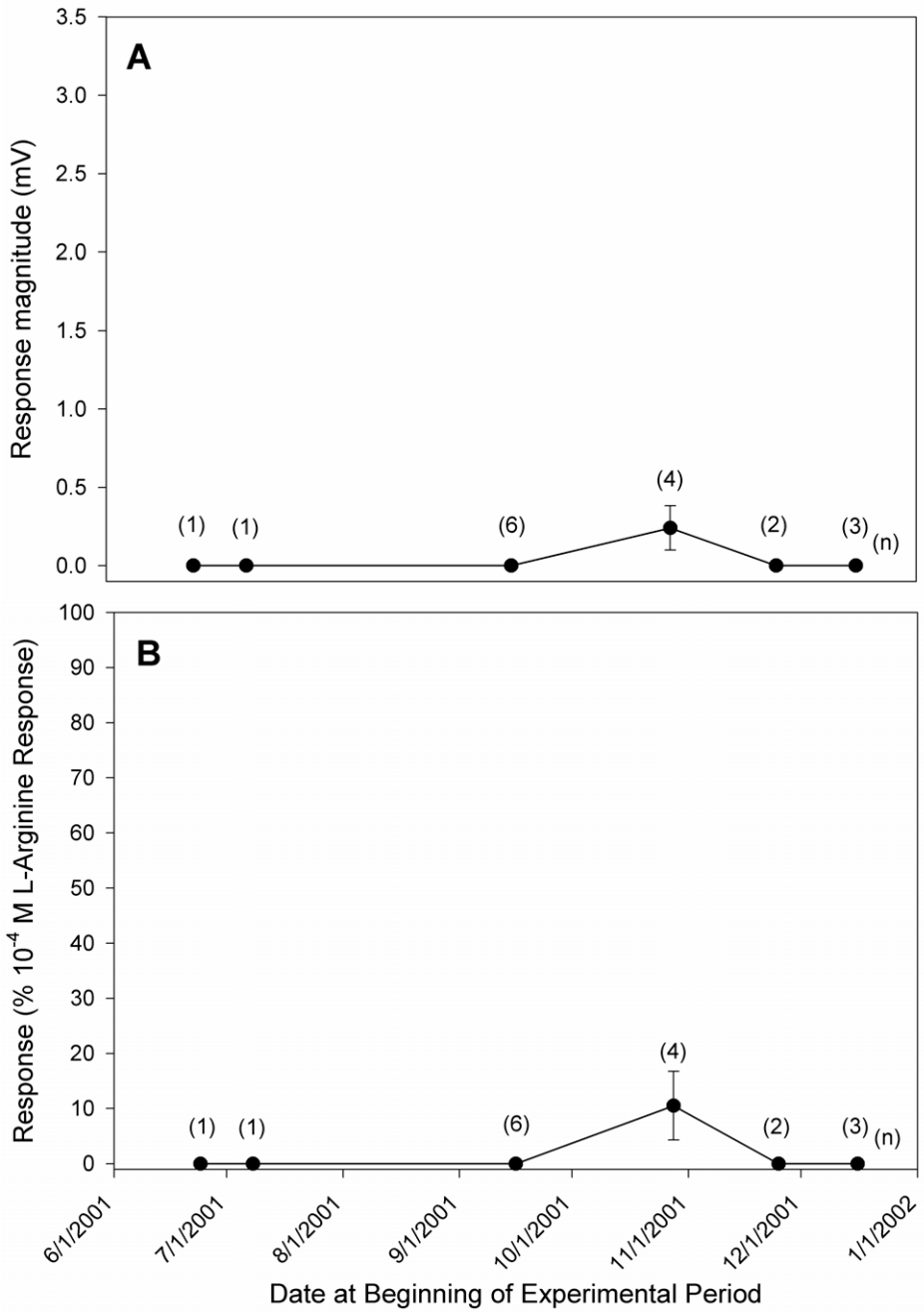

Figure 9. Olfactory responses of adult Pacific lampreys to 10^{-6} M allocholic acid (ACA) from June to December 2001. A) Response magnitudes in millivolts (mV), and B) Responses expressed as percent 10^{-4} M L-arginine standard response. Numbers above points are the sample sizes for each point. Error bars are ±1 S.E.

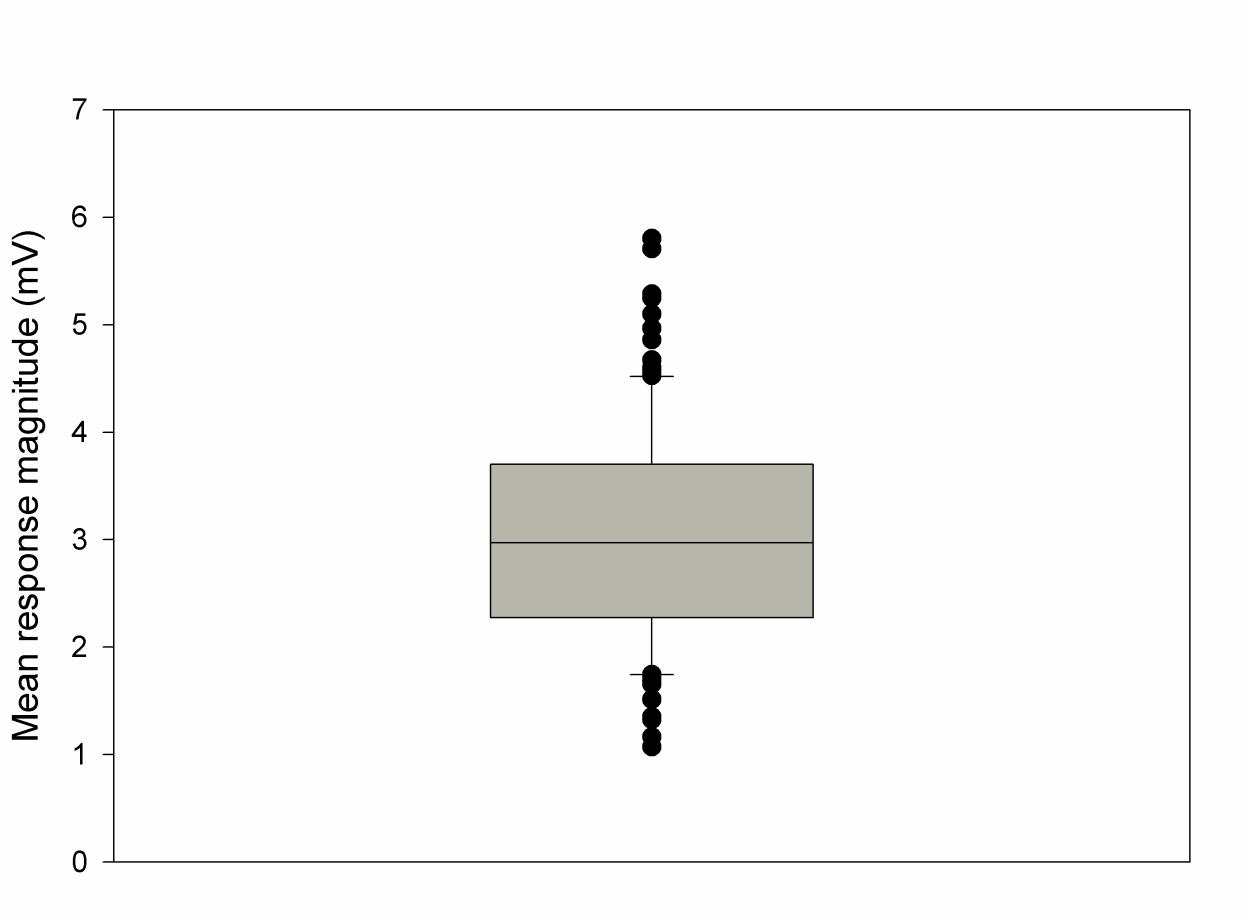

Figure 10. Box plot of mean olfactory responses of adult Pacific lampreys to 10^{-4} M L-arginine from June to December 2001. Response magnitudes in millivolts (mV).

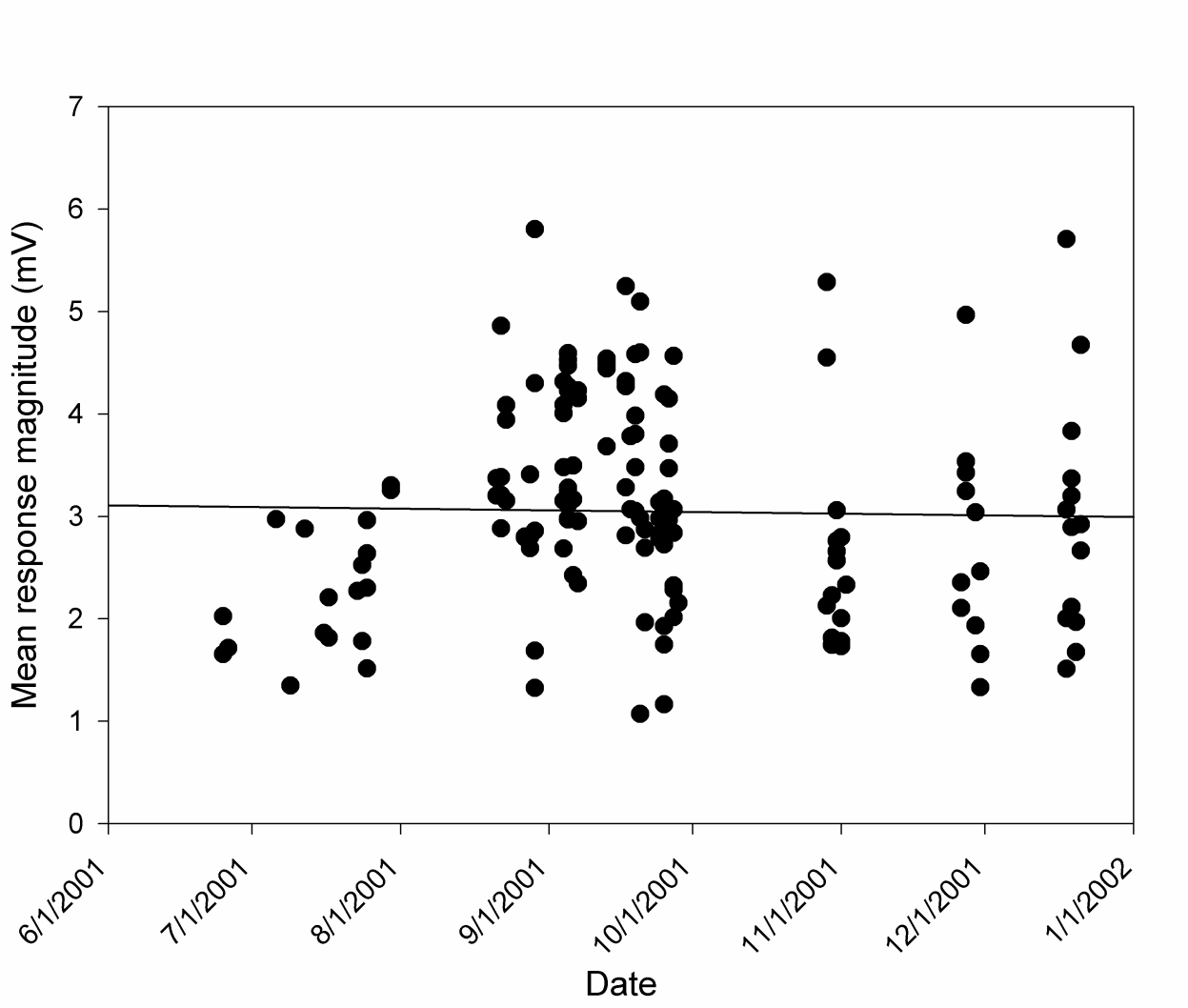

Figure 11. Scatter plot and regression line of mean olfactory responses of adult Pacific lampreys to 10^{-4} M L-arginine from June to December 2001. Response magnitudes in millivolts (mV).

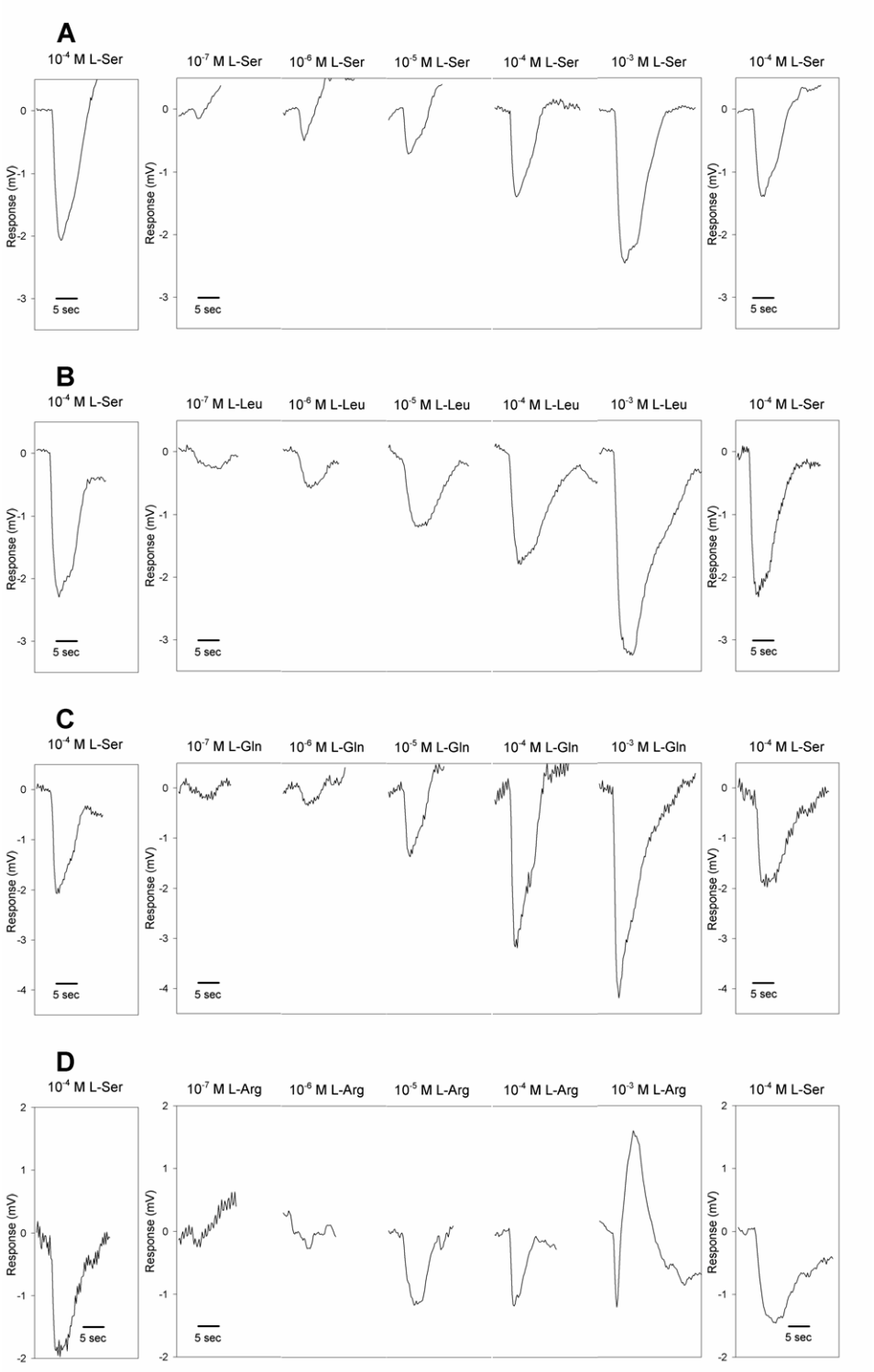

Figure 12. Electro-olfactogram (EOG) recordings from olfactory responses of juvenile steelhead to L-amino acids in July 2001. A) L-serine (Ser), B) L-Leucine (Leu), C) L-glutamine (Gln), and D) L-arginine (Arg).

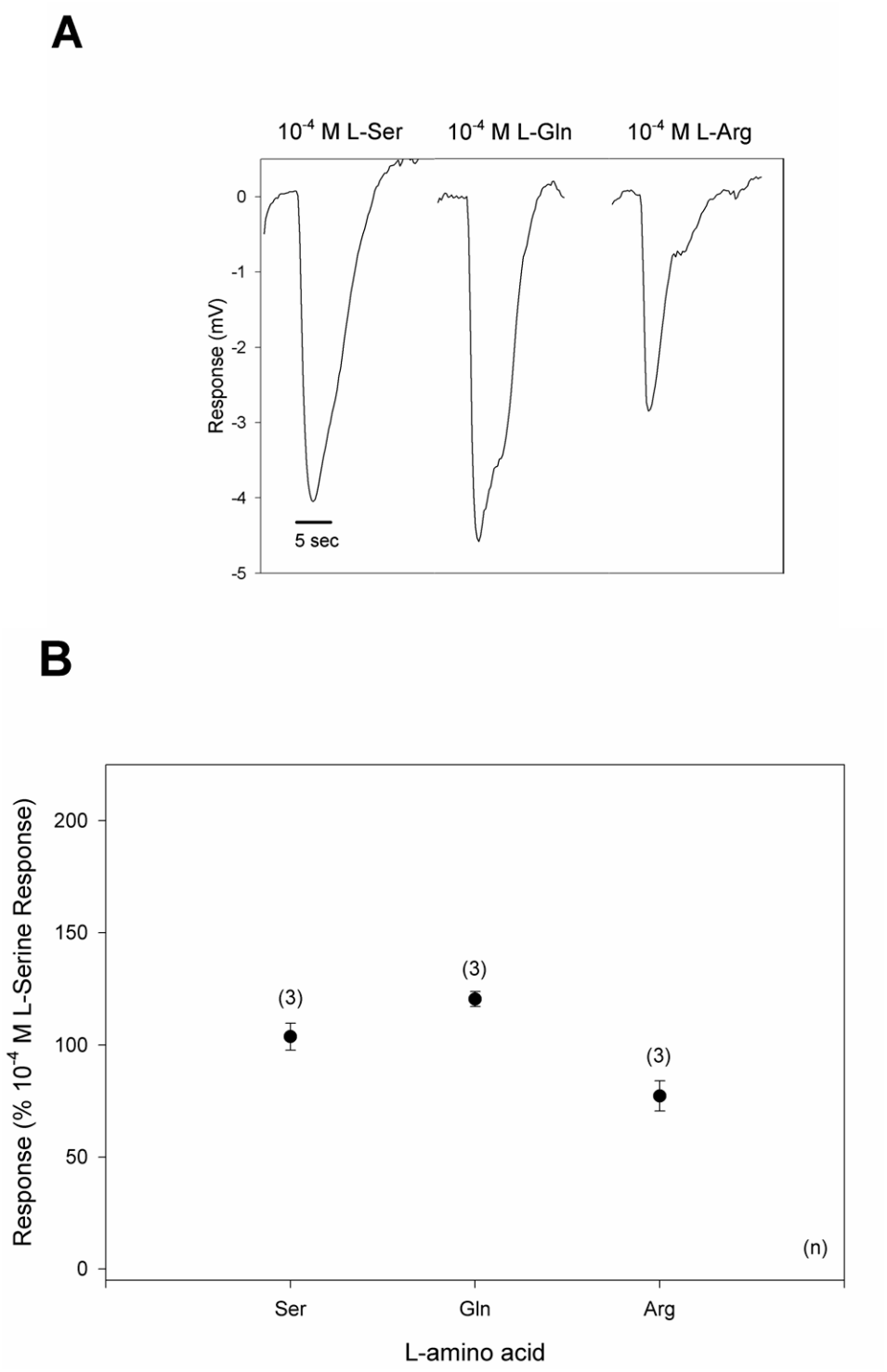

Figure 13. Olfactory responses of juvenile steelhead to 10^{-4} M L-amino acids in October 2001: A) Electro-olfactogram (EOG) recordings of responses to L-serine (Ser), L-glutamine (Gln), and L-arginine (Arg). B) Olfactory responses expressed as percent 10^{-4} M L-serine standard response. Numbers above points are the sample sizes for each point. Error bars are ±1 S.E.