

USING PHEROMONE TO ATTRACT FEMALE SEA LAMPREY (*Petromyzon  
marinus*) IN THE PRESENCE OF AMBIENT MALES

A Thesis Presented

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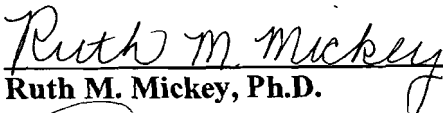
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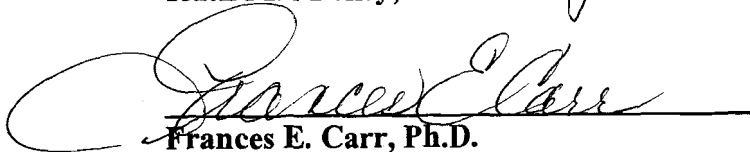
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## Abstract

Female sea lamprey (*Petromyzon marinus*) seek males through chemical cues of a sex pheromone. Female choice experiments in the Great Lakes indicated females were responsive to sex pheromone in half of the trials and could differentiate among concentrations of sex pheromones emitted from traps; i.e., females entered traps with higher concentrations. Those results were corroborated in experiments with Lake Champlain sea lamprey. However, none of these studies conducted experiments in the presence of background pheromone, which would be present under natural conditions. We conducted female choice experiments in which we stocked mature males (n = 3-9) for the purpose of providing competing pheromone in a 50-m stream enclosure in Malletts Creek, a tributary to Lake Champlain,. The equivalent of pheromone released by 0, 1, 3, 9, or 27 males was pumped through a lamprey pot 35 m upstream of a release cage containing a female. In each trial, lasting a maximum of 1 hour, a female was released and her behavior and movements were recorded through visual observations and antenna readings. Females swam to a pheromone source or swam upstream moving rocks in 17 of the 38 trials. Six of those females approached ambient males, six approached the lamprey pot, and four entered the lamprey pot. The greatest proportion of females approached the lamprey pot when the pheromone we applied was greater than that produced by ambient males. Although females were attracted to male pheromone in 17 trials, the positive response rates in these trials were lower (< 50%) than in previous experiments without ambient males. We conclude that a portion of females could be attracted to traps in an effort to provide some reduction in population sizes of sea lamprey. However, the ability to adequately capture the majority of females in this manner remains elusive.

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## **Chapter I: Testing female response to traps baited with male sex pheromone in the presence of background male sex pheromone.**

### **Abstract**

Female sea lamprey (*Petromyzon marinus*) seek males through chemical cues of a sex pheromone. Female choice experiments in the Great Lakes indicated females were responsive to sex pheromone in half of the trials and could differentiate among concentrations of sex pheromones emitted from traps; i.e., females entered traps with higher concentrations. Those results were corroborated in experiments with Lake Champlain sea lamprey. However, none of these studies conducted experiments in the presence of background pheromone, which would be present under natural conditions. We conducted female choice experiments in which we stocked mature males (n = 3-9) for the purpose of providing competing pheromone in a 50-m stream enclosure in Malletts Creek, a tributary to Lake Champlain. The equivalent of pheromone released by 0, 1, 3, 9, or 27 males was pumped through a lamprey pot 35 m upstream of a release cage containing a female. In each trial, lasting a maximum of 1 hour, a female was released and her behavior and movements were recorded through visual observations and antenna readings. Females swam to a pheromone source or swam upstream moving rocks in 17 of the 38 trials. Six of those females approached ambient males, six approached the lamprey pot, and four entered the lamprey pot. The greatest proportion of females approached the lamprey pot when the pheromone we applied was greater than that produced by ambient males. Although females were attracted to male pheromone in 17 trials, the positive response rates in these trials were lower (< 50%) than in previous experiments without ambient males. We conclude that a portion of females could be attracted to traps in an effort to provide some reduction in population sizes of sea lamprey. However, the ability to adequately capture the majority of females in this manner remains elusive.

## Introduction

Parasitism by sea lamprey (*Petromyzon marinus*) has contributed to population declines and difficult restoration of salmonids in Laurentian lakes (Cornelius et al. 1995; Elrod et al. 1995; Eshenroder et al. 1995; Holey et al. 1995; Marsden et al. 2003). Management of sea lamprey relies heavily on lampricides, but also employ trapping, migration barriers, and sterile male release to control these sea lamprey populations (Krueger and Marsden 2007). However, future sea lamprey management will rely on the development of new and alternative sea lamprey controls (Li et al. 2007) for reasons such as public scrutiny, increasing costs, and non-target mortalities associated with pesticides (Brege et al. 2003).

Sea lamprey sex pheromones are a promising sea lamprey control tool (Li et al. 2007). Sex pheromone consists of the bile acids 3-keto-petromyzonol sulfate (3kPZS) and 3-keto allocholic acid (3kACA) (Li et al. 2002; Siefkes et al. 2005) that are released through the gills of spermiating male sea lamprey (Siefkes et al. 2003b). At sexual maturity (ovulation), the olfactory system of female sea lamprey becomes highly sensitive to these bile acids (Li et al. 2002; Siefkes et al. 2005). A first step in testing the effectiveness of pheromones as a control was evidenced in two-choice maze experiments where female sea lamprey were attracted to and spent more time swimming in male pheromone than in blank water (Teeter 1980; Li et al. 2002; Li et al. 2003; Siefkes et al. 2003a, b; Siefkes and Li 2004; Siefkes et al. 2005). These initial results suggested that

attraction of ovulating females to pheromones could possibly be used to improve trapping effectiveness (Li et al. 2007).

The laboratory results led to experiments in streams that were free of sea lamprey and thus competing pheromone resources. Females were given a choice between two traps of equal distance from a female release cage. One trap contained a pheromone source (spermiating males or their washings) and the other did not (Johnson et al. 2005, 2006). Females did not enter traps devoid of a pheromone source, but entered pheromone traps at rates between 52% and 74%, demonstrating that male sex pheromone can attract ovulating females into traps in the field where stream dynamics and habitat complexity complicate female choice (Johnson et al. 2005, 2006).

Female choice experiments then advanced to using multiple pheromone sources of varying concentrations from traps placed equal distance from the female release cage, which tested for a threshold response to pheromone concentrations. Females that made a choice tended to choose the traps with the highest density of spermiating males. Trapping rates varied from 48% to 57% (Wagner et al. 2006). Next, females were exposed to a cumulative pheromone plume in experiments using three traps set in a downstream sequence ten, five, and one males (Wagner et al. 2006). Females tended to choose the first two pheromone sources encountered (one male and five males) over the upstream trap (ten males) with an overall trapping rate of 43% (Wagner et al. 2006). Thus, cumulative concentration of pheromones downstream can alter which traps females

choose to enter (Wagner et al. 2006). Interestingly, with increasing complexity of these pheromone experiments, trapping rates of females have declined

All of the previous field experiments were conducted in the Great Lakes where sea lamprey are an exotic species. Lake Champlain sea lamprey populations, likely native and genetically differentiated from Great Lakes populations (Bryan et al. 2005; Waldman et al. 2006), may respond differently in similar experiments. Starting in 2004, behavioral responses of female sea lamprey to pheromones were tested in simple female choice experiments similar to Wagner et al. (2006) in a Lake Champlain tributary. Females were given a choice of entering traps containing 0 vs 1, 1 vs 10, or 10 vs 20 spermiating males. Results showed that females always entered the lamprey pots containing the greater number of males (D.J. Hitchcock and D.L. Parrish, unpublished data). These experiments were all conducted in stream reaches with no competing background pheromone. The next obvious step was to test female choice between different concentrations of male pheromones in the presence of ambient male sea lamprey. Therefore, the goals of this study were (1) to determine if ovulating female sea lamprey would respond positively to male pheromone plumes from lamprey pots in the presence of freely swimming spermiating males, and (2) to determine which concentration of spermiating male washings was most effective at attracting females in this setting.

## Methods

### Experimental animals and pheromone collection

Migrating sea lamprey in Lake Champlain tributaries were caught in portable assessment traps (PATs) and by hand from a waterfall in April through May 2007. Sea lamprey were held instream, when possible, to encourage sexual maturation (Johnson et al. 2006). Animals were moved to laboratory flow-through tanks when they became stressed in the field by high temperatures. Females that displayed secondary sex characteristics (Applegate 1950) and released eggs upon gentle pressure to the abdomen were classified as mature (Siefkes et al. 2003b). Mature females were held in laboratory water  $\leq 8$  °C to slow senescence (Siefkes and Li 2004) or were used for experimentation. Males that displayed secondary sex characteristics and released ejaculate (Siefkes et al. 2003b) containing motile sperm were classified as mature (B. Young, USFWS, personal communication). These males were stocked in the site as ambient males or were used for obtaining pheromone washings

We collected washings under the assumption that mature males released pheromone at a rate of 250  $\mu\text{g}$  3kPZS/fish/h (Yun et al. 2002). We held pheromone-releasing males in 80 L of aerated lab water that ranged from 14 to 22 °C. Each batch, containing four to nine males, was held for a period of time (range = 17-45 h) that was estimated to obtain a concentration of 0.75 male-h/L. To calculate this concentration, we multiplied male density by the number of hours males were held and divided by 80 L. By using washings, we could imitate pheromone release of specific male densities, which we

defined as male equivalents. We attempted to imitate the pheromone release of 1, 3, 9, and 27 males. A peristaltic pump (Masterflex, Vernon Hills, IL) was used to deliver washings from a streamside container through the lamprey pot, which had an adjustable feed rate to accommodate the estimated concentrations. Two 1.5-ml water samples were taken from each batch of washings for analysis of 3kPZS concentration using Liquid Chromatography/Mass Spectrometry (assay) in the lab of Weiming Li at Michigan State University.

### **Experimental site**

Experiments were conducted in Malletts Creek (73° 8' 20.874" W, 44° 34' 19.287" N), a small tributary (0.09 m<sup>3</sup>/s) to Lake Champlain that flows into Malletts Bay. Malletts Creek contains a natural population of sea lamprey in the lower reaches, but not above a waterfall. However, our study site above the waterfall contained spawning substrate and was therefore considered capable of supporting sea lamprey. We stocked pheromone-releasing males within the site to provide a source of ambient pheromone. We maintained between three and nine ambient males within the site at all times.

The experimental site was a stream reach 50 m long and was approximately 12 m wide throughout most of the reach (Fig. 1). Both ends of the reach were enclosed by portable assessment traps (PATs, opening= 0.50 m x 0.50 m) with attached plastic mesh wings that formed a bank-to-bank barrier. Passive integrative transponder (PIT) antenna arrays (Oregon RFID, Portland, OR) were installed 1 m outside of the barriers. An opaque lamprey pot (aqua-colored PVC; length = 1.5 m, dia.=0.25 m) was placed in

spawning substrate in the middle of the stream, 15 m downstream of the upper barrier. A PIT antenna array was positioned horizontally on the substrate surrounding the lamprey pot. A release cage was placed 35 m downstream of the lamprey pot (Fig. 1).

### **Conducting experiments**

All experimental animals were surgically implanted with PIT tags (Texas Instruments®, Plano, TX), except for 10 deteriorating females during the last five days of trials. For an external mark to aid in visual observation of females, we inserted fluorescent flagging through the dorsal area. Twelve hours prior to experimentation, males used for background pheromone were released within the experimental site and females were placed in an acclimation cage.

We tested PIT antenna arrays daily and downloaded their data to monitor approaches to the lamprey pot and to record animals that escaped the site. We also recorded tag numbers of ambient males captured by hand and in PATs daily, to monitor how many males were present within the entire site. Water velocities were recorded daily (Swoffer ® model 2100 flow meter, Seattle, WA) at the entrance and 0.5 m below the entrance to the lamprey pot (funnel), and directly in front of the release cage (Fig. 1). Stream temperatures were recorded at the start of each trial.

We ran trials in a randomized block, each consisting of a single trial at each concentration of male equivalents (1, 3, 9, and 27) and a control of blank laboratory water. All trials within a block used washings from the same batch, which ensured that we created the proposed concentrations of male equivalents. When a block was begun,

we continued running trials until washings were depleted. If the washings were not fully consumed, we began a new block of trials that day, running between two and seven trials each trial day. If we did not have females for additional trials, the remaining washings were disposed of.

Trials were conducted between 0900 and 2000 h from 2 June to 15 July 2007. Stream temperatures ranged from 20.0 to 27.4 °C. Before each trial, a single female was acclimated for 30 min in the release cage to a randomly chosen male equivalent. Upon release, a female was given a maximum of 1 h to respond. In previous experiments, females usually entered traps within 40 min of release within 65 m of a trap or lamprey pot (Johnson et al. 2005, 2006; D.J. Hitchcock and D.L. Parrish, VTCFWRU, unpublished data). We observed female behavior and recorded whether a female swam within 0.50 m of an ambient male or the lamprey pot and if the female remained with an ambient male or entered the lamprey pot. Females were considered responsive if they exhibited searching behavior or swam to a pheromone source (Li et al. 2002; Siefkes et al. 2003a, b). Unresponsive behaviors consisted of lying motionless in the release cage or on the substrate immediately after leaving the release cage, searching the downstream barrier, entering the lower PAT, or emigrating from the site. If we did not have five mature females to independently test each concentration within a block, we reused females. Responsive females were chosen for reuse over unresponsive females to determine if they would respond differently to other concentrations of pheromone. When



responsive females were unavailable, unresponsive females that were energetic or first used in a control trial were reused.

## **Results**

We conducted 38 trials, which included pheromone applications (N=32) and controls (N=6). In 27 trials, females were used for the first time and 11 trials used females run more than once. Behavior of multi-use females tended to differ among uses, indicating there were no learned responses. Consequently, we pooled data from first-use and multi-use females in the analysis. Females responded positively in 17 trials and were unresponsive in 21 trials (Table 1). Females approached ambient males in seven trials, remained with ambient males in six trials, approached the lamprey pot in seven trials, and entered the lamprey pot in four trials. Over half (59%) of the responsive females remained with ambient males or entered the lamprey pot.

Of the unresponsive females, 16 of the 21 left the release cage, but either rested on the substrate (n = 9) or searched the lower barrier (n = 7). All 17 responsive females left the cage within 20 min of opening the release door. Females who approached the lamprey pot swam directly upstream without approaching any ambient males. However, one female left an ambient male and swam toward the lamprey pot. The seven females that approached ambient males either swam directly to an ambient male or swam upstream and then returned back downstream to an ambient male. In three trials, females were designated as responsive because they exhibited behaviors such as rock moving and

persistent upstream movement, but they did not approach an ambient male or the lamprey pot.

There was no relationship ( $R^2 = 0.008$ ) between our estimated 3kPZS concentrations in the male washings and those estimated concentrations in samples analyzed by LC/MS post-experiment (Fig. 2). Consequently, we could not use male equivalents in the behavior analysis. Therefore, we used the ratio of applied instream pheromone to ambient pheromone as the measure of concentration. We calculated the ratio of the instream concentration of applied pheromone (assay) to the instream concentration of pheromone created by ambient males. We estimated instream concentrations of ambient pheromone by multiplying the number of males present by the average male release rate of 3kPZS. Females approached the pot at higher pheromone ratios than they approached ambient males (Fig. 3). At ratios  $< 1$  more females approached ambient males, and at ratios  $> 1$  more females approached the pot (Table 1). The only female to leave an ambient male was exposed to a pheromone ratio  $> 1$ .

Females showed responsive behavior at discharges between 0.02 and 0.25 m<sup>3</sup>/s. Females swam within 0.5 m the lamprey pot at water velocities immediately below the pot (approach velocity) between 0.17 and 0.7 m/s, but did not enter the lamprey pot when approach velocities were  $> 0.2$  m/s. Females approached the lamprey pot at funnel velocities between 0.07 and 0.4 m/s, but did not enter the pot at funnel velocities  $> 0.09$  m/s. Velocities at the release cage, ranging from 0.03 to 0.18 m/s, did not have any detectable effects on female responsiveness.

We tested for differences in the amount of time females took to approach or search around the lamprey pot in relation to water temperature, velocity, and discharge, and pheromone concentrations. There were no differences in approach or search times related to water temperature, velocity, or discharge. There were also no differences in search times around the lamprey pot related to pheromone concentration. However, longest search times generally occurred at high water velocities at the funnel (entrance to the lamprey pot) and at approach velocities. Search times tended to increase with increased densities of ambient males (Fig. 3), increased application rates of 3kPZS (Fig. 4), and instream concentrations of applied 3kPZS (Fig. 5).

### **Discussion**

Our results show that mature females will approach and enter a lamprey pot emitting male sex pheromone in the presence of mature ambient males. Seven females (18%) approached a lamprey pot and four (11%) entered. However, the remainder of responsive females (16%,  $n = 6$ ) chose an ambient male. In experiments with no background pheromone, 74% of females entered a trap emitting pheromone instead of one that did not (Johnson et al. 2005), however, only 43% of females entered traps when given a choice of three traps exuding pheromone (Wagner et al. 2006). A similar percentage of the females were responsive (45%) in our experiments, suggesting that the addition of multiple sources of pheromone; i.e. the presence of ambient males, reduces the likelihood of mature females selecting a single source in any trial.

Female choice of ambient males or the lamprey pot was marginally related to pheromone concentration. In previous studies, responsive females typically chose a greater concentration of pheromone rather than a lesser one (Wagner et al. 2006; D. Hitchcock and D. Parrish, VTCFWRU, unpublished data). Applied concentrations (assayed) greater than ambient concentrations (estimated) attracted the greatest number of responsive females to the pot. In contrast, when ambient instream pheromone concentrations (estimated) were greater than applied instream concentrations (assayed) more females approached ambient males. In experiments testing female response to pheromone applied in pulses, females exhibited a loss of orientation by swimming side to side during the off cycle (Johnson et al. 2006). Three females in this study exhibited behavior similar to the on-off cycle experiments when ambient concentrations were greater than applied concentrations and during one control trial. The downstream side-to-side behavior ended when females approached an ambient male, suggesting that the females lost track of a pheromone plume or detected a decrease in pheromone concentration after swimming upstream of an ambient male. The idea that females seek the greatest concentration of pheromone is further supported by the observation that females returned downstream to an ambient male (i.e., the greater pheromone concentration).

However, females did not choose the greatest concentration in every trial. Interactions among pheromone plumes, temperature, and stream velocity may have also affected female choice. For instance, a female directly below both an ambient male and

the lamprey pot may detect both pheromone sources as one. This is described as an additive effect in experiments where females were given a choice among three male baited traps (Wagner et al. 2006). The traps were set in a downstream sequence containing ten, five, and one males. Females entered the first two traps encountered, with 1 and 5 males, more frequently than the upper-most trap baited with ten males.

High temperatures and velocities may affect the perception, and thus, responses of females to various sources of pheromone. Females in trapping experiments are likely most responsive to applied pheromone at temperatures from 20 to 23 °C (N. Johnson, Michigan State University, pers. comm.). No females approached the lamprey pot at temperatures > 23.9 °C, but 2 (14 %) responsive females approached ambient males swimming below the lamprey pot at temperatures at 26.9 °C and 27.4 °C. As for water velocity, female choice between ambient males and the lamprey pot did not vary with velocity, and females often spawn in velocities as high as 1 m/s (Applegate 1950). However, the two females who approached the lamprey pot and did not enter experienced funnel velocities more than four times greater than the four females who entered the lamprey pot. High funnel velocities were also associated with long search times.

Long search times of the lamprey pot were also associated with high densities of ambient males and high concentrations of applied pheromone. Ambient males were not detected in close proximity to the lamprey pot when females searched the entrance, indicating that ambient males were unlikely to have affected search time. However, females often stop upstream movement and initiate search behaviors upon approaching a

constant source of pheromone. Searching is generally concentrated on outside walls and in the entrance of a trap with mesh walls (Johnson et al. 2005, 2006); however, the lamprey pot we used had solid walls. Consequently, pheromone was only exuded through the entrance, which explains why females spent time searching the entrance specifically. However, the searching behavior at the lamprey pot does not explain long search times at high pheromone concentrations. A specific concentration of pheromone or a threshold may exist that affects female searching behavior, but our observations suggest that high velocities through the entrance funnel were an obstacle and increased search times. Pheromone thresholds need further exploration and pots or traps should be designed to reduce high velocities in the entrance. The time females spend in spawning habitat needs to be minimized because the likelihood of successful spawning increases as time spent in the spawning grounds increases.

Clearly, sex pheromone can be used to attract mature females to a trap or lamprey pot; however, response to the pheromone is extremely complex. We chose to use the washings of mature males because live males were not consistently available, synthetic 3kPZS is very expensive (Krueger and Marsden 2007), washings of mature males are more attractive to females than synthetic 3kPZS (Siefkes et al. 2005), and, similar to synthetic 3kPZS, application of washings can be administered at calculated rates (Johnson et al. 2006). However, variations in our presumed concentrations of pheromone using a standard value supports findings that spermiating males release a range of pheromone concentrations (Yun et al. 2002). Many factors, e.g., high water temperature,

timing of maturation, and handling stress of animals must affect how much pheromone a male produces over a short time period.

Our results indicated that female sea lamprey exhibit lower positive responses to male sex pheromone emitting from a lamprey pot in the presence of background pheromone than when background pheromone was not present. Thus, any anticipated use of male pheromone in management scenarios to attract and trap females prior to spawning will need further development of methods to collect predictable concentrations of male sex pheromone. Possibly, an inexpensive synthetic pheromone that contains properties similar to male washings would be a better option for management applications because of allowing for the control of pheromone concentration. In addition, an assay to quickly and accurately estimate 3kPZS concentrations produced by ambient males would improve the likelihood of applying accurate concentrations of pheromones needed to attract females. We estimated ambient 3kPZS concentrations similarly to washing estimates, by multiplying known male densities times an average male release of 250  $\mu\text{g}$  3kPZS/fish/h (Yun et al. 2002) and then calculating instream molarity. The ability to measure actual instream 3kPZS concentrations would allow managers to apply synthetic sex pheromones at concentrations higher than those produced by ambient males.

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*Table 1. Responsive females and their behaviors during control and at four categories of ratios of instream 3kPZS concentrations from washings (assay) to instream 3kPZS concentration released by ambient males based on an average release rate of 250 µg 3kPZS/fish/h.*

<b>Applied:Ambient</b>	<b>Approach ambient</b>		<b>Remain w/ambient</b>	<b>Approach lamprey pot</b>		<b>Enter lamprey pot</b>
<b>Control</b>	2	→	2	0	→	0
<b>0.0001-0.33</b>	1	→	1	2	→	2
<b>0.34-0.99</b>	1	→	1	1	→	1
<b>1.00-3.00</b>	2	→	1	2	→	1
<b>3.01-12.288</b>	1	→	1	2	→	0
<b>Responsive total</b>	7			7		

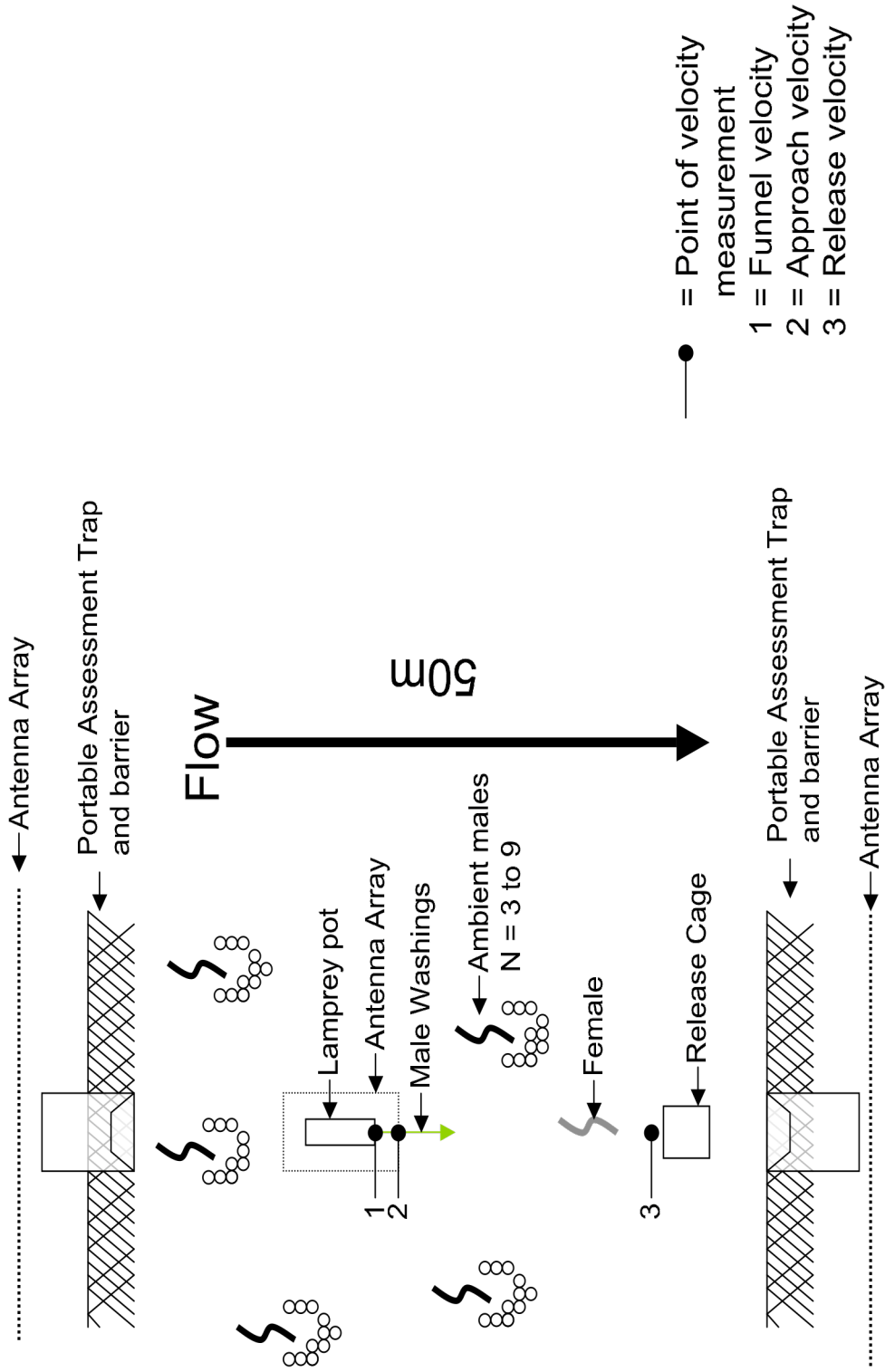
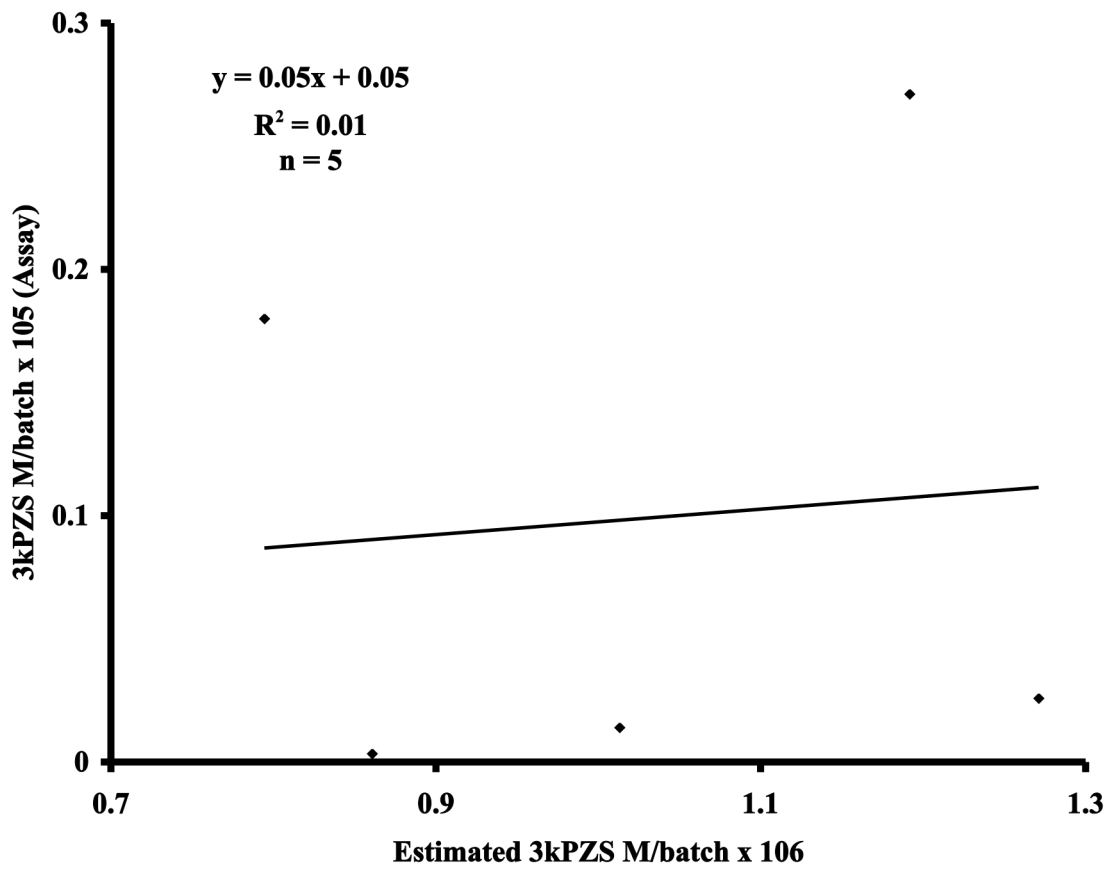
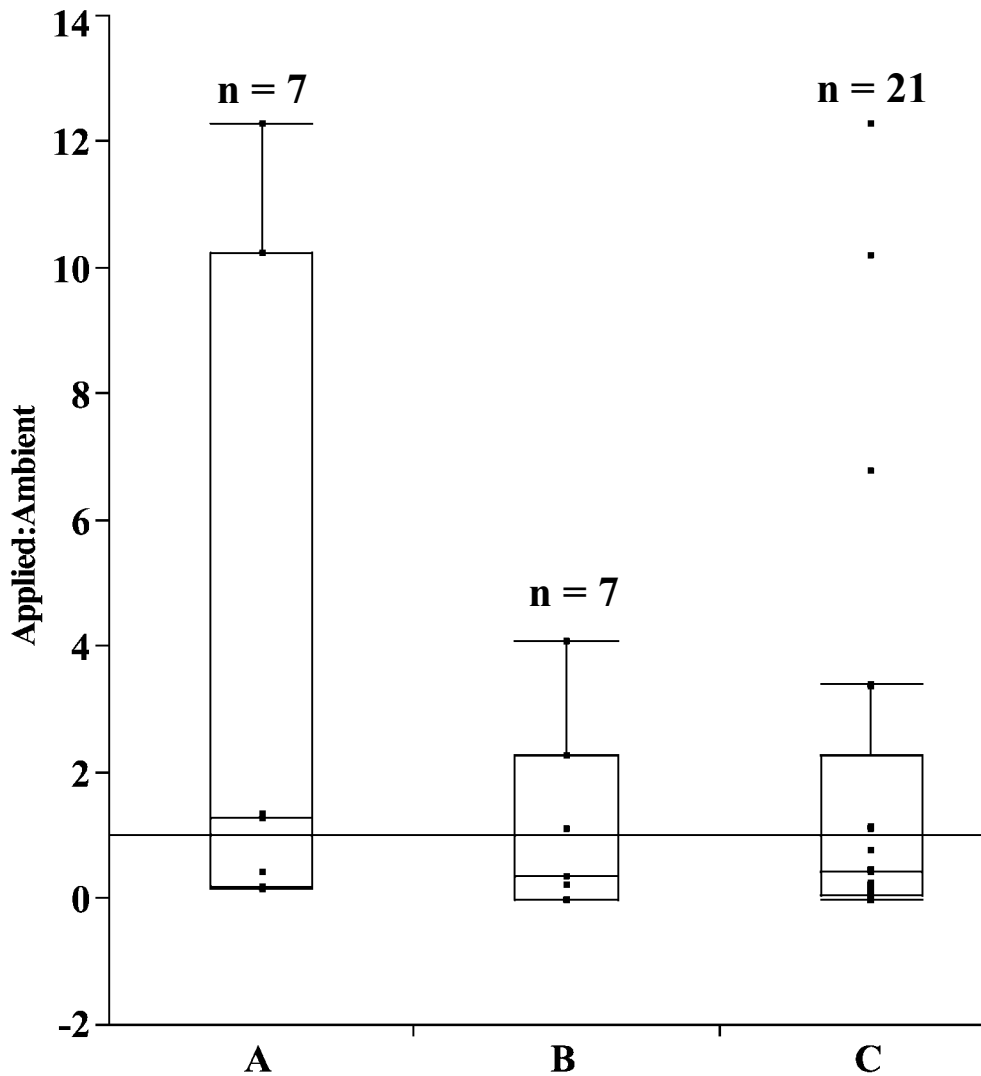


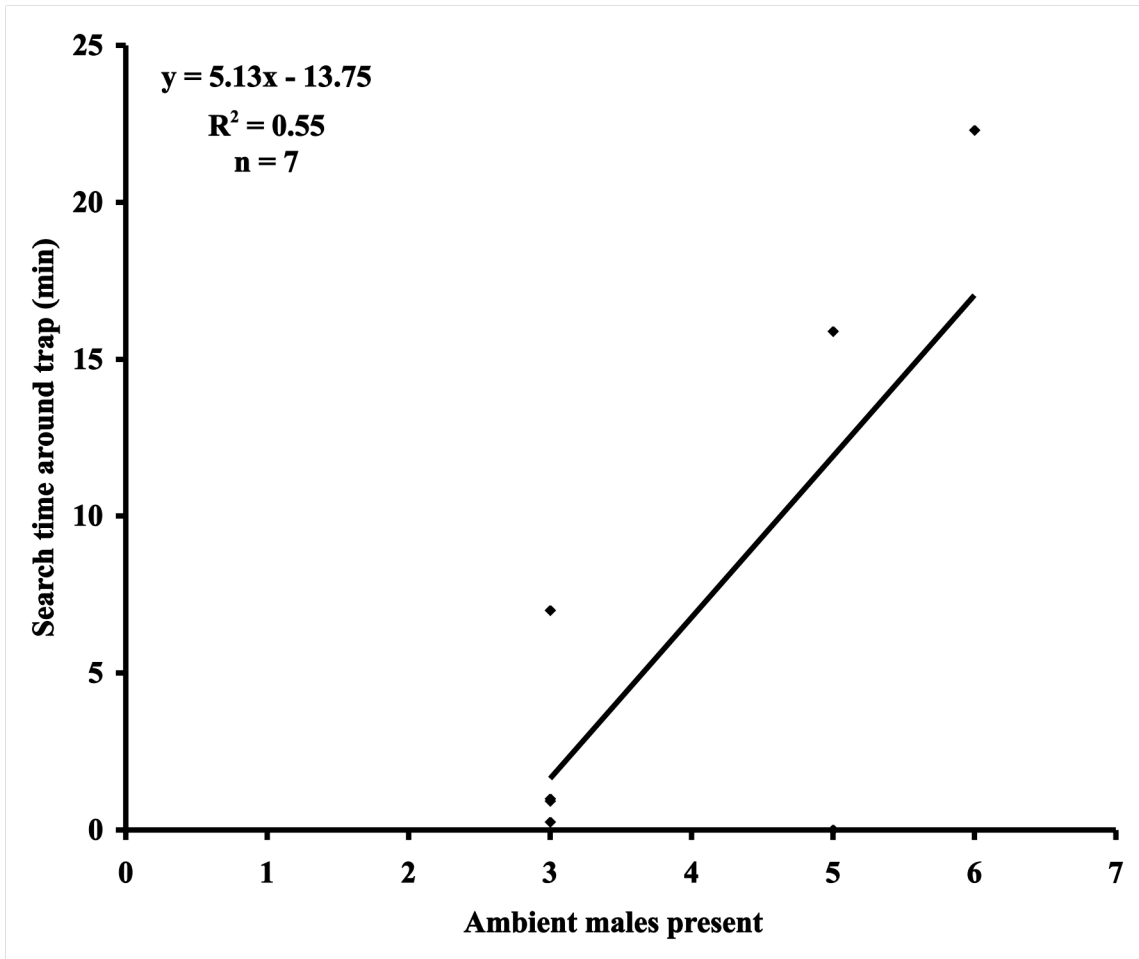
FIG. 1. Conceptual diagram of the study site showing the placement of the pot relative to the release cage. Numbered points refer to locations of stream velocity measurements. Dashed lines refer to antenna arrays. Ambient males and their nests are shown to be distributed throughout the site as they may be during the experiment.



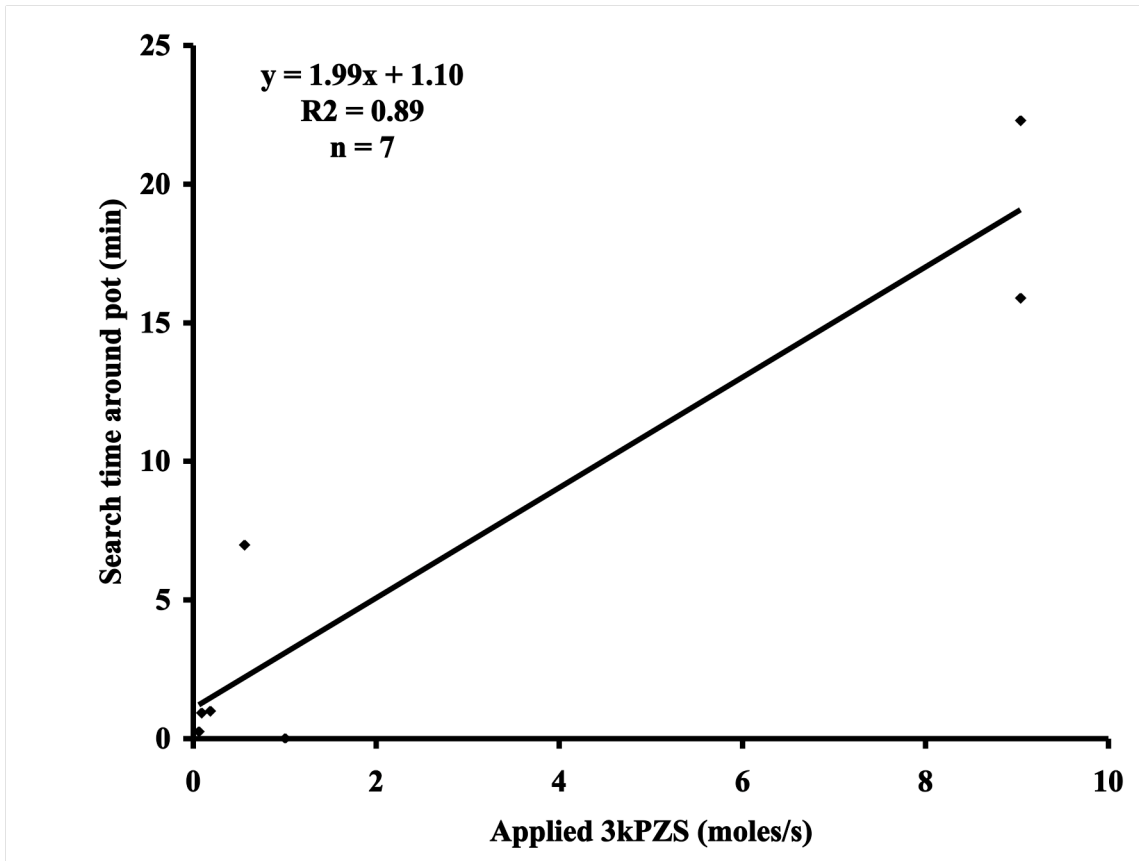
**FIG. 2.** *Estimated 3kPZS M (250 µg 3kPZS/fish/h) in relation to the assayed 3kPZS M in each batch of male washings.*



**FIG. 3.** Ratio of instream 3kPZS concentration created by washings (assay) application to instream 3kPZS concentration released by ambient males (250 µg 3kPZS/fish/h) in trials where females approached the pot (A), approached ambient males (B), and were unresponsive (C). For reference, a horizontal line is where applied:ambient equals 1. The points are individual female responses. The horizontal line through the box is the median. The ends of each box are the 25<sup>th</sup> and 75<sup>th</sup> percentiles. The end of the upper whisker is the 90<sup>th</sup> percentile and the end of the lower whisker is the 10<sup>th</sup> percentile. All points beyond the whiskers are outliers.

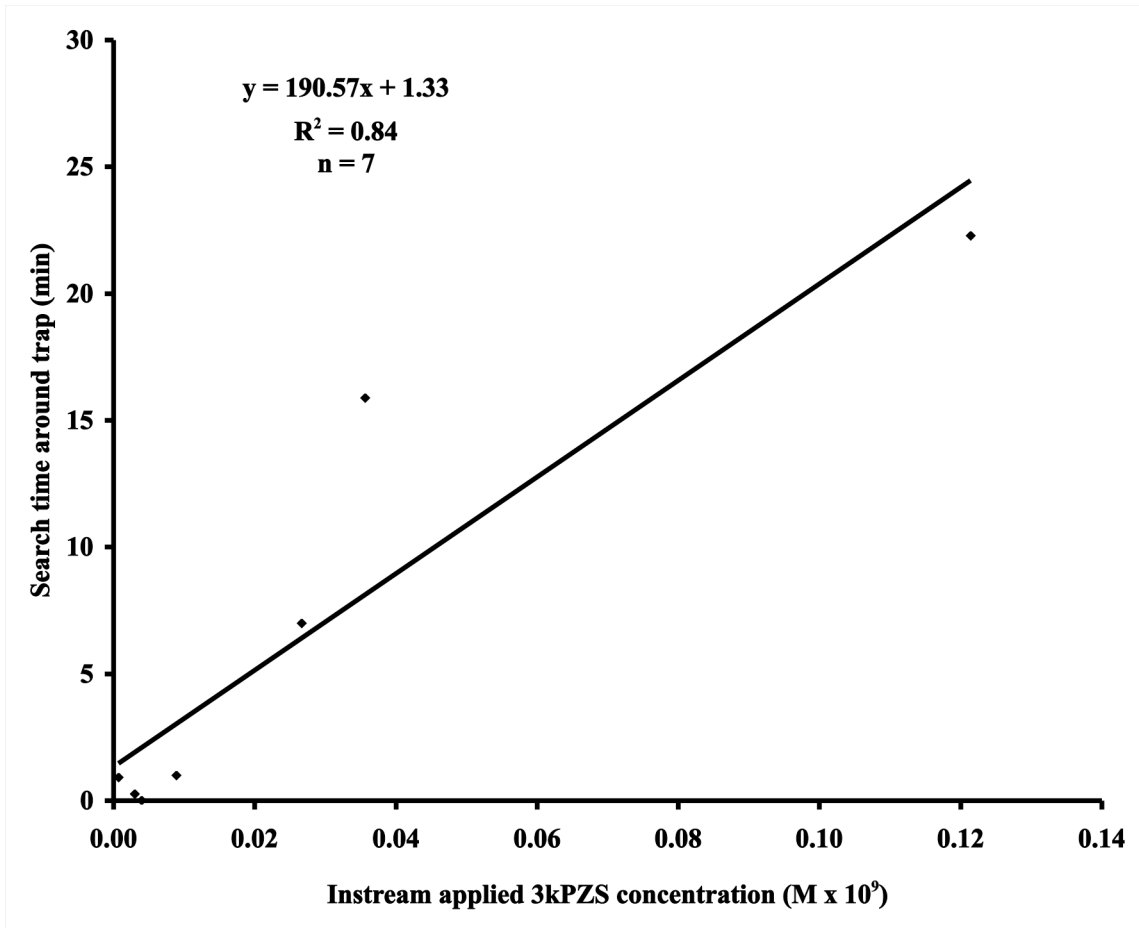


*FIG. 4. Female search time (min) around the lamprey pot in relation to the number of ambient males present in the stream. Number of trials were 7.*



*FIG. 5. Female search time (min) around the lamprey pot in relation to the application rate of 3kPZS (moles/s  $\times 10^9$ ).*





**FIG. 6.** Female search time (min) around the lamprey pot in relation to instream concentration of applied 3kPZS (M x 10<sup>9</sup>).

## Literature Review

### Introduction

Agnatha are a primitive group of jawless hagfishes (Myxiniiformes) and lampreys (Petromyzoniformes) (Hubbs and Potter 1971) that appeared in the fossil record in the mid-Pennsylvanian period (Bardack and Zangerl 1971). The roughly 40 species of Petromyzoniformes comprise three families: Mordaciidae and Geotriidae of the southern hemisphere, and Petromyzonidae of the northern hemisphere (Hubbs and Potter 1971). Petromyzonidae have a mostly holarctic distribution (Hubbs and Potter 1971) with *Lampetra geminis* and *L. spadicea* making the exception in the highlands of central Mexico (Hubbs and Potter 1971).

The anguillid body form of lamprey lacks paired fins and the seven external gill openings are deprived of opercle protection and pumping efficiency. The body is completely void of osseous tissue and cartilage protects the brain and notochord (Hubbs and Potter 1971). The jawless mouth, or buccal funnel, is sub-terminal and lined with rows of teeth. The tongue protrudes the center of the funnel and is plated with two rows of teeth on its dorsal side (Hardisty and Potter 1971b).

The simplistic design that has sustained lamprey populations through 280 million years of global changes is not currently maintaining stable populations. The Pacific lamprey (*L. tridentate*), known as *ksuyas* to Pacific coast tribes, is a protected species in the state of Oregon (Close et al. 2002). The northern brook lamprey (*Ichthyomyzon fossor*)

is endangered in the state of Vermont (Lake Champlain Fish and Wildlife Management Cooperative 1999) and even sea lamprey (*Petromyzon marinus*) numbers are diminishing in some native habitats (Oliveria et al. 2004). However, sea lamprey do not require protection in all systems and their populations are controlled in the Great Lakes and Lake Champlain.

### **Sea lamprey life history**

Sea lamprey have a multi-stage life cycle similar to that of Pacific salmon; beginning life in a stream, developing in a large body of water, returning to a stream to spawn and die. Larval lamprey spend three to seven years in the stream (Hardisty and Potter 1971a), filter feeding microorganisms through the oral hood that protrudes from their burrow (Applegate 1950). Towards the end of the larval period, sea lamprey experience several transformations. The gill openings are modified and the open oral hood closes to form a suction disc or buccal funnel (Applegate 1950; Hardisty and Potter 1971a). A new foregut forms to accommodate the new feeding mechanism (Hardisty and Potter 1971a). The eye becomes highly developed and the dorsal fin becomes more pronounced, and there is a change in coloration (Applegate 1950).

Starting in late October, high stream discharges spur the newly transformed sea lamprey to begin a downstream migration (Applegate 1950). Anadromous, coastal populations end their migration in the ocean, while landlocked potamodromous populations end their downstream migration in large lakes such as the Laurentian Great

Lakes and Lake Champlain (Applegate 1950). After the out-migration, transformed sea lamprey begin the third life stage.

The third phase is a parasitic phase where lamprey target large-bodied fish. Potamodromous sea lamprey have a preference for lake trout (*Salvelinus namaycush*), but they also feed on several other species of fish (Hardisty and Potter 1971b). The parasitic phase lasts 12 to 20 months, after which sea lamprey begin their single spawning migration (Applegate 1950).

The spawning migration begins in early April when they search for streams containing suitable spawning habitat (Applegate 1950; Teeter 1980; Li et al. 1995). Once they find a stream and before the onset of spawning activities, they spend six to eight weeks avoiding daylight by hiding under substrate and stream features (Applegate 1950). Males reach the spawning grounds first and initiate nest building in sand and gravel substrates with stream velocities between 0.4 m/s and 1.6 m/s; sea lamprey then pair, spawn, and die (Applegate 1950).

### **Justification of sea lamprey management**

The loss of large predators can cause many changes in the trophic structure of a system and result in the establishment of large stocks of introduced or invasive species (Jude and Leach 1999). Introduced or invasive fishes can facilitate additional invasions as with the invasional meltdown model of the Great Lakes (Simberloff and Von Holle 1999; Ricciardi 2001). Lake Champlain fishes including lake sturgeon (*Acipenser*

*fulvescens*), landlocked Atlantic salmon (*Salmo salar*), and lake trout (*Salvelinus namaycush*) were over harvested in the 1800's (Halnon 1963; Carlson 1995) and faced other challenges such as dam construction. Lake trout and landlocked Atlantic salmon were extirpated by the late 1800's and are now maintained by stocking (Carlson 1995; Marsden et al. 2003).

Until recently, it was thought that sea lamprey were an invasive species in Lake Champlain. Lamprey were not recorded in Lake Champlain until 1841 and sea lamprey were not confirmed in the lake until 1929 (Greeley 1930; Halnon 1963; Marsden et al. 2003). Routes of possible entry included the Champlain and Chambly canals or even introduction of ammocoetes used for bait by fishermen (Daniels 2001). Despite the invasive characteristics of Lake Champlain sea lamprey, genetic studies suggest that they are native (Bryan et al. 2005); entering Lake Champlain through the St. Lawrence River after the glaciers of the Wisconsin Age receded (Underhill 1986; Waldman et al. 2006).

Twenty-two Lake Champlain tributaries currently contain larval sea lamprey (Howe et al. 2006). It is suggested that deforestation in the Lake Champlain watershed may have increased sediment loads that are suitable for ammocoetes. Deforestation in the Lake Ontario watershed created optimum substrate for larval lamprey, which coincided with sea lamprey population increases in the lake (Jude and Leach 1999). As with most semelparous fishes, Lake Champlain sea lamprey are highly fecund, producing 70,000 eggs/female (Smith and Marsden 2007). It is possible that human influences

have given the highly fecund sea lamprey a reproductive boost resulting in the invasive behavior of the population.

Parasites generally cause little damage to their hosts and never immediate death, but host fatalities increase with infection rate (Moore 2002). Sea lamprey wounding rates in Lake Champlain approached 75 wounds per 100 lake trout in the 1990s and are currently nearing 100 wounds per 100 lake trout (Lake Champlain Fish and Wildlife Management Cooperative 1999; Marsden et al. 2003). An individual lamprey can destroy up to 18 kg of fish during its parasitic phase (Waldman et al. 2004) and the estimated probability of a single sea lamprey attack killing a lake trout in Lake Champlain is 0.26 (Madenjian et al. 2007). Literature does not contain actual lamprey-induced mortality rates in Lake Champlain or effects of wounds transferred from a lake trout to its fry. However, the population declines and failed restoration of Great Lakes salmonids have been attributed in part to parasitic lamprey predation (Cornelius et al. 1995; Elrod et al. 1995; Eshenroder et al. 1995; Holey et al. 1995).

The Lake Champlain Fish and Wildlife Management Cooperative identified sea lamprey as the obstacle to lake trout and Atlantic salmon restoration and started the eight-year Experimental Sea Lamprey Control Program in 1990 (Marsden et al. 2003). The estimated cost:benefit ratio of the program was 3.48:1, which was estimated to generate 21 million dollars profit (Marsden et al. 2003). Profit is expected to increase if the sea lamprey management program is continued (Marsden et al. 2003).

## **Lamprey management**

Fisheries managers in the Great Lakes use an Integrated Pest Management (IPM) framework called the Integrated Management of Sea Lamprey (IMSL) (Christie and Goddard 2003). IPM is “a decision support system for the selection and use of pest control tactics, singly or harmoniously coordinated into a management strategy, based on cost/benefit analyses that take into account the interests of and impacts on producers, society, and the environment” (Kogan 1998). IMSL aims to maintain sea lamprey at levels where management costs do not outweigh benefits through the use of chemical, biological, and alternative means in a way that combines all available knowledge in a standardized way to strategize management actions and their evaluations (Sawyer 1980; Christie and Goddard 2003).

The sea lamprey life cycle requires multiple forms of control at different stages for successful management. Sea lamprey are currently managed with 3-tri-fluoromethyl-4-nitrophenol (TFM), sterile male releases, stream barriers, and trapping. Sterile male releases are extremely expensive, trapping alone does not control populations, and the Great Lakes Fishery Commission hopes to reduce TFM use by 50% by 2010 (Great Lakes Fisheries Commission 2001). TFM application has also proved to be socially unpopular in Vermont’s tributaries to Lake Champlain. Alternative control methods, such as pheromone attractants, must be explored to meet the serious need for management while considering environmental and budgetary constraints.

## **Pheromone**

A pheromone is a chemical released into the environment that affects the physiology

or behavior of conspecifics (Karlson and Luscher 1959; Sorensen and Stacey 1999; Sorensen and Vrieze 2003). Many animals live in mediums where movement is difficult, visibility is low, and closely related species are numerous. Sex pheromones can be highly advantageous in finding conspecifics efficiently while avoiding hybridization (Sorensen 1996). Pheromones are often emitted as conspecific attractants, examples include insects such as male green lacewing, *Chrysopa nigricornis* (Zhang et al. 2006) and in female cactus moths, *Cactoblastis cactorum* (Heath 2006). Female goldfish, *Carassius auratus*, release pheromones that induce reproductive changes in males (Sorensen 1992; Kobayashi et al. 2002).

Sea lamprey are not homing species or efficient swimmers, and can be carried many miles from their natal stream by their hosts (Bergstedt and Sleelye 1995; Howe et al. 2006). Additionally, searching for spawning grounds is energetically costly after a migration and visual selection of mates is impossible due to the onset of blindness by the start of spawning (Applegate 1950; Manion and Hanson 1980; McKeown 1984). Conspecific pheromones make it possible for sea lamprey to find natal streams and suitable mates successfully (Teeter 1980; Li et al. 1995; Bjerselius et al. 2000; Li et al. 2002; Johnson et al. 2005). These pheromones have been studied for the last two decades and have been identified as migratory pheromones and sex pheromones. The pheromones are bile acids, which are typically used for lipid digestion and absorption in vertebrates (Larson 1980). The identified acids are excreted by larval ammocoetes and sexually mature males (Li et al. 1995; Li et al. 2003). Lamprey cease feeding during



their single migratory and spawning bout making the bile acids unnecessary for digestion at this point (Larson 1980; Li et al. 2003). Consequently, the bile acids can be reallocated through the gills of mature males in high quantities making the acids an ideal sex pheromone source that can be detected from a large distance (Li 2005). The pheromones flow through the nasopharyngeal pore where they are detected on specific receptor sites in the olfactory epithelia of conspecific lamprey (Li et al. 1995; Vrieze and Sorensen 2001; Siefkes and Li 2004; Johnson et al. 2006)

### **Migratory pheromone**

Migratory sea lamprey prefer water in which larval lamprey have been held, relative to water without larvae (Teeter 1980). During the larval phase, sea lamprey excrete bile acid that contains the pheromones petromyzonol (P), petromyzonol sulfate (PS), allocholic acid (ACA), petromyzonamine disulfate (PADS), and petromyzosterol disulfate (PSDS) (Teeter 1980; Yamamoto et al. 1986; Li et al. 1995; Sorensen et al. 2005). Electro-olfactograms show that olfactory systems of migrating sea lamprey are sensitive to PS, ACA, and PSDS and are extremely sensitive to PADS (Li et al. 1995; Sorensen et al. 2005). Despite olfactory sensitivity to the individual pheromone components, whole larval extract induces the largest behavioral response of migratory lamprey (Sorensen et al. 2005). The pheromone indicates suitable spawning and rearing conditions for larval lamprey, thereby attracting migratory lamprey to streams exuding the pheromone (Teeter 1980; Bjerselius et al. 2000; Sorensen and Vrieze 2003; Sorensen et al. 2003; Sorensen et al. 2005; Wagner et al. 2006). However, as the migration

progresses, olfactory sensitivity shifts from migratory pheromone to sex pheromone (Li 1994).

### **Sex pheromone**

Spermiating male sea lamprey secrete large amounts of the bile acid 3-ketopetromyzonol sulfate (3kPZS) and small amounts of the bile acid 3-ketoallocholic acid, (3kACA) (Li et al. 2002; Siefkes et al. 2005). Adult lamprey do not have gall bladders, bile acids are likely produced in the liver where they travel through the hepatic veins, to the heart, and to the gills where they are released through gill epithelia (Youson 1985; Yun et al. 2002; Li et al. 2003; Siefkes et al. 2003b; Li 2005). Electro-olfactograms show that 3kPZS or 3kACA alone stimulates ovulatory females, however they are not as stimulatory individually as water conditioned by spermiating male sea lamprey (Siefkes and Li 2004). The bile acid 3kACA has the same odor as ACA and may play a minor role inducing ovulatory females, retaining females on the nest, and possibly promoting sexual maturation in conspecifics (Li et al. 2002; Siefkes and Li 2004; Li 2005; Siefkes et al. 2005). However, 3kPZS has a unique odor that is 100 times more potent than 3kACA, which explains why ovulating female sea lamprey display preference behaviors for water conditioned by spermiating males and spermiating male washings over blank water (Teeter 1980; Li et al. 2002; Li et al. 2003; Siefkes et al. 2003a; Siefkes and Li 2004; Siefkes et al. 2005). This understanding of male sex pheromone and its components has instigated further research into uses and methods of application.

### **Determination and control of maturation**

Identifying the correct stage of maturation is very important when conducting sea lamprey pheromone research. Sexually mature males develop a dorsal rope from the gills to the dorsal fin (Applegate 1950) and release milt (Li et al. 2002; Siefkes et al. 2003b). Ejaculation of motile sperm (spermiating) is often used as an indicator of male sex pheromone release (B. Young, USFWS, personal communication, February 2007). Sexually mature females have an inflamed keel and will release eggs when gentle pressure is applied down the swollen abdomen (Applegate 1950). Sexual maturation of males and females can be encouraged by holding them in stream water (Johnson et al. 2006). If necessary, lamprey senescence can be delayed by holding them in temperatures at or below 8° C (Siefkes and Li 2004).

### **Pheromone application**

There are three methods of applying male sex pheromones for experimental purposes: spermiating males, spermiating male washings, and synthetic pheromone components (Table 1). When applying sex pheromone through the direct use of spermiating males, the male or males are placed up current of the response subject in a flow-through cage (Li et al. 2002). The use of spermiating male washings requires more preparation than applying through spermiating males, but washings can be frozen and stored (Siefkes and Li 2004). Male washings are collected by placing spermiating males that exude pheromone through their gills into a known volume of aerated water for an

exact amount of time (Table 2). Spermiating male washings and synthetic pheromone can be applied by peristaltic pump at a rate that will expire the volume at the end of an experiment or at a rate that imitates the pheromone release of a specified number of males.

### **Pheromone trapping experiments**

Most in-stream experiments allowed females to approach separate pheromone plumes simultaneously by setting their origins side-by-side and releasing females downstream, where plumes have mixed. In these experiments, females entered portable assessment traps baited with spermiating males significantly more than those baited with fewer or zero spermiating males (Johnson et al. 2005; Wagner et al. 2006). When three portable assessment traps were set parallel to the channel in a downstream sequence containing ten, five, and one spermiating males, females encountered a cumulative pheromone plume and tended to choose the stronger pheromone source (Wagner et al. 2006). Unlike results where females approached separate plumes simultaneously, these females chose the first two traps with less pheromone 88% of the time with an overall trapping rate of 43% (Table 3).

### **Future research**

Spermiating males and their washings both induced search behaviors significantly more than blank water in behavioral experiments in a two-choice maze (Siefkes et al. 2005). Ovulating females were also attracted to different applications of male sex

pheromone in streams naturally devoid of sea lamprey (Table 3, 4). An experiment has not been conducted to compare the trapping rates or responses of ovulating females to spermiating males, spermiating male washings, and synthetic 3kPZS (Johnson et al. 2006). It is also unknown how females will respond to applied pheromone when spermiating males are swimming freely amongst traps, if spawning pairs are present, or larval pheromone is present. Attraction rate and trapping rates decreased gradually as experiments were complicated by the addition of stream conditions, alternative pheromone sources, and multiple pheromone sources (Table 3, 4). This suggests that the addition of ambient pheromone would interfere with applied pheromone and our ability to attract ovulating females into a pheromone baited trap. We must explore the effects of ambient pheromone on trapping success.

### **Passive Integrated Transponders**

Tags should not affect physiology, behavior, or survival of a fish (Guy et al. 1996). Little research has been published on the effects of tagging on sea lamprey. It is known that the ventilation rates of Pacific lamprey stabilize within one hour of handling and surgical implantation of a radio tag (Close et al. 2003). Glucose levels of Pacific lamprey implanted intraperitoneally with 3.4-g radio tags did not differ from control subjects one hour after surgery, but it took as long as four days to recover from implantation of tags as large as 7.4 g (Close et al. 2003). Swim time to exhaustion was significantly less in fish tagged with 7.4-g radio tags one hour after surgery, but not after 24 h compared to control

fish (Close et al. 2003). These results suggest that implanting of small tags intraperitoneally is no more stressful than handling, but animals should be given more than one hour to recover (Close et al. 2003).

Sea lamprey female choice experiments have recently adopted the use of radio frequency identification technology (RFID) to monitor movements of females tagged with passive integrated transponders (PIT) (Wagner et al. 2006). PIT tags are glass-encapsulated transponders that send a unique alphanumeric code to an RFID-reader when activated by pulsed (half-duplex) or continuous (full-duplex) inductions sent by a reader through an antenna (ORFID 2007). Data loggers within the readers can store up to 8-million records that contain tag number, read time, read duration, and date that are in turn transferable to a personal digital assistant and spreadsheet program (ORFID 2007). As tag size increases, read range (Zydlewski et al. 2006) and antenna size can increase (Bond et al. 2007). In addition, larger, half-duplex tags and their reader systems are considerably cheaper (Bond et al. 2007). While stress levels seem to increase with tag size (Close et al. 2003), large half-duplex PIT tags are several magnitudes smaller than the smallest tags tested in Pacific lamprey weighing 0.8 g and are only 31.2 mm x 3.85mm. Half duplex tags are an ideal tag for female choice experiments with lamprey, with minimal physiological effects and allowing us to monitor lamprey movements past specific points in our experiments.

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*Table 1. Studies that have used spermiating males, spermiating male washings, and Synthetic 3kPZS as a pheromone source that elicited responses from ovulatory females.*

Spermiating males	Spermiating Male Washings	Synthetic 3kPZS
Li et al. (2002)	Teeter (1980)	Siefkes et al. (2005)
Johnson (2005)	Johnson (2006)	
Siefkes et al. (2003a)	Wagner (2006)	
Siefkes et al. (2005)	Siefkes et al. (2003a)	
	Siefkes et al. (2003b)	
	Siefkes and Li (2004)	
	Siefkes et al. (2005)	

*Table 2. Methods used to collect washings from spermiating males as pheromone is exuded through gills and the rates they are applied. The following numbers describe experiment types: 1 = maze experiment; 2 = instream experiment; 3 = electro olfactogram experiment; 4 = chamber experiment.*

Studies	Number of Males	Water Volume (L)	Time (hr)	Application rate (ml/min)
Teeter (1980)	1	1	1	instant
Li et al. (2002)	1	10	4	75
Siefkes et al. (2003a)	1	10	4	75
Siefkes et al. (2003b)	1	7	1	n/a
Siefkes and Li (2004)	1	10	4	unknown
Siefkes et al. (2005)	1	10	4	75
Siefkes et al. (2005)	5	100	2	200
Johnson (2006)	5	25	2.5	167

*Table 3. Trapping rate of ovulatory females exposed to spermiating males (SM) and spermiating male washings (SMW) in stream experiments devoid of ambient pheromone from spermiating males and larval ammocoetes. A single asterisk (\*) identifies a multiple trap experiment where the traps were aligned longitudinally in respect to streamflow, while traps in all other experiments were aligned latitudinally in respect to the streamflow.*

Trapping Studies	Application Method	Number of pheromone sources	Trapping Rate
Johnson (2005)	SM	1	0.74
Johnson (2006)	SMW	1	0.52
Wagner (2006)	SM	3	0.57
Wagner (2006)	SM*	3	0.43
Hitchcock and Parrish unpublished	SM	2	0.48



*Table 4. Preference rate and attraction rate of ovulating females exposed to various methods of pheromone application in two-choice maze experiments and two-choice stream experiments. Asterisk (\*) indicates that spermiating males were sterilized and spermiating male washings were collected from sterilized males. Double asterisks (\*\*) indicate that washings were only collected from anterior half of male. Triple asterisk (\*\*\*) indicates that females were attracted to the release site, but did not remain at the site.*

Preference Studies	Two-choice maze		Two-choice stream	
	Application Method	Preference rate	Application Method	Attraction rate
Li et al. (2002)	SM	1.00		
Li et al. (2002)	SMW	0.88		
Siefkes et al. (2003a)	SM*	0.92	SM*	0.67
Siefkes et al. (2003a)	SMW*	0.90		
Siefkes et al. (2003b)	SMW**	0.79		
Siefkes et al. (2005)			SMW	0.70
Siefkes et al. (2005)			Synthetic $10^{-12}$ M 3kPZS***	0.70