GREAT LAKES FISHERY COMMISSION

2003 Project Completion Report[1]

A first step towards developing a field test to determine whether a larval pheromone can be used in sea lamprey control: ascertaining its effects on adult behavior in a lake and characterizing the complete pheromone

by:

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Completion Report for the Great Lakes Fishery Commission

Project Title: A first step towards developing a field test to determine whether a larval pheromone can be used in sea lamprey control: ascertaining its effects on adult behavior in a lake and characterizing the complete pheromone.

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Project Dates: April 1, 2001- March 31, 2003

TERMS OF THE PROJECT AS SPECIFIED IN THE CONTRACT PROPOSAL

Problem Statement and Objectives as Stated in the Contract Proposal: 'Our previous research has demonstrated that migratory adult sea lamprey locate spawning rivers using their sense of smell and that a critical component of river odor is the pheromonal odor of larval lamprey living in these rivers. We have also clearly demonstrated that two unique bile acids, petromyzonol sulfate (PS) and allocholic acid (ACA) are components of the larval pheromone. Here we seek to determine how the larval pheromone is used by free-ranging adults in a lake, t0 use this information to design and conduct the first field test of this pheromone, finish developing the means of measuring PS and ACA by ELISA so that this assay can be used in lamprey management and control, and to fully elucidate the complexity and chemical character of the pheromone's identity so that it might eventually be fully identified and a potent synthetic form produced.

Rationale and Relevance as Stated in the Contract Proposal: 'The G.L.F.C. is looking for new techniques to control the sea lamprey. Our biochemical, electrophysiological, and behavioral studies of sea lamprey olfactory biology have clearly demonstrated that adult sea lamprey, like many other migratory fish, rely on a water-borne chemical cue released by larval conspecifics (pheromones) to locate spawning streams. So important is this cue that adults which do not detect it do not find rivers to spawn. Because this cue is detected at extremely low (picomolar) concentrations, is environmentally benign, and has the potential to be easily applied, it has enormous potential for use in controlling the migration of adult lamprey. Studies to date have identified two components of this pheromone (petromyzonol sulfate and allocholic acid) but demonstrate that other component(s) exist. We now seek funding to determine how adult sea lamprey locate the larval pheromone from a Great Lake and to what concentrations they respond, thereby permitting us to design and conduct pilot tests of how larval odor might best be used in lamprey control. We also seek funding to continue biochemical elucidation of the complete larval pheromone because a synthetic pheromone is widely thought to have the greatest utility for

control. Funding to develop practical means of measuring the pheromone's distribution is also sought because this technology would have use in assaying the distribution of larvae, predicting adult migration, and guiding pheromone-based trapping.'

Deliverables as Stated in the Contract Proposal:

1) An understanding of how and when free-ranging adult sea lamprey locate river plumes, thereby giving us the knowledge to conduct experiments to determine if pheromone plumes will attract adult lamprey.
2) An understanding of whether adult sea lamprey might be attracted to a river using the larval pheromone.
3) An understanding of whether the distribution of PS and ACA can be used to identify larval lamprey populations in the Great Lakes Basin, and if ELISA is a practical tool to make this determination.
4) An understanding of the number of key component(s) in the larval pheromone, whether one of these might be a derivative of PS, and if not, what molecular feature might characterize it (them).

PROGRESS MADE TOWARDS COMPLETING THE DELIVERABLES:

Deliverable #1: To understand how and when free-ranging adult sea lamprey locate river plumes.

Summary.
We discovered that lacustrine sea lamprey searched for streams in an astonishingly active manner which involved rapid swimming in straight-line paths at night while performing extensive vertical movement throughout almost the entire water column (the top m was not used). When sea lampreys encountered a river plume, they adopted a circular pattern of swimming which often brought them into river mouths. These data demonstrate that if the migratory pheromone is to be used in sea lamprey control it need only be applied at night, it is best be added to rivers with plumes that extend to more than a meter in depth, and that if added to streams in an optimal manner it should attract sea lamprey which come from great distances.

Introduction.
To employ a pheromone as an attractant for lacustrine sea lampreys in an efficient manner, one must add in a manner that creates a plume that sea lamprey are likely to encounter, and then have the ability to orient into and enter a trap. To achieve this objective, one must know where sea lamprey are located in the Great Lakes as they search for odor plumes, when during the day they might exhibit these behaviors, and what types of plumes they are able to track. However, at present nothing was known about the whereabouts of adult sea lamprey prior to upstream migration, the behaviors they exhibit while searching for river plumes, or the distribution of plumes. This study used acoustic telemetry to answer these fundamental questions.

Methods and Results:

Lamprey were surgically fitted with acoustic transmitters (Lotek), released into Hammond Bay, Lake Huron (n=60), and then located and followed using a hydrophone mounted on a small boat. The position and depth of river water plumes was monitored simultaneously using a conductivity meter (river water has a higher conductivity than lake water). A laboratory experiment employed a Blazka-type swim tube to confirm that the swimming speeds of control, surgical-sham, and surgically implanted lamprey did not differ (N=24; ANOVA; P=0.62). Lampreys were released in several different manners to address two questions outlined below:

1) How do lampreys search for rivers from the open lake?

To address this question acoustically tagged adult sea lampreys were released at distances 0.5 km offshore of the Ocqueoc River plume in May - June of 2001 and 2002. Two sub-experiments were performed, the first to determine what time of day lampreys are most active, the second to determine the search paths and speeds of sea lamprey during the time that we found them to be active. Procedures and results are described below:

i) Determining what time of day do lampreys search for rivers

To answer this question we released 25 groups of 3-4 tagged animals at either 04:00h (n=6), 12:00h (n=10), or 20:00h (n=9) during the month of May in 2001 within a 1 km of the Ocqueoc River. Lampreys were then re-located over the next 12-14 hours with a 3h break at 0200h, permitting us to refuel the boat and have short break. Every half an hour we surveyed a grid outside the release point to locate a lamprey. Each time an animal was found, it was followed for 5 min. If it was found to be moving, it was then tracked for another half hour after which it was abandoned and a search initiated for another lamprey. In this way we were able to assess movement patterns of many lamprey throughout the day. Lampreys showed a distinct nocturnal activity pattern, with the relative number of active animals increasing dramatically after sunset (21:30h) and ending after sunrise (05:45h) (Figure 1). During the day (between 08:00h and 22:00h), animals were rarely active.

ii) Determining the search paths of lacustrine sea lampreys.

To address this issue individual lamprey were implanted with an acoustic tag that transmitted depth information (0.6m resolution, 0-30m range), released after sunset outside the Ocquoec River plume (as determined by conductivity measurements), and tracked continuously for 3-6 h throughout the night. This was repeated 14 times. Lampreys swam surprisingly quickly; the median ground speed of the individuals located outside the river plume was 1.5 km/hr, and the median maximum speed was 2.4 km/hr (Table 1). These animals moved in a relatively straight westerly to northwesterly bearing (the direction from which the lake currents were measured; Figure 2). Their median straightness index (straight line distance between first and last observations divided by distance of entire path) was 0.77 (Table 1). While swimming in this manner, lamprey actively moved from the top to the bottom of the water column on almost a continuous basis (approximately every 15 min). Thus, when the water column was divided into 10 equal sections for the purpose of analysis, animals were found in all sections with approximately equal frequencies, the only exception being that lamprey spent slightly less time at the surface and slightly more time near the bottom than otherwise expected (Figure 3a). Only rarely did lamprey come within 1 m of the surface, a notable finding because the river plume was often less than 1 m in depth in early summer when warm (Figure 3b). Notably lampreys frequently made substantial vertical excursions of 10m or more (Fig. 4). These data appear to

explain why many rivers cease catching adults in the late spring when their plumes are warm and thin. Our findings indicate that pheromone should be applied to rivers with relatively thick plumes (>1m) such as exist in the early spring. Animals with occluded nasopores also made extensive vertical movements, with no noticeable difference from control animals (Figure 5b), suggesting searching behavior was not driven by olfactory cues (but represented a search for them)**.**

iii) Do lamprey follow the shore or search from the open lake?

Lampreys released outside of river plumes did not appear to search for the shoreline, or shallower water, but if they encountered the shore, they often followed it. To determine an animal's movement relative to shore, a shore index was calculated. This was computed from the ratio of the change in an animal's distance to shore in a path segment over the length of the path segment weighted over the entire path by segment length (-1 represents straight movement away from shore; +1 represents straight movement towards shore; 0 represents movement parallel to shore). Of 11 animals, six were last observed farther from shore than their release and five were last observed closer to shore than their release (Table 1). However, only one animal exhibited significant movement in either direction (#77; Table 1). All five olfactory -occluded animals moved away from shore, three of them in a significant manner (Table 3; Figure 5a).

2. How do lampreys orient into odorous river plumes?

To determine how lamprey behave and orient once within river plumes, six sea lamprey were released in or very near the Ocqueoc River plume (n=6). These data were combined with data from animals which were released outside of the plume but later encountered it, allowing for a direct comparison of behavior before and after they entered the plume. Once again, all animals were tracked after sunset, when we knew lampreys were most active. Two animals released near the mouth of the Ocqueoc River located and swam into the mouth fairly directly (ex. Figure 7), other animals within the plume made large circular movements within the plume, taking some time to find the river mouth (Figure 6; Table 2). This circular swimming motion observed within the plume contrasted with unidirectional swimming of open-lake migrants; the median straightness index for all animals that spent some time in the plume was 0.61, lower than the value of animals located outside of the plume. Those animals that spent almost all their observed time within the plume had straightness scores less than 0.2 (Table 2) and exhibited extensive vertical migration throughout the water column (Figure 7). Two olfactory-occluded animals encountered the river plume but swam straight through it without changing course (data not shown). Sea lampreys appear to be extremely sensitive to river water odor containing pheromone which they locate using a form of orthokenesis; changing their bearing with odor concentration. A persistent pheromone plume will be needed to consistently attract lacustrine sea lamprey into rivers.

Deliverable #2: An understanding of whether adult sea lamprey might be attracted to a river using the larval pheromone.

Summary:
Two Creeks were identified in northern Michigan with good potential for a pheromone test. Both are of a moderate size, have attracted sea lamprey in large numbers in the past but presently do not now, presumably because they now lack larval lamprey and pheromone. Present understanding of both of these streams suggests that lamprey would likely be attracted to them were pheromone to be added to them.

Introduction and Methods:
We spent the past two years examining small streams near Hammond Bay Biological Station to determine their suitability test to whether free-ranging lampreys can be attracted into streams from the lake using the migratory pheromone. We sought to identify small streams that could attract sea lamprey (they have in the past) but do not at present, presumably because of a lack of larvae and pheromone which would be added as part of the test. Unfortunately, plans for a small-scale test involving adding larval holding water extract to a stream in year two were put on hold pending approval from the E.P.A. However, we did gather useful baseline information on several streams in preparation for a test upon E.P.A. approval. We assessed streams for adult lamprey, collected data on flow and temperature, collected water samples for future analysis for the presence of pheromone, and examined historical records. With the assistance of the Marquette Biological Station, fyke-nets and/or portable assessment traps were set in five streams near Hammond Bay Biological Station and monitored daily between May 9, 2001 and June 15 2001 (Greene Creek, Lone Pine Creek, Mulligan Creek, Three Creek, and Grace Creek; Figure 5). In 2002, assessment traps were again placed in Greene, Mulligan, and Lone Pine Creeks from May 7 to June 15. In addition, personnel from Hammond Bay Biological Station operated a trap in Black Mallard Creek between April 23 and July 25, 2001, and May and June 2002.

Results:
A large number of lampreys were captured in Greene Creek in 2001 (Table 4), but none were captured in 2002 following a TFM treatment in the fall of 2001. Likewise, many fewer adults were captured in the Black Mallard in 2002 following a TFM treatment in the fall of 2001, although that treatment left a significant residual larval population. The Black mallard may thus not be a good choice for a pheromone test. Although the only traps to catch lamprey were those located in the Greene and Black Mallard Creeks (Table 4), historical records report that Mulligan, Greene, and Black Mallard Creeks all supported significant, equivalent lamprey runs 10 years ago. The Mulligan was treated with TFM seven times between 1966 and 1994 and following its last treatment in 1994, only two sea lamprey larvae were found the next year, and none since. Notably, we caught no adults in the Mulligan in either 2001 or 2002. Some adult lamprey were caught in the Greene in 2001 but none after treatment late that year. Both the Greene and Mulligan are readily accessible from shore and in both cases landowners located at their mouths expressed a willingness to assist possible efforts to test pheromones. Thus, both Mulligan and Greene Creeks appear to be excellent candidates for a field test.

Deliverable #3: To determine if the distribution of PS and ACA can be used to identify streams with populations of larval lamprey in the Great Lakes Basin.

Summary:

Water samples were collected from nearly a dozen North American streams, some with sea lamprey larvae and some without, extracted them and their PS levels analyzed. We concentrated on PS because initial studies of ACA levels in larval holding waters in the laboratory found them highly variable and therefore unlikely to be of value. Because initial efforts to employ ELISA were severely complicated by cross-reactivity with unknown florescent compounds in river water, we used of chromatography-mass spectrometry (LC-MS) to measure concentrations of petromyzonol sulfate (PS). PS was found only in streams with larval lamprey (n=7), was absent in those which lacked lamprey (n=5); however, where PS levels were correlated with the abundance of larval lamprey (n=4), little apparent relationship was evident. Small sample size and sampling error makes this conclusion tentative. Suggestions of a seasonal trend in PS abundance in the St. Mary's River were evident. The possibility of measuring larval cues to assess larval density appears to have some promise, the nature of which might merit further study in much larger number of streams especially if compound '704' (another component of the pheromone; see below) can be included in this study.

Introduction.

The Great Lakes Fishery Commission spends a great deal of money monitoring the abundance of larval sea lamprey in streams. We now know that these larvae release a distinctive pheromone partially comprised of petromyzonol sulfate (PS). This study sought to determine whether their might be merit in measuring concentrations of PS in river waters to supplement direct (expensive) efforts to assess larvae.

Methods.

Water samples were collected from 13 streams and sent to the University of Minnesota for analysis. Five streams lacked larval lamprey of any species, and 7 had lamprey. In some cases, precise estimates of larval abundance were available for specific reaches of these streams, in which case, water samples were taken above these reaches and below them. For analysis, a liter of stream water was filtered through a C18 solid phase extraction sep-pak (Waters Inc, Mass), rinsed with 30% methanol (to remove extraneous material), and eluted with 70% methanol. The eluate was then fractionated/purified by HPLC to collect PS. Initial efforts to measured PS using ELISA met with limited success because antibody we produced was from petromyzonol and we experienced difficulties de-sulfating PS in ways that were quantifiable and did not interfere with the assy. Subsequently, we developed a way to quantify PS using selected ion monitoring (SIM) with a LCQ electrospray ionization mass spectrometer. Values were calculated relative to a standard curve which was constructed by injecting synthetic PS into river water samples. The detection limit for this assay was found to be 6×10^{-13} M in a river water background. Analyses were performed to: a) examine for presence/absence of PS in lamprey/non-lamprey streams; b) to look for possible correlations between PS concentration and larval abundance in lamprey streams, and c) to determine if there might be seasonal trends in PS concentrations.

Results:

a) Is PS present in lamprey streams and absent (not detectable) in non-lamprey streams?

We found PS levels to be below the detection threshold (6×10^{-13} M) in all 5 non-lamprey streams but detectable in 6 of 7 lamprey streams (Figure 8). In all streams where PS was measured the concentration fell between 10^{-12} M and 10^{-11} M.

b) Is there a correlation between PS concentration and larval density in streams?

Stream water samples were collected just downstream of four larval survey sites by staff of the Hammond Bay Biological Station in four well-studied streams in October of 1999. PS concentrations were measured in each of these streams (n=3/stream) by the methods outlined above and results can be seen Table 4. There was no apparent correlation between PS concentration and the number of larvae/discharge (m^3/sec) measured in these streams. This might be due to small sample size and vagaries of PS breakdown. More effort may be warranted using more streams, additional compounds (704) and improved techniques.

c) Is there a seasonal trend of PS concentration in the St Mary's river?

PS was also measured in the St Mary's river at 9 different periods throughout the course of about 1.5 years and in three more samples 2 years later. Samples were collected by Mr. Doug Cuddy at the dock outside the sea lamprey control center (D.F.O., Canada). Ten of the twelve samples yielded PS at ~10^{-12} M, and two samples in January and March greatly exceeded 10^{-11} M (Figure 9). These high concentrations suggest a possible trend for an increase in late winter but are not consistent enough to yield clear conclusions.

Deliverable #4: To identify and chemically characterize key missing components in the larval pheromone.

Summary:

The larval pheromone was extracted by C18 columns, fractioned and the olfactory and behavioral activity of these fractions elucidated. These analyses clearly demonstrated that pheromone is comprised of PS and another as yet unidentified compounds with a molecular weight of 704 (compound '704'). Initial chemical characterization of this compound demonstrated that it is sulfated and a derivative of cholesterol. Prospects for identification are excellent especially because we have developed means to isolate it in quantity. Allocholic acid has been ruled out as a significant contributor to the pheromone.

Introduction:

It is extremely important to know the complete identity of the migratory pheromone so that this cue can be measured accurately in river waters and synthesized. A decade of study has clearly shown PS to be component of the pheromone however a recent behavioral study suggested that other components might exist (Vrieze and Sorensen 2001). This work was complimented by another unpublished study (described in the contract proposal) which fractionated larval holding water C18 extracts and found that these extracts found to contain at least 6 odorants in addition to PS. The present study sought to determine: a) which of this HPLC fractions contained pheromonally-active odors; b) to characterize the odorous compounds found in this fraction; c) to determine if these compounds function as a mixture; and d) to determine if these compounds might be derived from PS or other bile acids.

Methods and Results

a) What are the HPLC fractions with the greatest behavioral activity?

Larval holding water was extracted with C18 Sep-Paks (a treatment known to remove all pheromonal activity), and fractionated in 20 fractions using a methanol/water gradient by HPLC. These fractions were then screened for olfactory activity using standard EOG recording. Only 5 of 20 fractions had notable olfactory activity (#1, 6, 7, 8, 9, 10, and 11). We then tested the behavioral activity of all 20 fractions by adding them to lake water in the Hammond Bay raceway mazes following procedure of Vrieze and Sorensen (2001). When tested in this manner, only fraction # 10 had significant behavioral activity (Figure 10). Subsequent efforts focused on this fraction.

b) Chemical characterization of the most active fractions: isolation, purification, and identification of the pheromone

Fraction #10 was re-injected onto the HPLC and fractionated into 20 sub-fractions using a shallow methanol gradient while monitoring eluted compounds with electrospray ionization - mass spectrometry (ESI-MS) (Fig. 11). The ESI-MS of Fraction 10 is shown in Figure 10. We then tested the 20 sub-fractions in Fraction 10 by EOG recording and found only one to possess significant olfactory activity. It was substantial (Figures 11 and 12). Close examination of sub-fraction #3 by ESI-MS found it to contain only 2 compounds, one with a moderate-sized peak and a molecular weight of 474 (after correcting for ionization), and another larger peak with a molecular weight of 704 ('Compound 704') (Figure 12). Injecting PS (which has a molecular weight of 474) onto the HPLC we have found that it co-elutes with the moderate sized peak; we conclude the later is PS. Also, when examined by mass spectrometry/mass spectrometry (MS/MS), both compounds show the same fragmentation patterns and possess moderate behavioral activity (see below). Initial chemical characterization of 704 with negative ion electrospray ionization has shown that the exact molecular weight is 704.3 (there is an M-H peak at 703.3) and MS/MS at 30% collision energy yields a peak at 605.3 (M-H-98), suggesting that 704 has at least one sulfate group. We have requested funding to determine the precise role of 704 in the pheromone and for chemical identification with nuclear magnetic resonance (NMR).

Olfactory and behavioral activity of PS and Compound 704 have now been established. The activity of Compound 704 is especially notable. EOG recording has confirmed that both extracted PS and Compound 704 have high activity (precise quantification is still needed), and that they stimulate different olfactory receptor mechanisms; cross-adaptation studies, when the sea lamprey olfactory epithelium was constantly perfused with a background odor of 10^{-10} M PS (so ALL olfactory receptors for PS are in use), the response to 704 remained unchanged while responses to 3-keto-PS were suppressed about a third (data not shown). Behavioral tests using the Hammond Bay Biological Station raceways (Vrieze and Sorensen 2001) confirmed the behavioral potency of compounds 474 (PS) and Compound 704. When tested on their own versus blank lake water control in the spring of 2002 using 12 groups of 4 migratory sea lamprey (n=48 for each test), compound 474(PS) was found to have moderate behavioral activity ($p\sim0.10$) – consistent with previous tests of PS by Vrieze & Sorensen (2001). Remarkably, 704 was found to possess consistently high behavioral activity ($p<0.05$; Figure 13).

c) Is the pheromone a mixture?

As described above, we have found two compounds in larval water (PS and 704) with pheromonal activity; the migratory pheromone is a mixture. To address whether these

components might synergize each others actions we tested them as mixture and found their activity to exceed that of 704 alone, suggesting that the pheromone is indeed a synergistic mixture (Figure 13). Further study is needed to elucidate the precise nature of this complex cue.

d) Are the missing component(s) actually metabolites of PS?

Studies have started to elucidate the chemical nature of the key missing component, '704'. Hypothesizing that 704 might be a derivative of cholesterol like PS, we have traced the fate of radiolabled cholesterol in larval sea lamprey to determine if it might produce this compound. Larval sea lamprey were each injected with 25 μCi of radiolabeled ^{3}H-cholesterol, placed into isolated tanks and their water extracted using C18 sep-paks every two days for 16 days. Extracts were then injected onto a C18 column using standard bile acid separation procedures (Polkinghorne et al. 2001). Fractions were collected very 2 min and counted. Two small radiolabeled peaks were observed, one with the retention time of PS, the other with the retention time of 704, suggesting that 704 (like PS) is a steroid derived from cholesterol (Figure 14).

Table 1. Summary of paths of lacustrine lampreys that did not encounter the river plume

ID	Release Time	Release Date	# Obs.	Path Linearity	Path Distance (m)	Ground Speed (m/hr)	Max Speed (m/hr)	Shore Index	Shore Significance
61	21:30	7/2/2001	14	0.854	14417	2464	2918	-0.029	0
19	22:03	7/13/2001	7	0.862	11536	2533	2975	0.063	0
33	21:59	7/14/2001	15	0.772	8248	1474	2806	0.138	0
104	22:57	7/18/2001	11	0.664	2265	466	691	-0.200	0
99	21:25	7/21/2001	10	0.493	1114	212	423	-0.078	0
77	23:02	6/6/2002	13	0.844	673	213	326	0.798	+
37	23:46	6/8/2002	10	0.518	1575	628	1064	-0.063	0
38	22:38	6/19/2002	12	0.791	6324	1666	2298	0.213	0
6	22:49	6/20/2002	14	0.891	8216	2413	3054	-0.052	0
91	23:06	6/25/2002	17	0.656	4852	1299	2660	0.001	0
27	23:24	6/28/2002	12	0.734	4900	1750	2383	-0.018	0
		Median	**12**	**0.772**	**4900**	**1474**	**2383**		

Table 2. Summary of paths of lacustrine lampreys that encountered the river plume

ID	Release Time	Release Date	# Obs.	Path Linearity	Path Distance (m)	Ground Speed (m/hr)	Max Speed (m/hr)	Shore Index	Shore Significance
81	10:30	6/7/2001	16	0.132	6278	1952	2773	-0.048	0
28	21:56	7/23/2001	10	0.773	4498	1397	1922	0.329	+
102	21:32	4/16/2002	6	0.640	1154	672	1694	0.200	0
45	21:13	4/23/2002	18	0.156	6329	1511	2211	0.085	0
1	21:39	4/29/2002	12	0.611	2613	947	1820	0.403	0
15	21:03	5/1/2002	20	0.223	9837	1886	2898	0.134	0
10	22:04	5/21/2002	15	0.699	3312	1030	2282	0.091	0
34	23:18	6/4/2002	11	0.706	3898	1314	2845	0.063	0
51	0:31	6/8/2002	18	0.187	4809	1348	2723	0.072	0
80	22:38	6/12/2002	16	0.172	3358	997	1540	-0.210	0
8	23:01	6/13/2002	14	0.834	6206	1605	2540	0.200	0
		Median	**15**	**0.611**	**4498**	**1348**	**2282**		

'Shore significance' .+ = significant movement towards shore; - = significant movement away from shore; 0 = no significant movement relative to shore.

Table 3. Characteristics of streams assessed for a possible field test of the pheromone.

STREAMS	Adults Trapped 2001	Adults Trapped 2002	Adults Trapped 1950-1951	Larvae last observed	Discharge (m^3/sec)[a]
Greene Creek[c]	289	0	2730	2001	0.027
Lone Pine Creek	0	0	0	never	0.006
Mulligan Creek	0	0	1227	1994	0.044
Three Creek	0	---	---	never	0.005
Grace Creek	0	---	84	1977[b]	0.018
Black Mallard[c]	186	12	2427	2001	---

[a] Discharges recorded on single date in late May, 2001
[b] Less than 10 larvae have been found since 1977
[c] Treated with TFM autumn of 2001

Table 4. Concentrations of PS measured in 4 lamprey streams relative to larval abundance

RIVER	[PS] (M) @ Upstream Site	No. of Larvae between Upstream and Downstream sites	[PS] (M) at Downstream site (mean+/-std err)	[PS] (M) at Downstream Site Less Upstream Site	Number of larvae/discharge (m/sec)
Big Garlic	$1.1x10^{-12}$ ±0.56	31,622*	$2.4x10^{-12}$ ± 0.84	$1.3x10^{-12}$	21,808
Misery	n.d.	179,114*	$3.2x10^{-12}$ ± 0.17	$3.2x10^{-12}$	1,990,156
Middle	n.d.	296,507*	n.d.	n.d	329,452
Rock	$3.6x10^{-12}$ ± 0.47	188,331	$1.4x10^{-12}$ ± 0.42	n.d.	1,448,700

n.d = not detectable
* more than 95% of larval lamprey were *P. marinus*

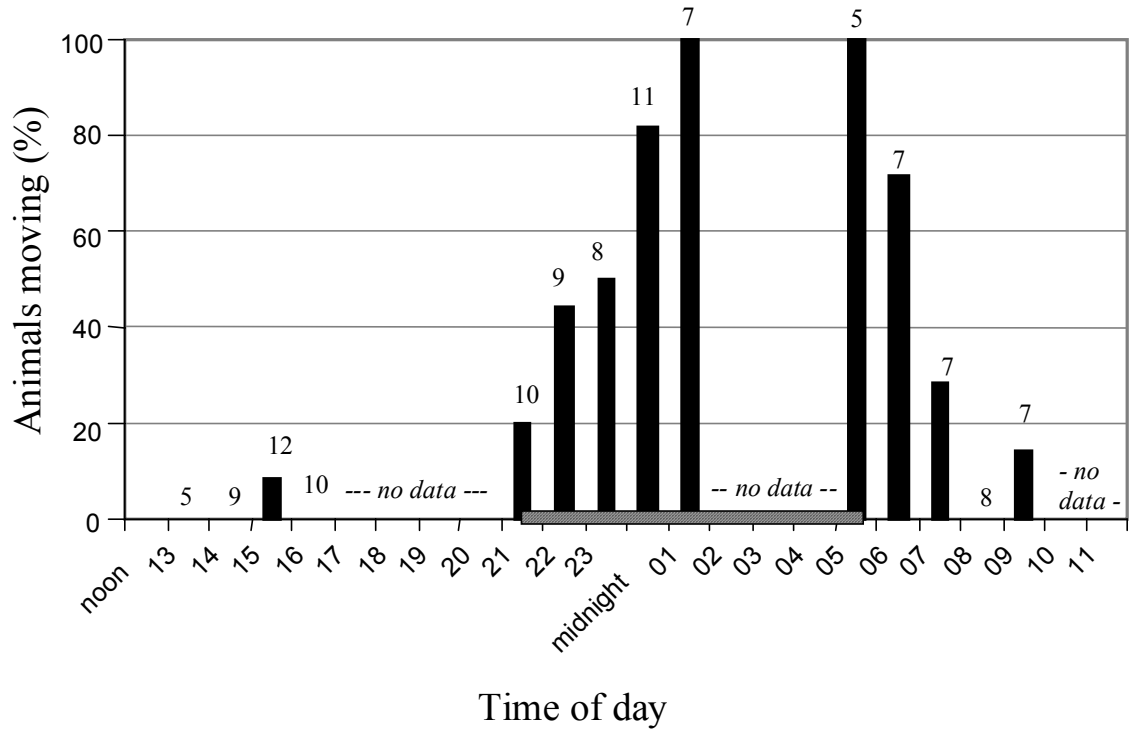


Figure 1. **Daily activity of lacustrine adult sea lampreys.** Percent migratory lampreys observed moving in Lake Huron each hour. Numbers above bars are the number of individuals observed. Cross-hatching on the x-axis indicates the period between sunset and sunrise.

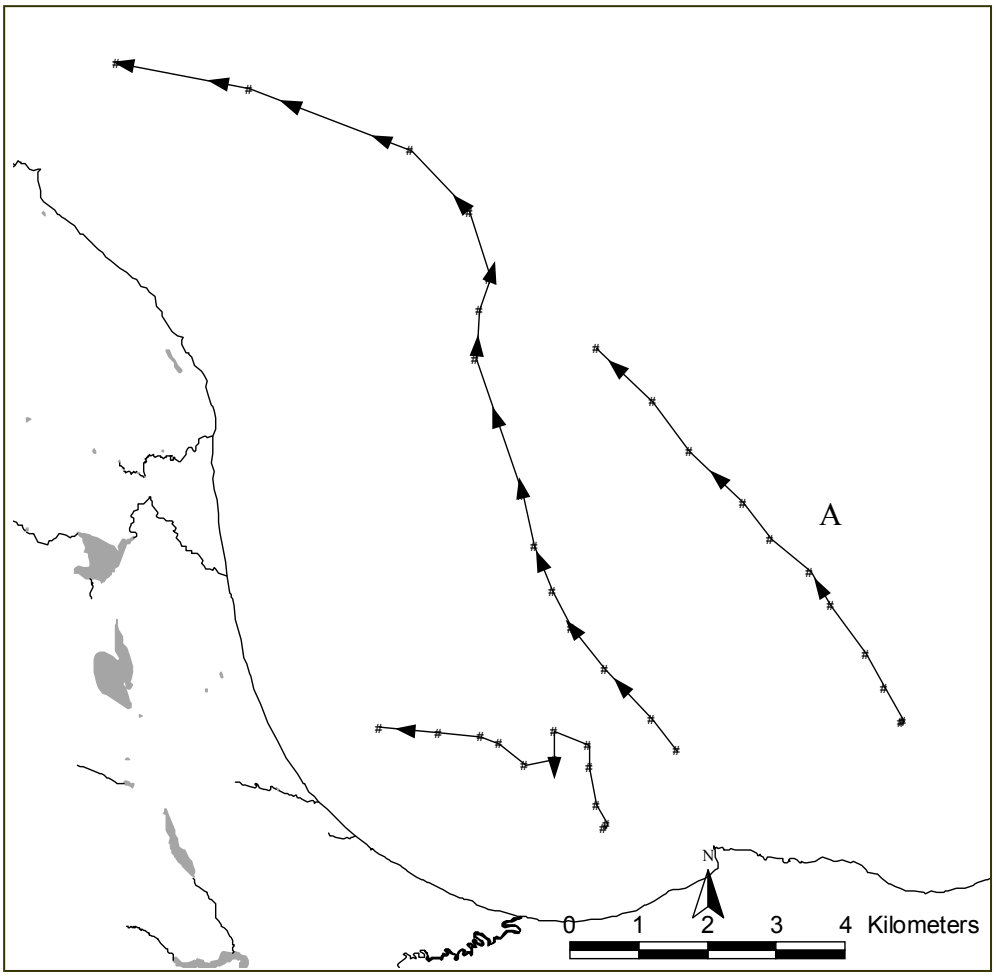


Figure 3. Horizontal paths of three representative migratory sea lampreys as they searched for tributaries in Lake Huron.

B

C

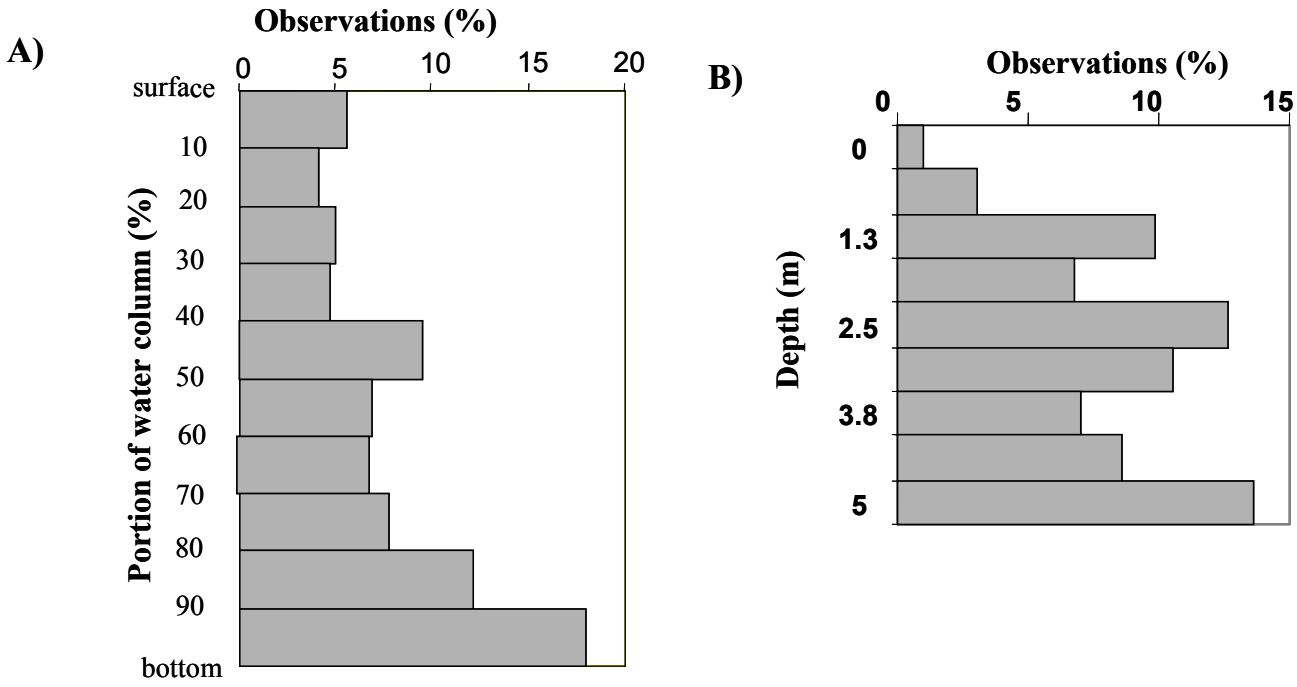
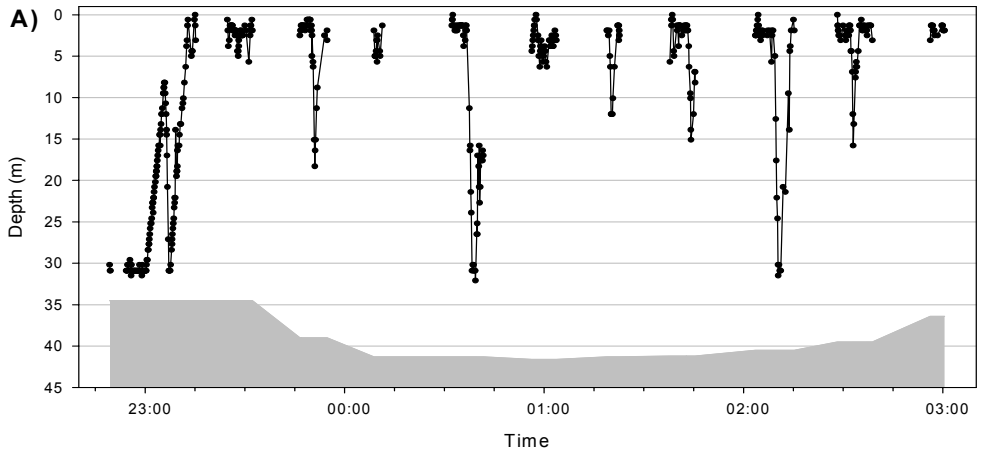
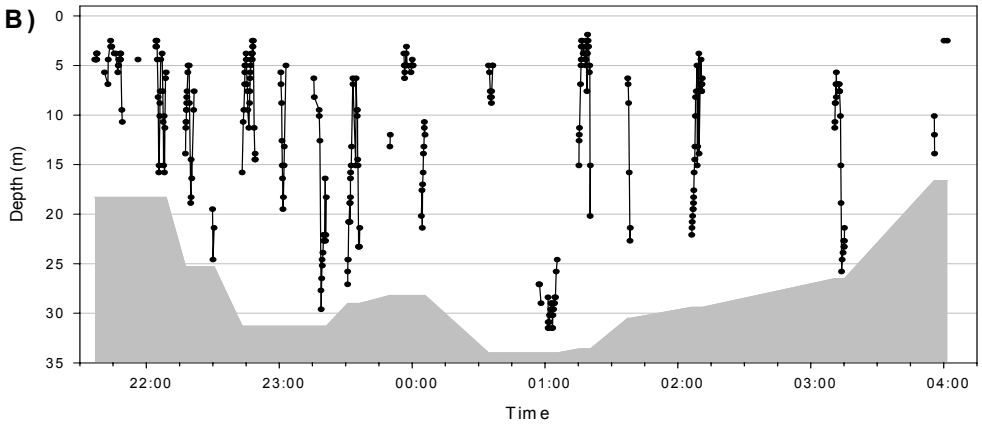


Figure 3. Depths occupied by lampreys in 2001 (2002 not completely analyzed yet). A) Frequency of time (median percent) that tagged lamprey were noted at different positions within the water column (n=6). **B)** Frequency of time these six lamprey spent at different intervals within the top 5M. Note that lamprey rarely ventured with a M of the surface. These data are from 2001.





C)

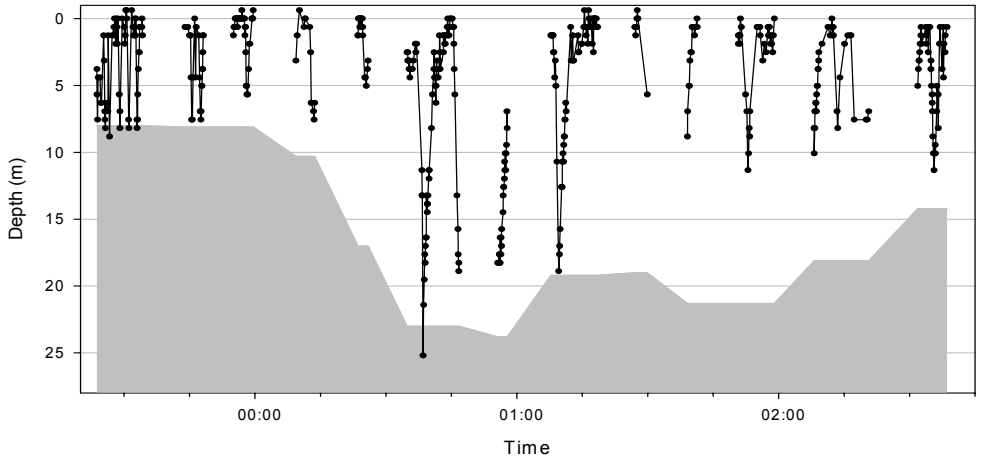
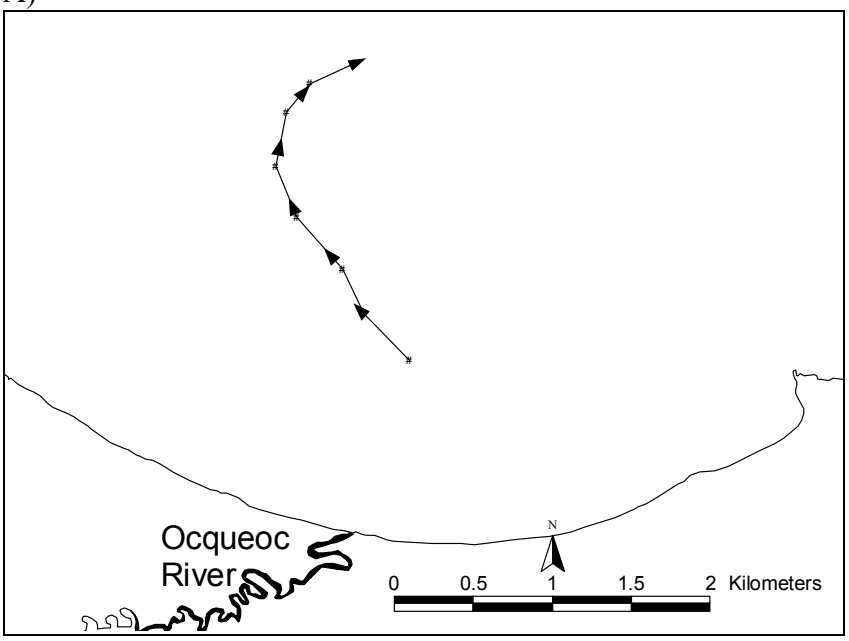


Figure 4. Vertical movements of three representative lampreys as they searched for spawning streams (individuals are matched to Figure 3 by corresponding letter). Shaded area represents lake bottom. Tags had a maximum depth range of 30 meters.

A)



B)

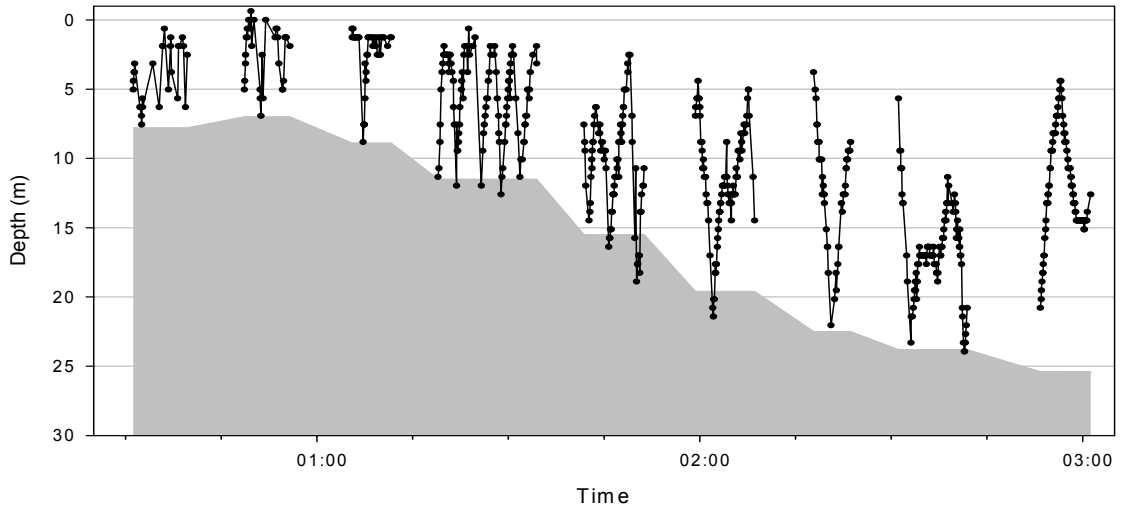


Figure 5. Example of a nasally-occluded sea lamprey movements in the: A) horizontal and B) vertical directions. Shaded area represents the lake bottom. This animal was released June 27, 2002.

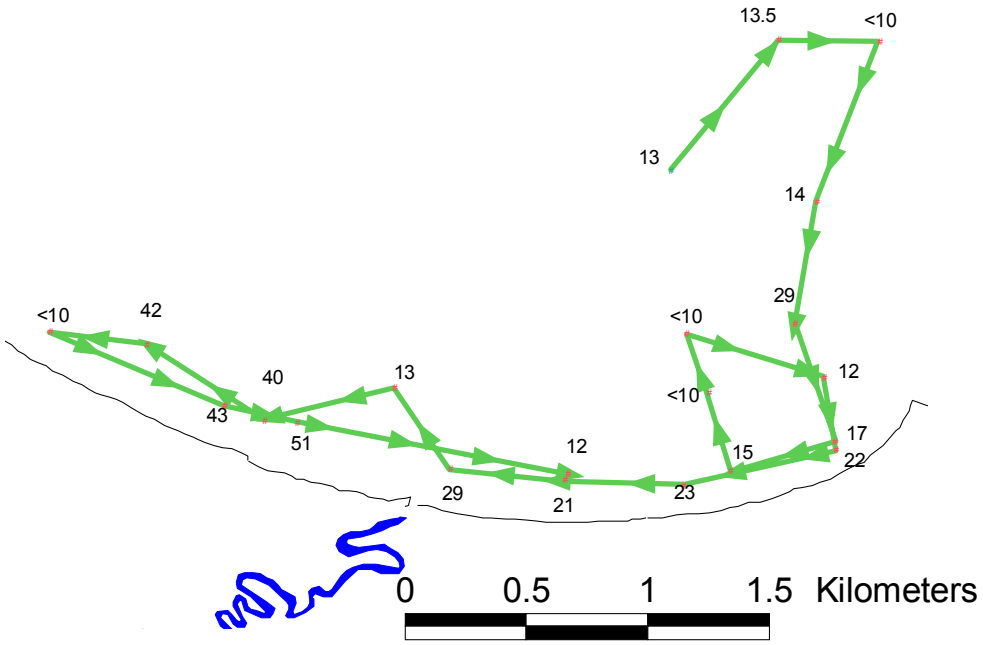


Figure 6. Representative path of an adult sea lamprey released within the Ocqueoc River plume in 2002 Numbers are the percent river water measured at each location.

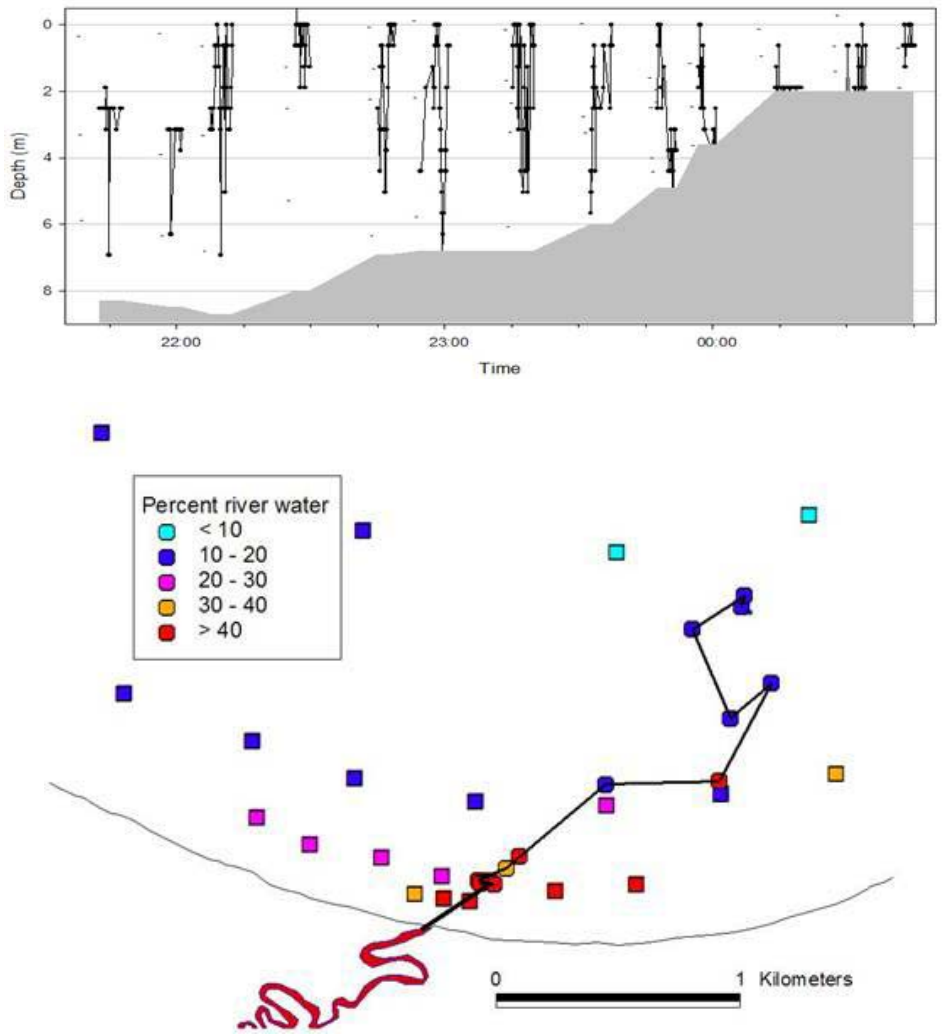


Figure 7. Track of a lamprey released in the Ocqueoc River plume which found that river.

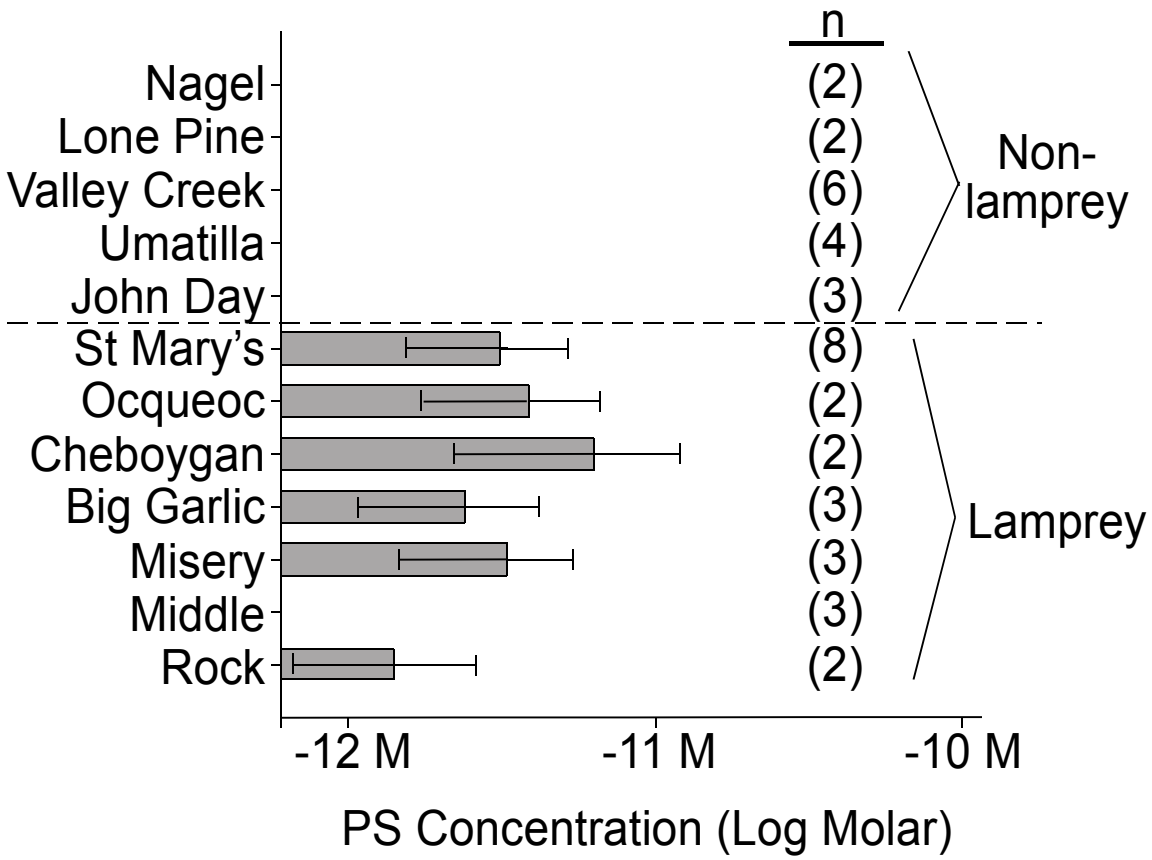


Figure 8. Concentrations of PS measured in 5 non-lamprey and 7 lamprey using electrospray ionization mass spectrometry. Bars represent average PS concentrations for the number of streams tested (n). The detection limit of the assay is 6×10^{-13} M and is the intersection of the 2 axes.

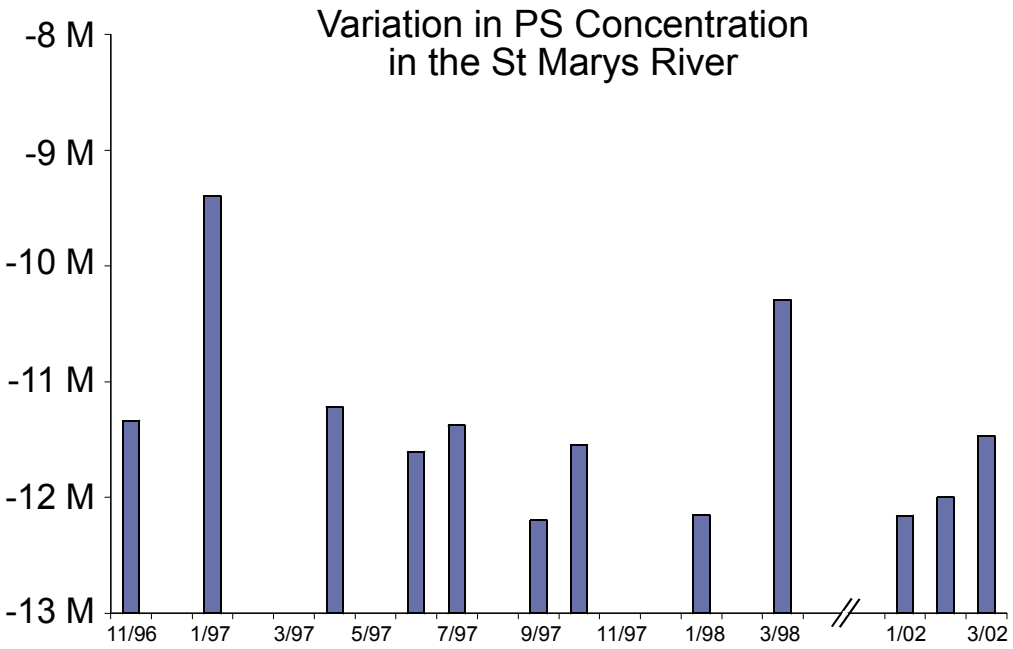


Figure 9. Annual variation in PS concentation in the St Marys River. n=1 for each sample date. The detection limit for the assay was 6×10^{-13} M.

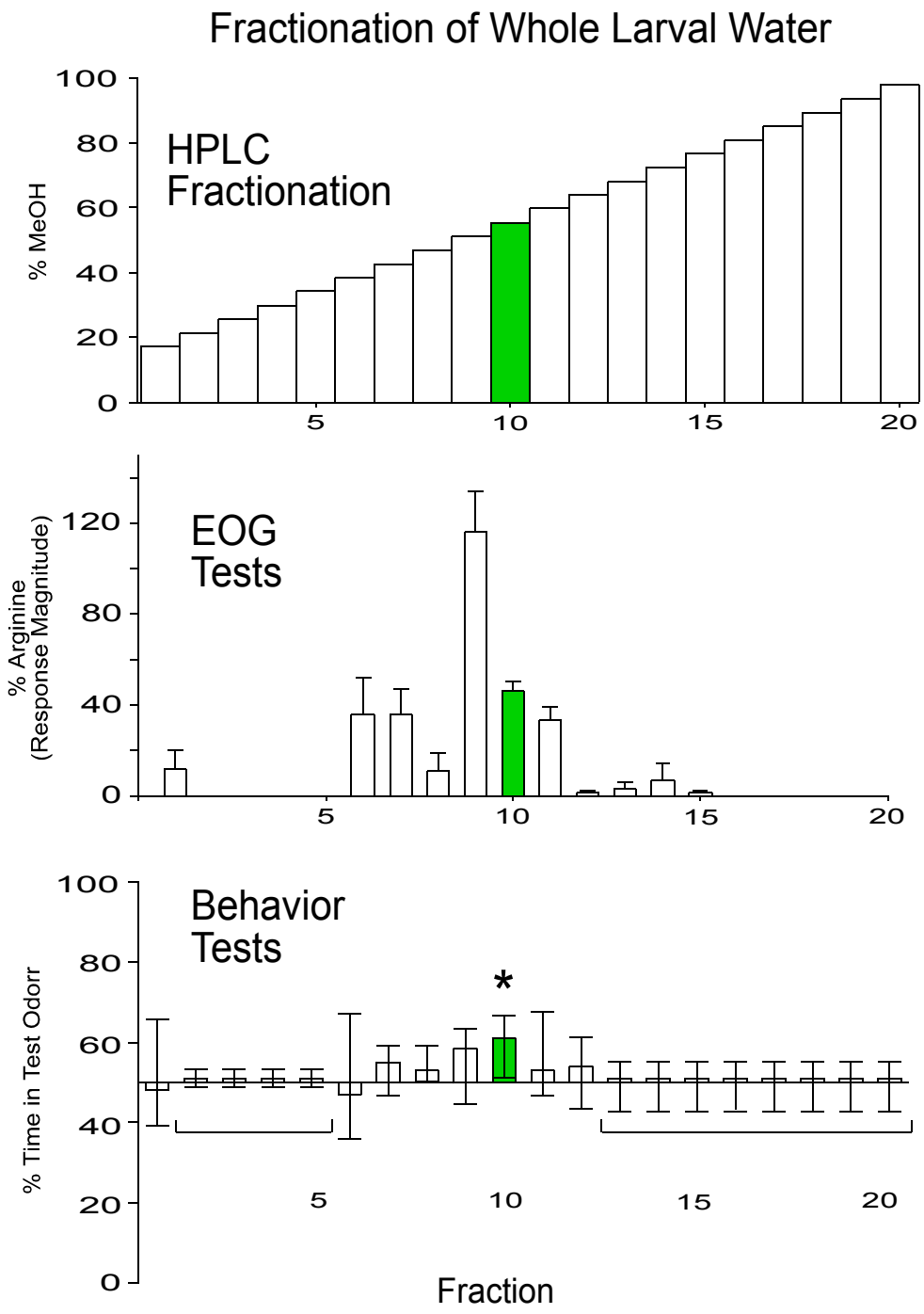


Figure 10. Initial fractionation larval sea lamprey holding water extract to characterize biological activity. A) Schematic of the fractionation scheme of whole larval water; B) EOG responses to these 20 fractions; and C) behavioral responses to individual fractions. Fraction 10 is highlighted as it is the only fraction with significant behavioral activity ($p < 0.05$). Fractions 2-5 and 13-20 were tested as groups because they lacked EOG activity.

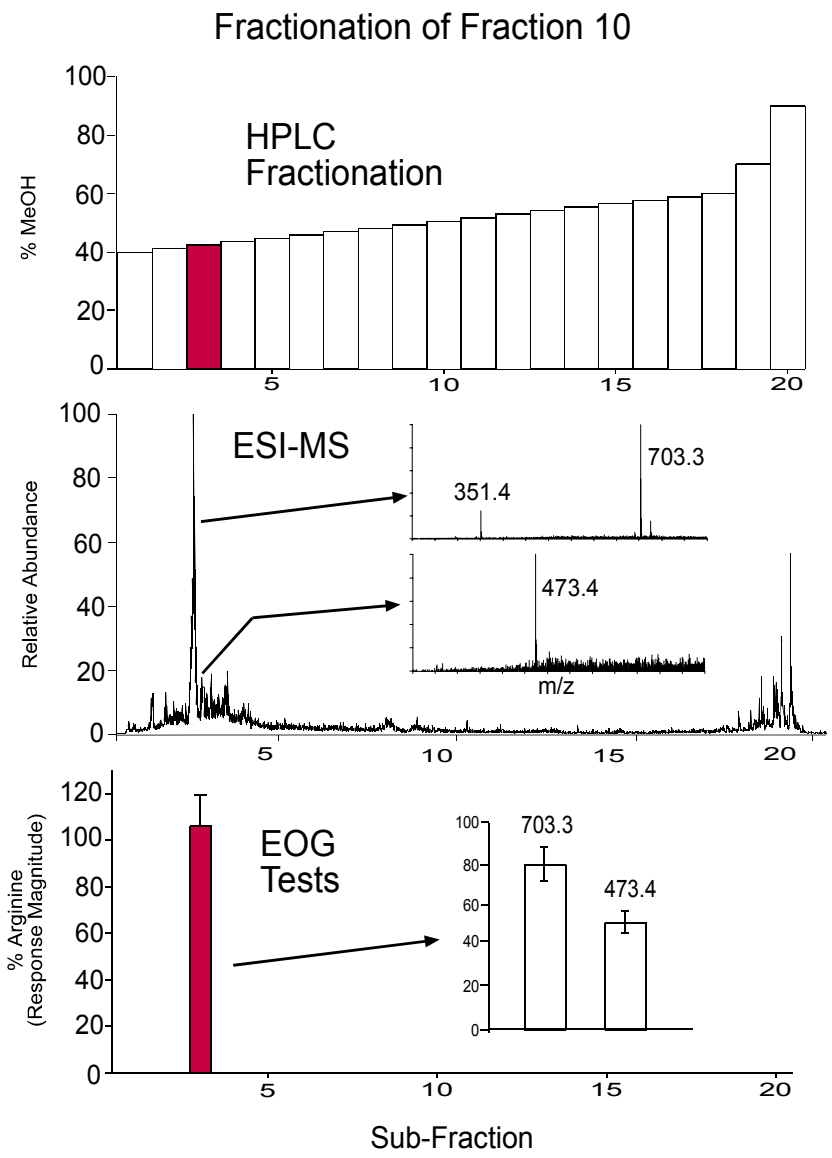


Fig 11. Subfractionation of 20 L of Fraction 10 to isolate compounds with biological activity. . A) Schematic of fractionation scheme; B) Chromatogram of LC/MS output, showing the two peaks in subfraction #3; and C) EOG responses to subfraction #3 and its components, PS (473.4) and 704, an unknown (inset).

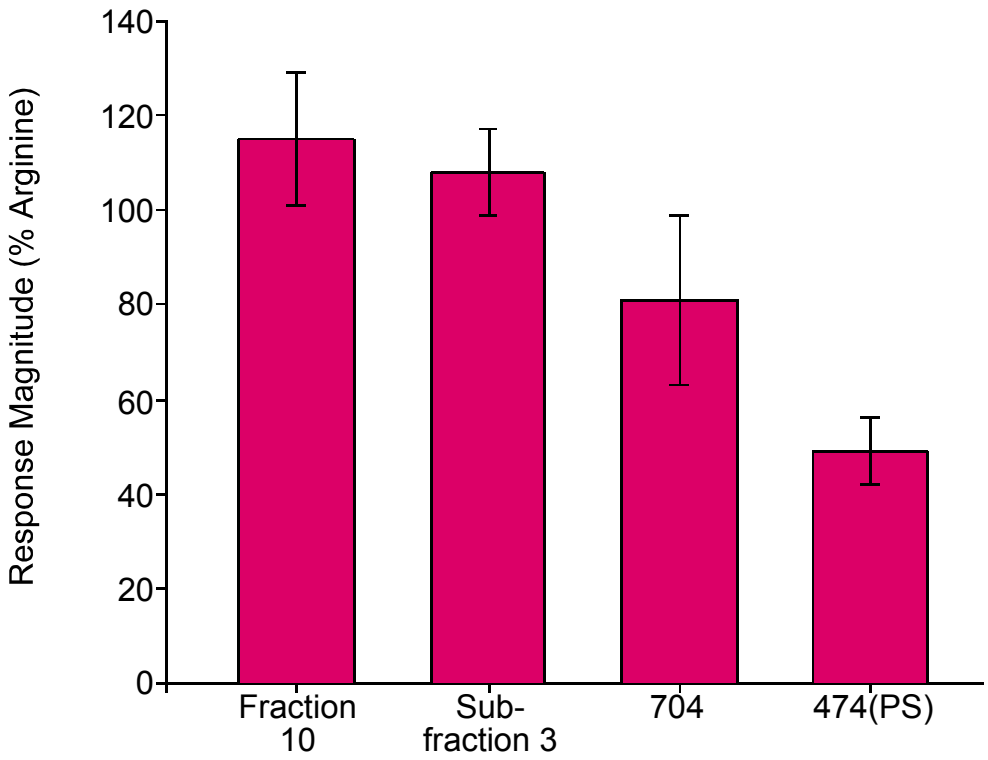


Figure 12. EOG responses to Fraction 10, subfraction 3 from fraction 10, and its components, 704 and PS (474). Columns are mean response (n=9) relative to arginine standard, error bars represent a single standard error.

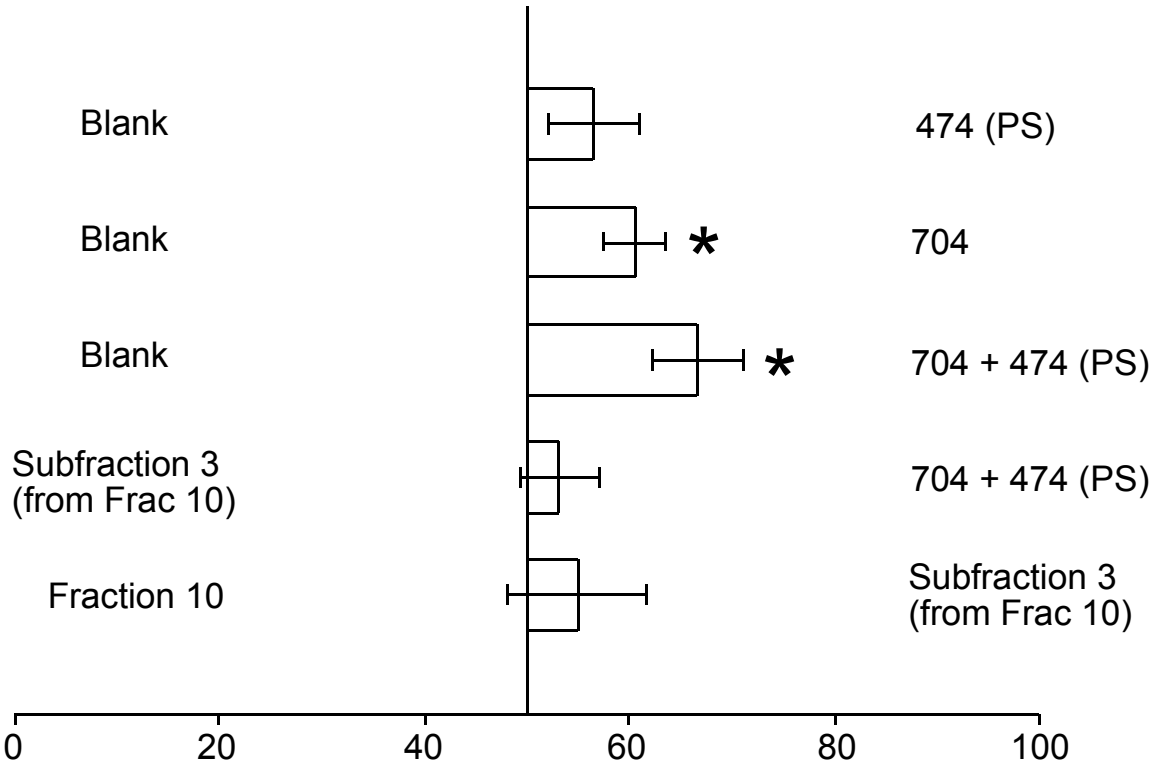


Figure 13. The compound with mw=704 purified and isolated from larval holding water is behaviorally attractive when tested alone, and in combination with PS accounts for all behavioral activity in fraction 10. Bars are means, error bars are standard error. * = $p < 0.05$.

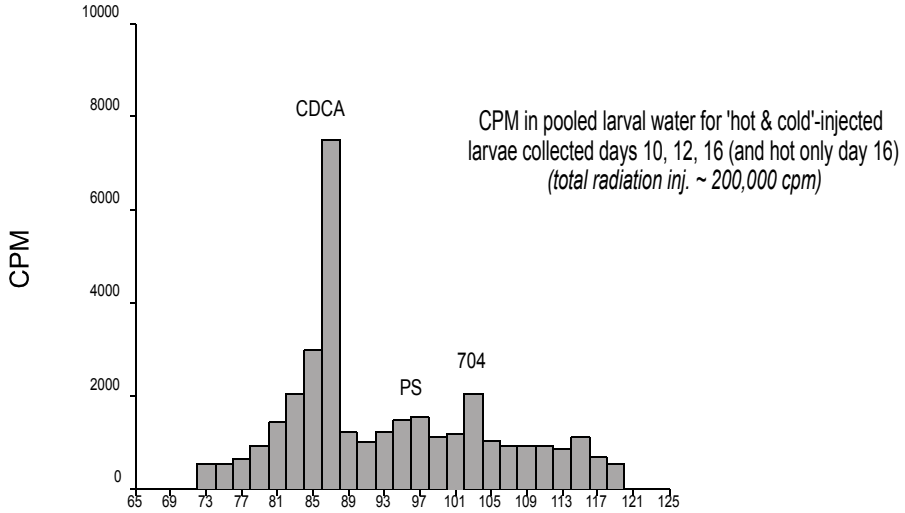


Figure 14. Counts per minute (cpm) of tritium in HPLC fractions of holding water from larvae injected with radio-labeled cholesterol. The retention time of PS in this run was 96 min and for 704 was 103 min as determined by standards. PS and 704 would both appear to be cholesterol derivatives.