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INVESTIGATIONS OF FEMALE PREFERENCE FOR MALE SEA LAMPREY ODORS THAT DIFFER IN CONCENTRATION AND COMPOSITION

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INVESTIGATIONS OF FEMALE PREFERENCE FOR MALE SEA LAMPREY ODORS THAT DIFFER IN CONCENTRATION AND COMPOSITION

By

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ABSTRACT

INVESTIGATIONS OF FEMALE PREFERENCE FOR MALE SEA LAMPREY ODORS THAT DIFFER IN CONCENTRATION AND COMPOSITION

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Fully mature male sea lampreys release a mating pheromone partially comprised of 3-keto-petromyzonal sulfate, and 3-keto-allocholic acid. I hypothesized that females prefer large pheromone signals, and that the most natural pheromone signals would be more attractive than partial signals. First, I tested female response to similar intensity pheromone signals from single males and multiple males in a behavioral maze and found no difference. Next, I tested female attraction to in-stream traps baited with one, three, and six males (10 male treatment), and to traps baited with two, six, and twelve males (20 male treatment) in a cross-channel alignment. Females preferred traps baited with 6 or more males ($F_{[2,48]}=34.63$, $P<0.0001$), indicating a preference for high concentration pheromone and a behavioral threshold for this preference ($F_{[2,48]}=4.15$, $P=0.02$). I tested the 1 vs. 3 vs. 6 male alignment with a diffuse pheromone background. The background signal did not affect female preference for the 6 male trap ($F_{[2,48]}=85.80$, $P<0.0001$). I compared traps baited with 3kPZS or 3kACA to a 10:1 (3kPZS:3kACA) mixture with and without a natural pheromone background. 3kPZS attracted females in all situations, while 3kACA did not. Females spent more time around the odor sources when no background was present ($F_{[1,150]}=21.13$; $P<0.0001$). Of the females that swam upstream, 75% were attracted to the traps in the background treatment. Females prefer high concentration signals, and 3kPZS may mediate this preference on spawning grounds. 3kACA does not attract or retain females near an odor source.

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I. Introduction

SEA LAMPREY LIFE HISTORY

The sea lamprey (*Petromyzon marinus*), an anadromous parasite of teleost fishes, is native to the Atlantic Ocean and invasive in the Laurentian Great Lakes. The sea lamprey life cycle begins in the tributary: as larvae, they burrow into the sediment and filter feed for 2-7 years (Youson 2003). Upon reaching at least 120 mm in length, the larvae leave the sediments, and metamorphose into the parasitic feeding stage (Potter 1980). Transformed larvae migrate downstream to the open ocean (or lake), locate a host, and begin to feed on its blood and body tissue. In the late winter or early spring, lampreys detach, and locate suitable spawning tributaries. Adult sea lampreys are attracted to three bile acids produced by the larvae in the stream that comprise the migratory pheromone (Sorensen et al. 2005), and swim up tributaries when the water temperature is 10-18°C (Applegate 1950).

Upon reaching full maturation, sea lampreys spawn at temperatures ranging from 15-23°C on riffle habitat with stream velocities ranging from 0.5-1.5 m/s (Applegate 1950). Males generally move to the spawning habitat and begin to build a crescent-shaped nest by moving rocks from the stream bed (Applegate 1950). Mature males release a mating pheromone to attract female sea lampreys to their spawning beds (Teeter 1980; Li et al. 2002). Ovulating females join the males on the nest and help with nest construction between bouts of spawning (Applegate 1950). Sea lampreys are semelparous, so the adults inevitably die after spawning (Hardisty and Potter 1971).

GREAT LAKES SEA LAMPREY MANAGEMENT

The sea lamprey became a pest species in the Great Lakes after the construction of the Welland Canal provided passage around Niagara Falls (Eschenroder and Burnham-Curtis 1999). Initial attempts to reduce lamprey populations using mechanical traps and electrical barriers were largely unsuccessful (Smith and Tibbles 1980). In the late 1950s, agencies began to use 3-trifluoromethyl-4-nitrophenol (TFM), a selective chemical lampricide, to kill larval sea lamprey (Smith et al. 1974). TFM treatments greatly reduced the population of sea lamprey basin-wide (Christie and Goddard 2003). Effective sea lamprey control allowed lake trout restoration to begin, and other salmonid sport-fisheries to thrive (Smith and Tibbles 1980). Initial success of TFM provided much optimism for eradication of sea lamprey from the Great Lakes basin (Christie and Goddard 2003). However, sea lamprey proved extremely difficult to remove from some stream systems, and eradication with current control techniques is probably impossible (Christie et al. 2003, Smith and Swink 2003, Smith and Tibbles 1980). Limitations of chemical control, technological advances in mechanical traps and barriers, and the discovery of a chemo-sterilant bisazir, prompted the Great Lakes Fishery Commission to adopt an Integrated Pest Management (IPM) approach to sea lamprey control (Sawyer 1980).

Integrated Management of Sea Lamprey (IMSL) uses the IPM principles of combining chemical, mechanical, and biological control tactics to reduce pest (sea lamprey) numbers to an optimal Economic Injury Level (EIL). Sea lampreys are now suppressed with lampricide treatments, electromechanical barriers in spawning streams, sterile male release, and mechanical trapping of adults (Christie and Goddard 2003).

Lampricides remain primarily responsible for effective control (Brege et al. 2003), but rising costs of TFM, the main control agent, and growing public concern over the use of pesticides mandate that additional alternative controls be implemented (Christie and Goddard 2003).

The sea lamprey mating pheromone has been targeted for use in sea lamprey control because it is highly attractive at low concentrations, is environmentally benign, and could be synthesized for widespread use in the Great Lakes basin (Li et al. 2003). Mating pheromones are common in pest insect species, and have been frequently used in insect IPM (Carde and Minks 1995, Chapman 2000, Smith and Swink 2003). Successful applications of mating pheromones in insect IPM and similarities between insect and sea lamprey pheromone communication suggest that the sea lamprey male mating pheromone may be used in IMSL to remove ovulating females from spawning grounds (Li et al. 2003).

The male sea lamprey was first suspected to release a mating pheromone by French fishermen who baited traps with mature males to increase their catch of females (Fontaine 1938). Recent studies have shown promise for the use of the male pheromone in sea lamprey control (Johnson et al. 2005; Johnson et al. 2006; Wagner et al. 2006). In one study, traps baited with spermiating males captured over 70% of females released, while traps baited with non-spermiating males captured none (Johnson et al. 2005). Furthermore, 52% of females released were captured in traps baited with spermiating male washings within two hours (Johnson et al. 2006). Finally, Wagner et al. (2006) found that traps baited with multiple males captured more females than those baited with single males, indicating that pheromone-baited traps may be able to attract females even when other pheromone sources are present on the spawning grounds. Effective implementation of trapping-based control strategies will require further information about female preference for male odors in settings that increasingly mimic natural spawning situations.

THE SEA LAMPREY MATING PHEROMONE

The sea lamprey mating pheromone indicates spawning readiness to mature females, is important for males in attracting females, and females in locating males. Teeter (1980) found only the odor from spermiating males was attractive to only mature females. Lampreys actively spawn both during the day and at night (Applegate 1950), and sea lamprey vision deteriorates during the spawning season (Manion and Hanson 1980). Therefore, olfaction is believed to be the primary sensory system used by females to locate spermiating males (Li et al. 2003). Field tests confirmed female attraction to and retention near spermiating male washings in otherwise pheromone-free spawning habitat (Siefkes et al. 2005), and females with an occluded naris had difficulty locating a source of males in a spawning tributary (Johnson et al. 2006).

Recent studies also indicate that the male pheromone may be important for female mate choice. Females prefer multiple male signals over those produced by single males (Wagner et al. 2006), and mate selection could be important for females in the lamprey mating system. This preference may arise from variable detection thresholds in females for higher concentration pheromone, female preference for higher concentration pheromone, or female preference for multiple males.

Genetic and observational research findings show that female sea lampreys range from monogamous to polyandrous (Applegate 1950; Gilmore 2004). Variable paternal contribution to the next generation (Gilmore 2004) indicates that it may be advantageous for females to choose quality male spawning partners, or invest more of their eggs with males that are believed to be higher in quality. The pheromone is released through the gills, and is probably actively pumped (Siefkes et al. 2003). Laboratory measurements of pheromone release rates indicate high variability between males (Siefkes et al. 2003; Siefkes et al. 2005). Because pheromone release may be costly, and likely varies between males, release rates could honestly indicate male quality. Quality males may provide direct or indirect benefits to their mating partners (Kirkpatrick 1982; Kirkpatrick and Ryan 1991). In the sea lamprey mating system, quality males could provide direct benefits such as enhanced fertilization rate, or access to better resources (nesting sites). Poor nesting sites have been postulated to reduce offspring survival (Applegate 1950). Indirect benefits of good genes could also play a role in female choice. However, links between pheromone production and offspring survival have not been established.

Li et al. (2002) discovered 3-keto-petromyzonal sulfate (3kPZS), the most prevalent compound in spermiating male washings. Two-choice maze tests showed that 3kPZS was attractive to ovulating females, and field tests with 3kPZS showed that it attracts females in a pheromone free environment, but does not retain them near the pheromone source (Li et al. 2002; Siefkes et al. 2005). Electro-olfactogram tests showed that female olfactory receptors were highly sensitive to 3kPZS (Li et al. 2002). These findings present the argument that 3kPZS is the main component of the male pheromone, but it is unknown how well 3kPZS performs in attracting females in a pheromone-rich landscape.

Yun et al. (2003) discovered a secondary compound with electro-olfactogram potency 3-keto-allocholic acid (3kACA) that was present only in spermiating male washings (Siefkes and Li 2004). Further tests confirmed that 3kACA was detected by specialized receptors (not those from 3kPZS) in the female sea lamprey (Siefkes and Li 2004). Li (2005) postulated that 3kACA may serve to increase lamprey retention or to function as a priming pheromone.

Here, I investigated the potential function of the mating pheromone in female choice. I sought to determine whether females preferred pheromone from multiple males or high concentration pheromone signals, and whether or not their preferences were based simply on limitations (variable detection thresholds). Secondly, I sought to determine which mixture of currently identified compounds was most attractive in a pheromone-free environment and in the presence of natural male odors.

FEMALE SEA LAMPREYS AS RESEARCH SUBJECTS

The sea lamprey is of particular interest as a subject of study because of its status as a valued food species in Europe, and an invasive pest in the Great Lakes. Knowledge about the sea lamprey mating pheromone and its mating system may be readily applied in control or restoration programs. I chose to investigate questions of female response for a two reasons. First, female choice is likely important for the sea lamprey mating system, potentially influencing male behavior and offspring survival. Second, control tactics using the male mating pheromone are potentially more effective if they target females for removal (Li et al. 2003). Female response to different odors is useful information for sea lamprey managers.

Furthermore, the sea lamprey pheromone is unique because bile acids are rarely found as mating pheromone signals. Hormonal steroids are commonly identified as mating pheromones in other fishes (Stacey 2003), and bile acids identified in other fishes have generally been found in migratory pheromones (Sorensen et al. 1998; Baker et al. 2006).

Finally, olfactory navigation presents a unique challenge for lampreys because they only have a single naris as opposed to paired receptors found in most fish, insects, and mammals. This restriction makes it impossible for sea lampreys to track pheromone gradients by comparing information from paired receptors. Instead, lampreys must use temporal differences in the signal to locate an odor source.

OBJECTIVES

With the following objectives, I sought to investigate the female response to the full male signal and pheromone components in increasingly complex pheromone environments. These studies will provide insight into the function of pheromone signals in natural environments. Biological understanding of the organism and how it uses pheromonal cues will provide managers with necessary information to successfully apply the mating pheromone in sea lamprey control tactics.

Objective 1. Investigate the attractiveness of natural male odors in various arrangements.

H1: Females prefer multiple male odors vs. similar intensity odors from single males.

Female sea lampreys are attracted to the traps with multiple males vs. a single male (Wagner et al. 2006). The pheromone signal may vary between males, providing females the ability to distinguish between a pheromone signal from one male, and that of multiple males mixed together. A choosy female would be more apt to investigate a source of pheromones coming from many males than that of a single male, because a multiple male signal would signify more potential mates. If in fact females prefer multiple male signals per se, I expect the mixed male washings would be more attractive than a similar intensity signal from single males.

H2: Females prefer high concentration pheromone signals vs. low concentration signals in a spawning stream.

In our experiment for hypothesis 1, I determined that females do not prefer multiple males vs. similar intensity signals from single males. Yet, females prefer traps baited with multiple males vs. single male traps (Wagner et al. 2006). The number of males in the trap influences the concentration of the pheromone signal coming from the trap (more total males=more total pheromone). Here, I investigated female preference for and trapping efficiency of high concentration (multiple male) signals in the stream. Females may prefer high concentration signals because of a variable detection threshold or because the pursuit of high concentration signals provides some advantage for female mating success.

H3: A diffuse background signal will reduce female preference for high concentration signals.

Wagner et al. (2006) found that when three traps were placed in a longitudinal arrangement (1 male, then 5 males, then 10 males), the capture rate appeared to be reflective of an additive quality of pheromone signals. After completion of the experiment for hypothesis 2, I tested whether a diffuse background signal causes female response to be less skewed toward the high concentration odors. In natural systems, females must be able to navigate to males even when upstream pheromone sources are present. Background signals may be additive or may obfuscate individual pheromone sources. Traps aligned to intercept females as they approach the spawning grounds would prevent them from mating prior to capture, and potentially additive signals may enhance the strength of a trap signal (Wagner et al. 2006). Optimal intercept trapping requires knowledge of how female response is affected by background pheromones.

Objective 2. Investigate the attractiveness of synthesized compounds in competing and background environments.

H1: 3kPZS+3kACA is more effective than either single component (3kPZS, 3kACA) in pheromone-free spawning streams.

3kPZS attracts females in pheromone-free spawning habitat, but does not retain them near the source (Siefkes et al. 2005). I expect that 3kACA, the minor component will increase retention near a 3kPZS source. Also, I expect that the most natural compound will be more attractive than either individual compound.

H2: In a diffuse background of pheromone, 3kPZS+3kACA will be more effective than 3kPZS and equally effective as 3kACA.

The natural pheromone signal upstream will produce enough 3kPZS to spur upstream movement. Females may rely on 3kACA for retention and for short range attraction in a pheromone landscape environment. Therefore, I expect the traps with 3kACA as part or all of the signal to attract and retain females.

II. Chapter 1: Evidence that females prefer large pheromone signals

ABSTRACT

Fully mature male sea lampreys release a mating pheromone that attracts females to their nests. In this study I investigated female preference for multiple male odors in various arrangements. I compared female preference for similar intensity pheromone signals from single males and multiple males in a behavioral maze, and found that females do not specifically prefer multiple male odors. Next, I compared female attraction to traps baited with one, three, and six males in a cross-channel alignment (10 male treatment). I also compared female preference for traps baited with two, six, and twelve males (20 male treatment). Females preferred traps baited with 6 or more males ($F_{[2,48]}$=34.63, P<0.0001), indicating a preference for high concentration pheromone and a behavioral threshold for this preference ($F_{[2,48]}$=4.15, P=0.02). Females oriented towards the higher bait traps up to 90 m downstream. I captured 69% of the females released in the 10 male treatment, and 55% in 20 male treatment (t=1.41, df=16, p=0.18). Finally, I tested the 1 vs. 3 vs. 6 male alignment with 9 males evenly spread out 30 m upstream of the traps. I found that the background signal did not affect female preference for the 6 male trap ($F_{[2,48]}$=85.80, P<0.0001), and 95% of the females that swam upstream approached a trap before moving on to investigate the background signal. Trap capture was reduced from 69% to 35% when the background was added (t=-2.85, df=16, P=0.01). My results indicate that the pheromone concentration is important for female choice in spawning streams. I also conclude that while traps baited with more males can catch more females, there are diminishing returns on increasing the concentration.

Finally, the pheromone can attract and capture females in a diffuse background, but traps must be highly efficient.

INTRODUCTION

Mating pheromones are emitted by various species to attract mates, signal mating readiness to conspecifics, and in sex or species recognition (Wyatt 2003). Although they are most well-studied in insect species, mating pheromones have been identified in various terrestrial and aquatic vertebrates as well. In some instances, pheromones are not only a generalized attractant, but are also important in mate selection. For example, females prefer larger male tiger moths (*Utetheisa ornatrix*), that produce more pheromone, and exhibit better offspring survival (Iyengar and Eisner 1999). In tomato fruit borer moths (*Neoleucinodes elegantalis*), males are attracted to the larger females, which also produce the best pheromone blend (not the most pheromone) (Jaffe et al. 2007). Female mice preferred the odors of dominant males over those of subordinates (Drickamer 1992). Mate selection is important for species where one or both sexes have the ability to choose, and gain some fitness benefit by choosing. Criteria for selection based on pheromone production generally falls into one of two categories: selection based on the amount of pheromone the signaler produces or selection based on the quality of pheromone produced. In either instance, choice based on pheromonal cues is only advantageous when pheromone production or quality serve as honest indicators of mate quality.

The sea lamprey (*Petromyzon marinus*), a voracious parasite of commercially important fishes in the Great Lakes, employs at least two pheromones to complete its life cycle. Larvae release a migratory pheromone that attracts adults to spawning streams (Sorensen et al. 2005), and sexually mature males release a mating pheromone to attract ovulated females to their nests (Li et al. 2002). Sea lampreys are of particular interest to fisheries managers because of their status as an invasive species in the Great Lakes, and are a convenient organism for study because of the conspicuous nature of their spawning activities. Spermiating males usually arrive first on spawning riffles, begin nest construction, and release the mating pheromone to attract females (Applegate 1950; Teeter 1980; Li et al. 2002). In particular, spermiating male sea lamprey release at least two unique compounds (3-keto petromyzonal sulfate, 3kPZS, and 3-keto allocholic acid, 3kACA) that are believed to partially comprise the male mating pheromone (Li et al. 2002; Siefkes and Li 2004; Siefkes et al. 2005).

In the sea lamprey system, females appear to have both the opportunity to choose a mate or mates and the opportunity to gain fitness for their offspring by choosing. In general, sea lamprey males cannot monopolize mating opportunities for females because of extensive available habitat and a relatively brief spawning period. In fact, several males commonly build nests on the same spawning riffles (Applegate 1950), indicating that females have the opportunity to choose a spawning partner or partners. Sexual dimorphism that occurs exclusively during the mating season often indicates that sexual selection is acting on a population (Lande 1980). Three secondary sexual traits in the male sea lamprey follow this model: mature males grow an enlarged head and oral disc (Hardisty and Potter 1971), develop a rope-like dorsal ridge (Applegate 1950), and specialized glandular cells (believed to be responsible for releasing the mating pheromone) appear on their gills (Siefkes et al. 2003b). These dimorphisms suggest that morphological and/or pheromonal cues may be important for female choice. In some systems where active choice is prevalent, different cues are used to narrow down the choice at each stage of the decision (Gibson and Langen 1996). In the sea lamprey, pheromonal cues probably operate over a fairly large active space because they are carried downstream by current, and could be used in preliminary screening. Visual or tactile cues would have to operate over a much shorter distance, and could be important in later stages of choice. Variable paternal contribution to the next generation has recently been confirmed, providing a motive for female choice (Gilmore 2004). Yet, no studies have specifically linked oral disc size, dorsal ridge size, or pheromone quality or quantity to better offspring survival.

Minimally, the male pheromone signals readiness to spawn to the females. Only fully mature males release the pheromone, and it is only attractive to fully mature or ovulating females (Teeter 1980; Li et al. 2002; Siefkes et al. 2005). This attraction has been shown to be highly robust in otherwise pheromone-free spawning environments (Siefkes et al. 2005; Teeter 1980; Li 2005), and females were unable to locate mature males during short-term olfactory occlusion (Johnson et al. 2006). Recent studies of the male pheromone provide preliminary evidence that odor cues could be important for female choice. Ovulating females are invariably attracted to male signals in pheromone-free environments. Furthermore, females appear to prefer signals originating from groups of males (vs. single males) (Wagner et al. 2006). However, because the technology to measure mating pheromone concentration remains elusive, the number of males operated as a surrogate measure of the actual pheromone concentration under experimental conditions. Consequently, when Wagner et al. (2006) used traps baited with live spermiating males to create the pheromone signal, they manipulated both the number of males and the quantity of pheromone emitting from traps. A female preference for large pheromone signals could arise either as a consequence of female choice, or the statistical phenomenon of variable detection thresholds for male pheromone (i.e. more females can detect strong signals than can detect weak signals). Alternately, females may simply prefer signals from multiple males because they represent more than one male from which to choose. The multiple male signal could indicate a higher probability of locating a high quality male (if male quality is ultimately judged by morphological traits, or pheromone purity) or the opportunity for spatial or genetic bet-hedging. There is recent genetic (Gilmore 2004) and previous observational evidence of polyandry in the sea lamprey mating system (Applegate 1950), and females may derive direct or indirect benefits from multiple matings.

In actual spawning stream conditions, the male pheromone operates in a pheromone landscape that often includes pheromone from other males upstream. Wagner et al. (2006) suggested that a larger pheromone signal upstream enhanced the attractiveness of a single male source. Upstream pheromone may have an additive effect on downstream point sources. Alternately, because Wagner et al. (2006) located pheromone sources in traps, the enhanced trap catch of the single male source may have been due to the statistical probability that females approach the downstream source first and only those that were not captured in that trap were available to investigate upstream signals.

Because the mating pheromone is a natural product and highly attractive to females, it has been targeted for use in the Integrated Management of Sea Lamprey in the

Great Lakes (Twohey et al. 2003). Therefore, my studies have been designed to enhance understanding of the sea lamprey mating system while providing valuable information to management agencies about the potential for the use of the pheromone in sea lamprey control.

I conducted three experiments testing female preference for male odors. My working hypothesis was that females prefer groups of males because they produce more pheromone than single males. Since pheromone concentration alone cannot be directly manipulated, I employed a two-choice maze experiment and determined that females do not prefer signals from groups of males vs. similar-intensity signals from a single male. Next, I conducted two in-stream experiments to answer the following questions: (1) Do females prefer large pheromone signals over small signals? (2) Does this preference change when the overall pheromone signal is doubled? (3) Do trap captures actually reflect female preference? (4) What effect does a uniform background have on female preference and capture rate? I expected more females to approach large signals even when the total signal was doubled. Secondly, I hypothesized that trap capture distributions would be similar to approach distributions. Finally, I expected a uniform background to reduce female affinity for the large signals by increasing the attractiveness of all signals, and reduce overall capture rate either through obfuscation or reduced female effort.

METHODS

Experimental animals

Adult sea lampreys were captured in U.S. Fish and Wildlife Service and Canadian Department of Fisheries and Oceans traps in Lake Huron and Lake Michigan tributaries. Individuals were separated by sex on site according to the procedures established by Vladykov (1949), transported to U.S. Geological Survey (USGS) Hammond Bay Biological Station (HBBS), and held in flow-through tanks fed by 10-20 °C Lake Huron water. I moved male and female lampreys from HBBS tanks to holding cages in local spawning streams to facilitate maturation. The density of lampreys in the holding cages was 20-90 lamprey/m^3. Stream water temperatures ranged from 15-27 °C. The lampreys in holding cages were checked daily for maturation (animals express eggs or sperm with light squeezing of the abdomen) according to the procedures described by Siefkes et al. (2003a). Mature lampreys were returned to HBBS and stored in tanks receiving Lake Huron water at 15-20 °C until needed for experimentation.

Maze Experiment: Are mixed male washings more attractive than single male washings?

I used two-choice mazes constructed according to the specifications provided by Siefkes et al. (2005). Briefly, the fully constructed maze was 2.44 m long and 1.22 m wide. The upstream portion of the maze was divided into two equal chambers, whereas the downstream portion was left undivided. Water flows through the maze at .07 m/s at a depth of 17 cm. These mazes were erected on the banks of the Ocqueoc River, MI, USA, upstream of the sea lamprey barrier.

The main objective was to determine whether odor from multiple males is more attractive than odor from a single male at equal concentrations. Because there are currently no precise methods for measuring pheromone concentration, I used a behavioral assay to test multiple male vs. single male washings. Laboratory studies show variability in pheromone release rates across males (Siefkes et al. 2003b). Statistically, I expected that the males were ranked 1, 2, and 3 in terms of pheromone production, and the pooled washings represent a mean pheromone concentration. If pheromone concentration were more important for female preference than the number of male odors, I expected results similar to Figure 1a or Figure 1b. However, if mixed male odors were more attractive than single male odors, I expected results similar to Figure 1c.

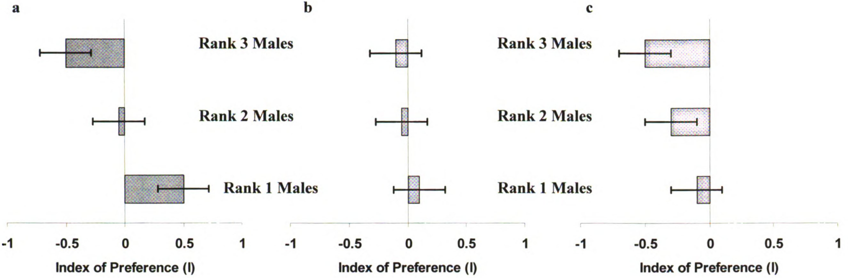

Figure 1. Three potential results for the two-choice maze experiment. Positive *I* values represent female preference for the single male odors; negative *I* values represent female preference for the mixed odors. Figure 1a shows the expected female response if pheromone concentration is more important to female response and there is large variability in pheromone release rates between males within each group. Figure 1b shows the expected outcome if pheromone concentration is more important than mixed male odors, and there is small variability in pheromone release rate between males within each group. Figure 1c shows the expected outcome if females prefer odors from multiple males over odors from single males.

Washings were prepared prior to trials each day. I filled three identical buckets with 24 L of water and placed them in a 17°C water bath. Each bucket held one spermiating male for 12 h. After 12 h, I removed the males from the buckets, and combined 12 L of water from each bucket to form the "mixed" washings pool. Each test compared 2 L of washings from each individual male to 2 L of mixed washings from each group of three. Because I mixed an equal volume of washings from each male, mixed washings have the average pheromone signal strength of the three males.

To start each trial, females were released into the maze and allowed to acclimate with no odors for 10 minutes. The time the female spent on each side of the maze was recorded for the next 20 minutes. Next, I pumped the washings into both sides for 25 minutes, and recorded the amount of time each female spent in each side of the maze for the final 20 minutes. Washings from each male were tested with 5 females (each time against the mixed washings). The procedure was replicated five times. Finally, I calculated an index of preference (*I*) for each female tested using the formula:

$$I = \frac{T_s}{T_{bs} + T_s} - \frac{T_m}{T_{bm} + T_m},$$

where T_s is the time spent on the single male side during the experimental period, T_m is the time spent on the mixed male side during the experimental period, T_{bs} is the time spent on the single male side before the experimental period, and T_{bm} is the time spent on the mixed male side before the experimental period. The index of preference (*I*) ranges from -1 (full preference for the mix) to 1 (full preference for the single male) and was used as the response variable for the data analysis because it was approximately normally distributed. I ranked the males from each group 1, 2, and 3 according to mean *I* score (males with the highest *I* were ranked 1 and so on); individual males were modeled as random effects nested within male rank. The full statistical model was a general linear mixed effects model with the fixed effect of male rank, and the random effect of male nested within male rank. T-tests (with a Bonferroni adjustment for multiple comparisons if the fixed effects for the model were not significant at alpha=0.05) were used to determine whether the mean *I* for each male rank was different from zero to determine the pattern of preference. Bonferroni adjusted LSD tests for significant differences were also used to test for differences between male ranks.

In-stream Experiment 1: Do females prefer large pheromone signals? How does more overall pheromone affect this preference? Does trap capture approximate female preference?

Trials for in-stream experiments were conducted in a 150 m section of the Ocqueoc River, MI, USA, upstream of the electro-mechanical lamprey barrier. The Ocqueoc River is a Lake Huron tributary with a history of sea lamprey spawning runs. I chose a location above the barrier to eliminate uncontrolled sources of pheromone. The 150 m stream section was measured along the left bank and each 10 m increment was marked. Nylon block nets with 1 cm mesh were placed across the channel at the upstream and downstream ends to isolate the section. An acclimation cage was placed at the downstream block net. I spaced three traps across the channel 120 m upstream of the acclimation cage. Passive integrated transponder (PIT) antennas were arrayed as shown in Figure 2.

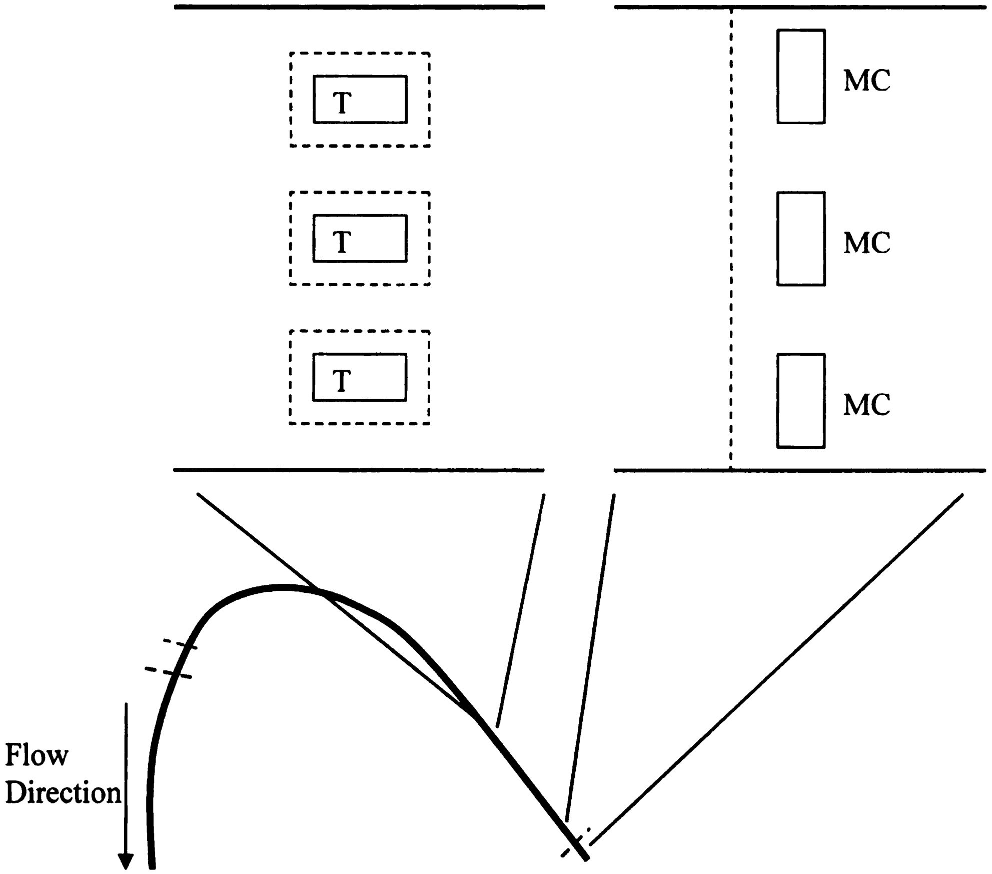

Figure 2. The 150 m stream section in the Ocqueoc River used for the in-stream experiments. The solid curved line shows the shape of the full 150 m section, while the insets show the trap and upstream male cage set-ups. Dashed lines represent PIT antennas, boxes indicate traps or holding cages. The acclimation cage is 120 m downstream of the traps (T), and 150 m downstream of the male cages (MC). The furthest upstream PIT antenna and the male cages were only in place for the background experiment.

One day prior to the start of each trial, 10-12 ovulating females were measured, weighed, and tagged externally with fin tags and PIT tags, (Oregon RFID, Portland, Oregon, USA). Males were measured, weighed, and tagged with fin tags. Twelve hours before the trial, the 10 most active tagged females were transported to the field site and placed in the acclimation cage. Males were placed in randomly assigned traps 1 h prior to the start of each trial. Females were released and monitored by PIT antennas for 12 h. The PIT antenna system recorded the date and time of contact with each antenna for each individual female. Females were visually tracked for the first two hours of each trial. At the end of each 12 h trial, the males and captured females were removed from the traps, and fin tag numbers were recorded.

Two treatments were tested according to the above procedure. The first treatment consisted of 10 males distributed between a high bait (6 males) trap, a medium bait (3 males) trap, and a low bait (1 male) trap. The second treatment consisted of 20 males distributed in the same ratio (12:6:2). Trap bait levels were arranged so that each bait level would be in each trap for 3 of the 9 trials for each treatment. Trials were conducted in random order from June 8 through June 21, 2005. Males were removed from the traps at the end of each trial, transferred to a holding tank, and visually inspected for declining health. Healthy males were reassigned to traps for the next trial, while those in poor condition were replaced. Newly acclimated females were released for every replicate. The experimental procedure was replicated nine times for each treatment.

Trap choice (the first trap a female swam to) was used as the main response variable to compare treatments and traps within each treatment. Two by three factorial ANOVA was used to test for overall bait level effects and trap bait level effects on trap choice for the 10 male treatment and the 20 male treatment. Fisher's protected LSD was used for mean separation. These analyses were also run with trap capture replacing choice as the response variable. Percent of females captured in the trap they first approached and capture efficiency was calculated for each treatment (# of females captured/# females that approached traps). I conducted t-tests on arcsine square-root transformed capture efficiency data to test for differences between the 20 and 10 male treatments. I used the visual tracks to determine whether females were in the high bait, medium bait, or low bait 1/3 of the stream at each 10 m transect. Percents of females in each 1/3 of the stream at each transect were calculated and graphed.

In-stream Experiment 2: Do females still prefer large signals in the presence of a background? Does a background reduce female capture rate?

I tested the effects of a diffuse background in the same experimental set up as the 10 male treatment; I added a nine male background signal 30 m upstream of the traps spread across the channel in three different cages and an upstream PIT antenna to detect female approaches to the male cages (Figure 2). I followed the same experimental procedure for the background treatment for nine trials from June 28 through July 13, 2005. The data were analyzed the same way for the background experiment, this time comparing the background experiment to the 10 male treatment.

The upstream antenna allowed us to calculate the mean amount of time each female spent in the trap area before moving upstream to the cages. I compared the time for lampreys that approached a trap and then moved upstream to the cages without investigating any of the other traps. Because not enough data was available on the low bait trap, I only was able to use data from the medium and high bait traps. These data were square root transformed (to satisfy normality assumptions), and were compared using a t-test.

Dye Test

I conducted a rhodamine dye study on June 7, 2005 to determine how the pheromone is carried from the traps by the current. I measured the discharge of the stream using the method described by Murphy and Willis (2006) and calculated the pumping rate necessary for a fully mixed within stream dye concentration of 5 ppm. Before sampling, dye was pumped into each trap for 10 min to establish an equilibrium plume. The plumes from each trap were sampled separately, to determine the part of the pheromone plume specifically attributable to each trap. Five evenly spaced (across the channel) samples were taken at each 20 m transect, starting 10 m below the traps and continuing downstream to 10 m upstream of the acclimation cage. Samples were taken in 5 mL cuvettes from the middle of the water column, and were placed in a 17 °C water bath until the dye concentration could be measured using a field fluorimeter. I waited 30 min between the sampling of each plume to ensure that the dye from the previous sampling period was discharged downstream of the study site.

RESULTS

Maze Experiment

Female sea lampreys did not prefer the mixed male odor over the odors from individual males of any rank (t-test on *I* Rank 1 males, t= 1.00, df=12, P = 0.34; Rank 2 males, t = -1.25, df=12, P = 0.24; Rank 3 males, t = -2.62, df=12, P = 0.022; $\alpha/3$=.016667

Figure 3). Rank 1 males were not significantly more attractive than either rank 2 or 3 males (F-test for fixed effects; $F_{[2,12]}=3.35$; P=0.07).

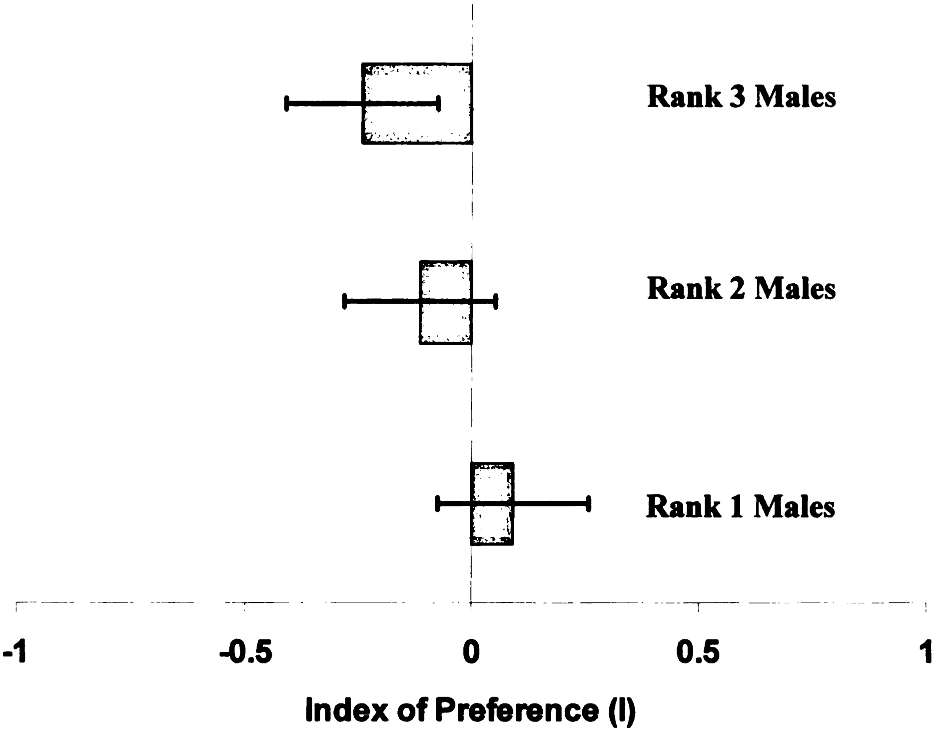

Figure 3. Results from the two-choice maze test (mean I-score ±1 SE, N=5) indicate no preference for mixed-male odors vs. single male odors (all t-tests non-significant). A positive score indicates preference for the single male odor, a negative score indicates preference for the mixed-male odor.

In-stream Experiment 1

There was no significant difference in the number of females approaching the traps between the 10 (mean 7.4 ± SE 0.65) and 20 (mean 8.0 ± SE 0.50) male treatments (F-test for treatment effects, $F_{[1,48]}=0.11$, P=0.75); in both cases >70% of the tagged females approached the traps.

Females strongly preferred traps baited with 6 or more males (Figure 4). In the 20 male treatment, both the high (12 males) and medium (6 males) bait levels attracted females (F-test for bait level effects, $F_{[2,48]}=34.63$, P<0.0001; protected t-tests within 20 male treatment, low bait vs. medium bait, t=-2.72, df=48, P=0.0092; low bait vs. high bait, t=-4.75, df=48, P<0.0001; medium bait vs. high bait, t=-2.04, df=48, P=0.047), whereas only the high bait trap (6 males) was attractive in the 10 male treatment (F-test for bait level effects, $F_{[2,48]}=34.63$, P<0.0001; protected t-tests within 10 male treatment, low bait vs. medium bait, t=-0.56, df=48, P=0.57; low bait vs. high bait, t=-6.68, df=48, P<0.0001; medium bait vs. high bait, t=-6.11, df=48, P<0.0001). Traps baited with 3 or fewer males were essentially ignored (Figure 4). Because the medium-bait trap became attractive in the 20-male treatment, I observed a significant interaction between the treatment and bait level effects (F-test for bait level by treatment interaction, $F_{[2,48]}=4.15$, P=0.02).

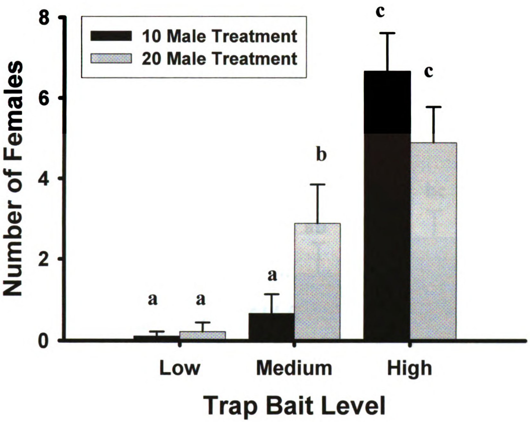

Figure 4. Results from the first in-stream experiment (mean ± SE, N=9) indicate female preferences for bait levels of 6 or more males. Letters show mean separation between all bars ($\alpha=0.05$).

When captured, females were typically caught by the first trap they approached (76 % for 10 male treatment, mean total captures=5.1 ± SE 0.68 ; 75% for 20 male treatment, mean total captures=4.6 ± SE 0.85). Consequently, the distribution of captures among traps appears similar to the distribution of trap approaches (Figures 4-5). The 20 male treatment had a lower overall capture efficiency (55 % of approaching females captured) than the 10 male treatment (69 % of approaching females captured); the difference was not statistically significant (t-test, $t=1.41$, $df=16$, $p=0.18$).

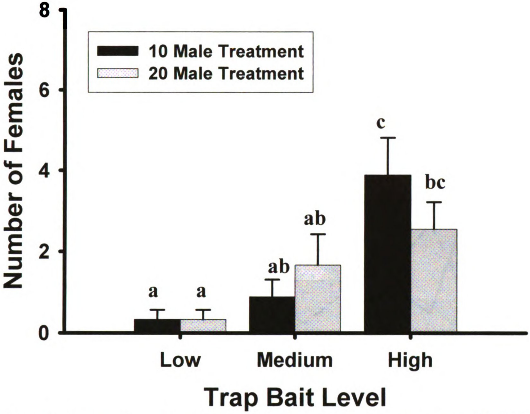

Figure 5. Captures in each trap for the first in-stream experiment (mean ± SE, N=9). More females are captured in the higher bait level traps. Letters show mean separation between all bars (α=0.05).

Upon release the majority of females quickly began swimming in the portion of the channel corresponding to an odor of 6 or more males (high and medium bait "lanes" in the 20 male treatment, high bait "lane" in the 10 male treatment, Figures 6-7). In both cases this decision occurred greater than 100 m downstream of the traps and below a bend in the river (i.e., out of sight of the traps). When physical habitat features caused lamprey to be guided into the middle "lane" of the stream (i.e. a log partially blocking part of the stream 30 m downstream of the traps), the females quickly returned to their previous cross-channel distribution (within 10 m of passing the obstruction) (Figures 6-7).

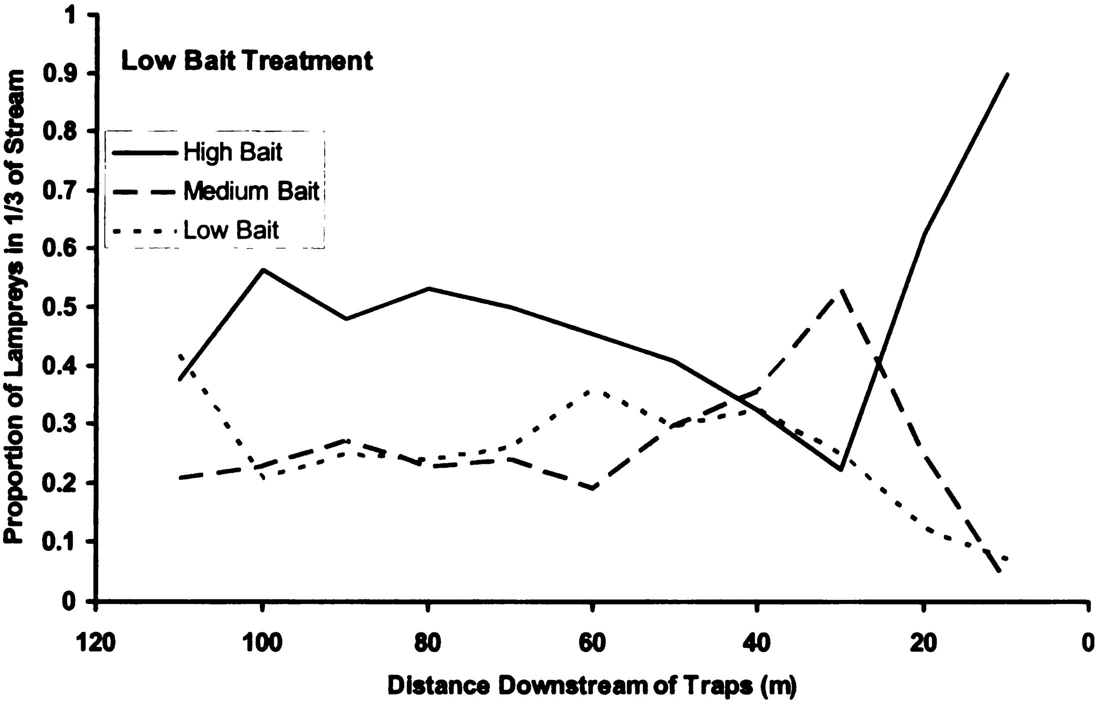

Figure 6. Proportion of females observed swimming in each third of the stream for transects downstream of the traps for the 10 male treatment. Females preferred the high bait lane.

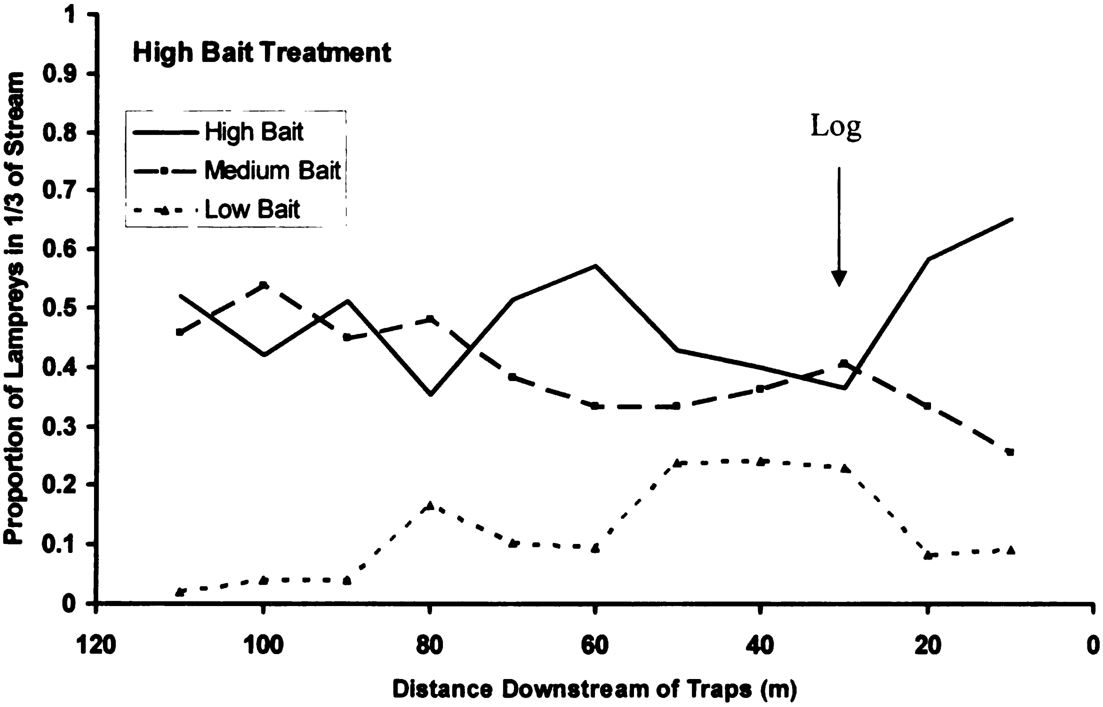

Figure 7. Proportion of females observed swimming in each third of the stream for transects downstream of the traps for the 20 male treatment. Females preferred the high and medium bait lanes.

In-stream Experiment 2

Adding a background of male odor did not alter the number of females approaching the traps (10 male mean 7.4 ± SE 0.65; 10 male + bkg mean 8.2 ± SE 0.52; F-test for treatment effects, $F_{[1,48]}=0.07$, P=0.80) or the strong preference for first approaching the 6-male trap (F-test for bait level effects, $F_{[2,48]}=85.80$, P<0.0001; Figure 8). However, the number of captures was reduced from 5.11 ± SE 0.68 to 2.89 ± SE 0.51 in the presence of the background odor (Figure 9). This represented a 50% reduction in overall trapping efficiency (from 69% to 35%; t-test, t=-2.85, df=16, P=0.01). Only 4 out of the 74 total females that swam up as far as the traps continued to the background cages without first investigating the traps. Forty-two of the 48 females that were not captured in the background trial eventually swam upstream to investigate the male background cages. Interestingly, females most strongly oriented to the high bait trap in the presence of a background, acquiring the 6-male lane 100 m downstream and maintaining the distribution until reaching the traps (Figure 10).

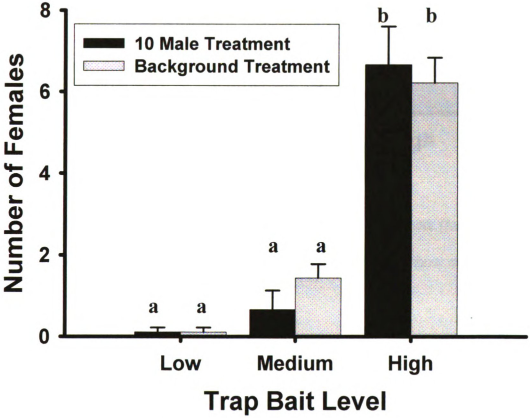

Figure 8. Results from the second in-stream experiment (mean ± SE, N=9) indicate no effect of background on female approaches. Letters show mean separation between all bars (α=0.05).

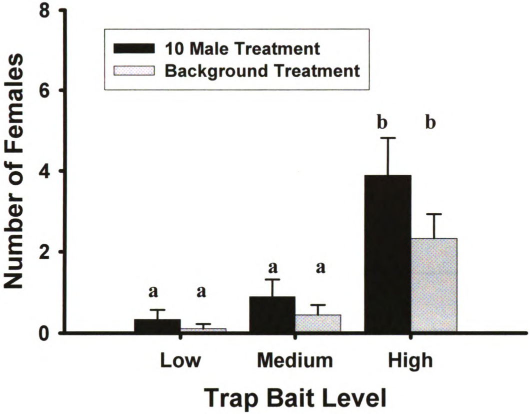

Figure 9. Captures in each trap for the second in-stream experiment (mean ± SE, N=9). More females are captured in the higher bait level traps. Letters show mean separation between all bars ($\alpha=0.05$).

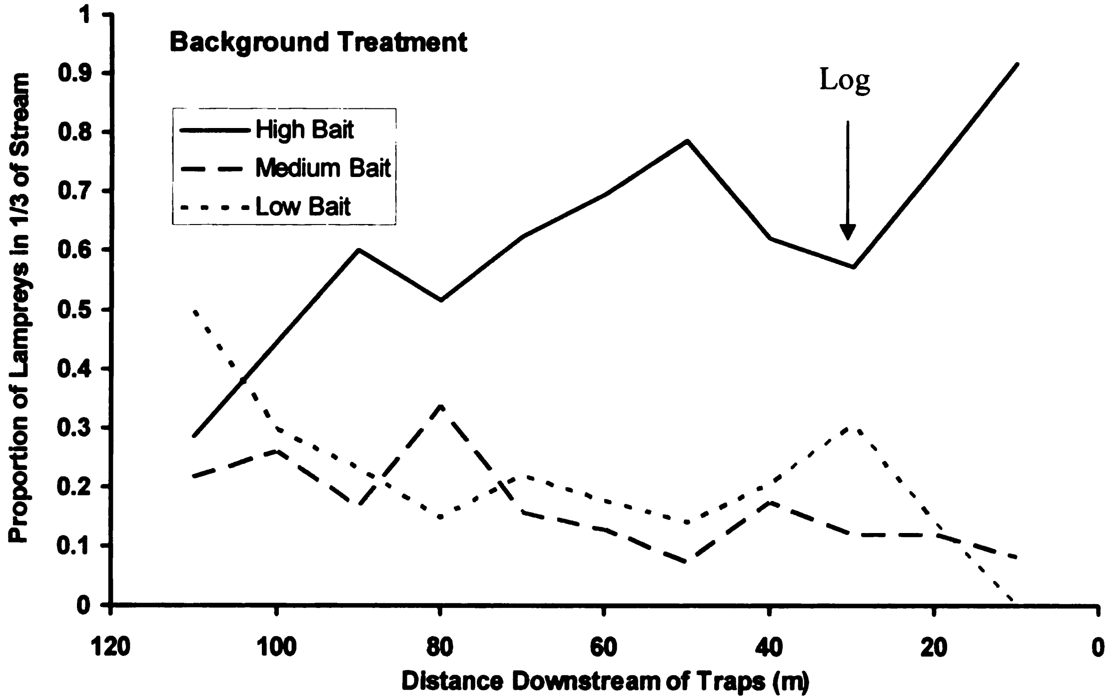

Figure 10. Proportion of females observed swimming in each third of the stream for transects downstream of the traps for the background experiment. Females preferred the high bait lane.

Female retention near the high bait trap was higher on average than retention near the medium bait trap though this difference was not significant (high bait mean 7901 s ± SE 1448; medium bait mean 3791 s ± SE 1450; t-test on square root transformed data t_{22}=1.37, P=0.18)

DISCUSSION

The data in this study confirm my central contention: females prefer groups of males because they produce more total pheromone than individual males. Two lines of evidence support this conclusion. First, when presented with competing odors of similar concentration (single male vs. mixtures of three males), females did not prefer the mixture (maze experiment). Second, females consistently approached and were captured in traps baited with 6 or more males, and the decision occurred at least 100 m downstream of the traps (field experiment 1). Given the physiography of my study site, odor was the only male cue available to the females at this distance.

Two competing hypotheses may explain the female preference for large pheromone signals. Conceivably, my in-stream results represent a statistical phenomenon of variable lower detection limits in females. Lamprey sensory systems are degrading during this late life stage (Applegate 1950), and the physical measure of ovulation I used to choose females allows for variance in the physiological state. While I can not exclude the above hypothesis, five lines of evidence indicate that females could detect all the signals presented in my experiments. Females preferred high bait lanes at least 100 m downstream of the traps where the small signal would be least detectable; however, the fully mixed concentration of pheromone released by one average male (Siefkes et al. 2003b) on the highest discharge day (1.7×10^{-13}M) was higher than the levels needed for behavioral response (Johnson unpublished). Second, the dye study showed that the pheromone plumes largely converged and six-fold differences in concentration near the traps were reduced to two-fold differences at the point where most females chose a lane (near full mixing by the time the pheromone reached the acclimation cage; data not shown). Also, females returned to their original preference 20 m downstream of the traps (where more would be able to detect the low bait signals) after a log caused them to follow the thalweg (Figures 6-7, 10). The six male signal was highly attractive in the low bait treatment (clearly detectable), yet more females approached the

12 male trap than the 6 male trap in the high bait treatment (Figure 4). Finally, preference for the high bait trap did not change when I added a background signal, which increased the pheromone concentration throughout the stream (Figure 8).

Females should prefer high concentration odors if pheromone production is a good indicator of male quality. Because sea lamprey males vary in pheromone release rate (Siefkes et al. 2003b), and females are highly sensitive even to very low pheromone concentrations (Siefkes and Li 2004), females may be very capable of choosing a mate based on pheromone production. Handicap theory suggests that honest signals are costly to the signaler (Zahavi 1975). 3kPZS is released through the gills, and the presence of specialized glandular cells in the gill epithelia of spermiating males suggests that it is actively pumped (Siefkes et al. 2003b). Given that males cease feeding at least 2 months prior to spawning (Applegate 1950), pheromone synthesis and release may be costly and linked to energy stores in males. In other aquatic and terrestrial mating systems, males that produce more pheromone provide better resources, presumably leading to better survival in offspring (Fisher and Rosenthal 2006; Iyengar and Eisner 1999). Sea lamprey die shortly after spawning, and there is no parental care of the eggs or young. Still, high quality sea lamprey males may acquire preferred nesting sites. Males construct nests on certain areas of riffle habitat before others (Applegate 1950), and poor nesting sites may result in higher egg mortality due to predation or suffocation (Applegate 1950). Indirect benefits of good genes (i.e. offspring survival) have been widely cited as impetus for female choice, and pheromone production rate has been linked to better offspring survival in some moths (Iyengar and Eisner 1999; Wyatt 2003). A direct association between offspring survival and male pheromone production in sea lamprey remains to be investigated.

Despite the findings that females cannot distinguish a group of males based on the chemical make-up of the pheromone signal, females may still be attracted to groups of lampreys based on pheromone production. Females may use high concentration plumes as a preliminary screening tool to increase the probability of locating a quality mate, by increasing male encounters. Extra encounters could increase probability of selection of a high quality male (based on shorter range assessment) or increased opportunities for multiple matings. Female lampreys do not respond to the male mating pheromone until the onset of ovulation (Li et al. 2002), and typically die within the following week (Applegate 1950). The semelparous nature of their spawning effort combined with the brevity of the spawning season requires that females maximize their reproductive value in a short period of time. Mating with several males may increase egg fertilization (Godfray 1994), maximize genetic diversity in offspring (genetic bet-hedging, Jennions and Petrie 2000), or mitigate the impact of stochastic nest failure as a consequence of environmental disturbance (Jennions and Petrie 2000). Attraction to high concentrations of pheromone would be advantageous for females to gain the potential benefits of multiple mating.

Females were strongly attracted to traps baited with six or more males, and the 12 male trap was only moderately more attractive than the six male trap. I believe this indicates the potential for a behavioral response threshold. This threshold could result from the presence of an upper detection limit (i.e., females may simply be unable to discriminate between pheromone concentrations above the equivalent production of six average males. However, the highest overall estimated pheromone concentration in my experiments was 5.86×10^{-12}M (20 male treatment on the lowest discharge day) and electro-olfactogram experiments failed to show sensory adaptation even at 10^{-8} M concentrations of 3kPZS (Siefkes and Li 2004). More likely, the response represents an upper behavioral response threshold: females simply "conclude" that 6 males is a sufficiently large signal to warrant attention, while a 3 male signal is not. My data and previous studies support a behavioral threshold of attraction of around 5 males (Wagner et al. 2006; Figure 4). Previous measures of pheromone release rates by males have shown variation in pheromone production that could account for five-fold differences (Siefkes et al. 2003b), which may represent the difference between a robust male and a puny male. However, I observed no trials where the one or two male traps contained a "supermale" that could draw away from the high and medium bait traps. Also, the results from the maze data indicate low variability in the pheromone concentration of the male washings. There is genetic evidence that the maximum number of males a polyandrous female mates with is around five (Gilmore 2004). Therefore, the threshold could represent some maximum number of mating opportunities that a female seeks.

Female capture rate was reduced when I added a diffuse background upstream of the traps. Two different mechanisms might explain this finding. First, the signal may have obfuscated the trap bait signals to the females (i.e. they were unable to distinguish distinct pheromone sources in the background setting). The data indicate that this was not the case in my study because 95% of the females that swam upstream approached a downstream trap, and the female approach distribution did not change from the 10 male treatment with no background (Figure 8). More likely, the females spent less effort (probably time) attempting to get at the males in the traps, and investigated the pheromone source upstream. Unfortunately, I only had the upstream PIT antenna in the stream for the background treatment, but I did note that females generally spent more time near the traps if they initially investigated the high bait trap. In my visual observations, I never observed any females go upstream past the cages in the no background treatments, but saw several individuals swim upstream in the first two hours of the background trials. Secondly, of the 48 females that did not enter the traps in the background trials, 42 swam upstream to investigate the cages. Because female sea lamprey must operate on a limited energy budget, and are also limited in time before death, there must be some point at which they "give up" on a pheromone source to pursue other options upstream. Quality of the initial signal investigated should play a role in how long a female stays before giving up. I found no significant difference in retention between high and medium levels, but the trend was towards more time near the high bait trap. The availability and quality of an upstream signal, stage of ovulation, and energy stores available to the female also would be expected to affect this choice.

MANAGEMENT IMPLICATIONS

Integrated sea lamprey management (IMSL) currently suppresses Great Lakes populations with lampricide treatments, electromechanical barriers in spawning streams, sterile male release, and mechanical trapping of adults (Christie and Goddard 2003). Two lampricides are primarily responsible for effective control (Brege et al. 2003), but rising costs of TFM (3-trifluoromethyl-4-nitrophenol), the main control agent, and growing public concern over the use of pesticides require additional alternative controls to be implemented (Christie and Goddard 2003). The sea lamprey pheromones have been targeted for use in alternative control because pheromones are environmentally benign, effective at low concentrations, and could be synthesized at low costs (Li et al. 2003).

Pheromones are employed in pest management mainly for monitoring of pests, mass trapping, or mating disruption (Wyatt 2003). My data shed some light on the potential for each of these uses in the sea lamprey control program.

The mating pheromone would be an effective tool for monitoring previously un-infested streams for lamprey invasion, and areas upstream of barriers for lamprey passage. A reasonably high baited trap (i.e. 5-6 male equivalent) in a low competition environment (as would be expected in the above two situations) is highly effective at attracting the majority of females.

The mating pheromone offers several advantages in mass trapping scenarios as well. First, large pheromone signals increase the trap encounter rate. Previous studies have shown that un-baited free standing traps are ignored by ovulating females (Johnson et al. 2005). My data show that increasing the bait level of a trap also increases its attractiveness. However, because of the threshold effect, traps with large signals would be most effective in the absence of other large signals. In a high competition environment, increasing trap density will likely be more effective than increasing the bait level of one trap (i.e. 6 traps at a 12 male equivalence would likely be more effective than one trap at a 72 male equivalence). Secondly, pheromones also increase the probability of capture upon encounter. During my visual observations, females were commonly seen circling and searching around, against, under, and above the trap for the pheromone source. Previous studies with male washings have shown that females will remain in the vicinity of a pheromone source even without males present for hours (Johnson et al.

2006). However, capture probability is decreased by a diffuse background of pheromone because females will give up on locating the pheromone source or trap entrance sooner in the presence of a background. Because of this, traps must be highly efficient when spermiating males are available upstream. Finally, pheromones likely increase trap retention. Often, I observed lamprey around or even under the traps at the end of the trials. Clearly, if lampreys could be retained in the area when not in the traps, they would likely be retained in the traps as well. Within the traps, lampreys were often observed trying to get at the males in the holding cages behind the traps. If more time is spent attempting to get at a pheromone source, the lampreys are less likely to be searching for a way out of the trap.

Employing the male mating pheromone to disrupt the mating system appears to be more difficult. Previous work has shown that females have difficulty locating spermiating males in a spawning stream without the use of their olfactory organ (Johnson et al. 2006), lending promise to this technique. However, a diffuse background had no effect on the female ability to find the large pheromone signal in my experiment. Extremely high (potentially expensive) levels of background pheromone would likely be needed to successfully carry out this type of strategy.

III. Chapter 2: Efficacy of 3-keto-petromyzonal-sulfate and 3-keto-allocholic acid in attracting and retaining female sea lampreys

ABSTRACT

Two bile acids partially comprise the sea lamprey male mating pheromone, 3-keto-petromyzonal sulfate, and 3-keto-allocholic acid. I directly compared the attractiveness of traps baited with each single odor to a 10:1 combination of the two both with and without a natural pheromone background. 3kPZS+3kACA was more attractive with and without a background than 3kACA alone ($F_{[1,28]}=43.00$; $P<0.0001$). Females did not prefer 3kPZS+3kACA over 3kPZS alone, with or without a background ($F_{[1,28]}=1.18$; $P=0.29$); they preferred the right side of the stream during these treatments ($F_{[1,28]}=31.11$; $P<0.0001$). Females spent more time around the odor sources when no background was present ($F_{[1,150]}=21.13$; $P<0.0001$), but were only retained for around 134 s. About 75% of the females that swam upstream in the background treatment approached the traps, but only 2% of the total lampreys released were captured. 3kPZS is an important compound for female mate-finding on spawning grounds, and is sufficient to attract females to odor sources in a diffuse background. 3kACA does not attract or retain females near an odor source. Unidentified additional components may improve retention of females near the odor source. Capture efficiency can be improved by more efficient trap design or by identification of new compounds. When pheromone odors are considered equal, physical habitat features may be important to females navigating to a nest.

INTRODUCTION

The sea lamprey mating pheromone, released by spermiating males to attract females to their nests (Li et al. 2002), may be important in mate choice. Mate selection is common among females of freshwater fishes with varying levels of polyandry or monandry (Barbosa and Magurran 2006), both of which have been displayed in the sea lamprey (Applegate 1950; Gilmore 2004). Female sea lampreys probably invest in mate choice because they are semelparous, males vary in their contribution to the next generation (Gilmore 2004), and several males are often available on spawning grounds simultaneously (Applegate 1950). Females often use male traits or courtship displays to assess male quality (Darwin 1871). Mature male sea lampreys develop a distinct rope-like dorsal ridge, an enlarged oral disc, and specialized glandular cells on the gills which release the mating pheromone. Any or all of these characteristics could be important to the female for assessment of male quality. However, specific evidence indicates that pheromonal cues are important for female decisions. Female sea lampreys prefer large male pheromone signals over smaller ones, and make this decision up to 90 m downstream (Luehring Chapter 1). Also, the presence of pheromone signals upstream most likely reduced the time females spent investigating male odor sources (Luehring Chapter 1). Finally, sea lampreys are primarily nocturnal (Hardisty and Potter 1971), though they spawn day and night (Applegate 1950), and sea lamprey visual ability is widely believed to deteriorate during the spawning season (Manion and Hanson 1980). Olfaction has thus been hypothesized to be the primarily sensory system used in mate-finding (Li et al. 2003; Johnson et al. 2006).

Recently, 3-keto-petromyzonal sulfate (3kPZS) was identified as the primary pheromone constituent (Li et al. 2002). 3kPZS is most likely the main component females use to locate potential mates. Seven out of 10 females swam within 1 m of a 3kPZS point source and paused before moving upstream in an otherwise pheromone free spawning stream (Siefkes et al. 2005). In trials conducted with spermiating males, females chose to follow the highest concentration pheromone signals up to 90 m downstream of the source where use of other sensory modalities would be improbable, and detection of minor pheromone components would be less likely (Luehring Chapter 1). Potentially, 3kPZS concentration is used by females to make preliminary decisions about what part of the spawning stream is most likely to contain quality mates. In fact, the only deficiency 3kPZS has shown in pheromone free environments as compared to spermiating male washings is poor retention near the pheromone source (females only paused by it before moving upstream); spermiating male washings attracted and retained females for extended periods (Siefkes et al. 2005). Siefkes et al. (2005) postulated that this difference indicated that other compounds in the male washings are necessary to retain females near the nest. If this were indeed the case, males may gain a fitness advantage by releasing high levels of retentive compounds to keep females at the nest.

Alternately, three lines of evidence suggest that 3kPZS acts as an attractant which invokes an odor-conditioned rheotaxis in ovulating females. (1) 3kPZS is released from the gills at a high rate (0.5 mg/male/h, Siefkes et al. 2003b) and is detected by females at a low concentration (at least 10^{-12} M, Siefkes and Li 2004). Both of these features indicate that 3kPZS probably has a large active space in spawning streams necessary for long range attraction. (2) Females swam upstream at a higher ground speed when presented with 3kPZS than when presented with spermiating male washings (i.e. the absence of other components may have signaled that the males were far away) (Siefkes et al. 2005). (3) Females continued swimming past 3kPZS, but were retained near spermiating male washings (Siefkes et al. 2005). If 3kPZS is only important for stimulating rheotaxis, other pheromone components or sensory cues (i.e. vision, electroreception) might be important to the female for specifically locating and choosing a male in a spawning ground. Thus, females may be attracted to secondary compounds only after 3kPZS spurs upstream movement to find a male. No studies to date have tested the attractiveness or retention of females near 3kPZS odors in a spawning stream with natural pheromone present.

A second compound, 3-keto-allocholic acid was identified shortly after 3kPZS (Yun et al. 2003). 3kACA is also found only in the washings of spermiating males, is similar in chemical structure to 3kPZS, and is released at about a 1:10 ratio to 3kPZS (Yun personal communication). 3kPZS and 3kACA are physiologically distinct odors for the lamprey olfactory system (Siefkes and Li 2004). Females were not attracted to 3kACA in pheromone-free spawning streams (Johnson unpublished data), and Li (2005) postulated that it may serve to physiologically advance maturation in conspecifics or augment the response to 3kPZS.

Current knowledge of 3kACA is consistent with the hypothesis that 3kACA retains females on the nest. 3kACA is released at a much lower rate than 3kPZS (Li et al. 2003; Yun et al. 2003), and is detected by the olfactory organ at higher concentrations (10^{-10} M vs. 10^{-12} M for 3kPZS) (Siefkes and Li 2004). Male sea lampreys create a crescent-shaped depression for their nest on the spawning grounds (Applegate 1950), conceivably concentrating odors. Although no studies of pheromone constituents on the nest have been conducted, females may only be able to detect 3kACA in close proximity to the pheromone source. In this scenario, 3kACA concentration could inform the female as to whether she should stay and mate or move on to another male. Alternately, the above properties of 3kACA also support the hypothesis that it may be important for short-range attraction when 3kPZS is already present in the spawning stream. If this is the case, 3kACA might need to be synergized by the presence of 3kPZS or other components because it has not proven attractive on its own (Johnson unpublished data). Often in insect pheromones, synergism occurs between two or more compounds that are not attractive on their own (Silverstein 1977). However, 3kPZS has already proven to be attractive to females (Siefkes et al. 2005), indicating that synergism is an unlikely mechanism for sea lamprey pheromone function.

Sea lampreys are an invasive pest species in the Laurentian Great Lakes, and managers have targeted the male pheromone for use in control. Twohey et al. (2003) suggested several possible applications of the mating pheromone for Integrated Sea Lamprey Management including trapping and removal strategies. Any control strategy involving trapping and removal of females from the spawning grounds will have to contend with natural male interference around the trapping site or sites. In previous field studies conducted with spermiating males, females usually investigated the first pheromone source available to them (Luehring Chapter 1). Yet, female efforts to pursue these sources were reduced when a background signal was available upstream, and total captures were reduced from 70% to 35% when a background was added (Luehring Chapter 1). Logistical issues preclude the use of the live males in large-scale management applications. Will either synthesized compound or a combination of the two be effective in attracting females to traps? How will their efficacy change in the presence of natural male signals? The efficacy of 3kPZS and/or 3kACA in attracting and retaining females in the presence of a natural background signal must be investigated for their effective implementation in sea lamprey control.

In this study, I directly compared ovulating female attraction to 3kPZS and 3kACA to a 10:1 mixture of the two (3kPZS+3kACA) with and without a diffuse background of natural male odor in a Lake Huron tributary. I expected that 3kPZS would serve as the main attractant for females both with and without a pheromone background, and that 3kACA would increase retention near the 3kPZS+3kACA odor source. My alternative working hypothesis was that 3kPZS would invoke odor-conditioned rheotaxis in females and 3kACA would be synergized to serve as a short-range attractant when 3kPZS was already present. If this were the case, I expected to see 3kPZS+3kACA attract more females than 3kPZS alone with and without a background, and 3kPZS+3kACA attract more females than 3kACA alone without a background, and equally attractive in the presence of a background. I hypothesized that females would spend less time investigating pheromone sources with a natural male signal upstream. I dispensed point source odorants into traps to determine whether synthesized components would effectively trap females in a pheromone landscape. Consequently, I expected a reduced capture rate when the pheromone background was added.

METHODS

Experimental animals

Adult sea lampreys were captured by traps in Lake Huron and Lake Michigan tributaries. Individuals were separated by sex on site according to the procedures established by Vladykov (1949), transported to the U.S. Geological Survey Hammond Bay Biological Station (HBBS), and held for several days in flow-through tanks fed by 10-20 °C Lake Huron water. Male and female lampreys were moved from HBBS tanks to holding cages in local spawning streams to facilitate maturation. Stream water temperatures ranged from 15-27 °C. The lampreys were checked daily for maturation (animals release eggs or sperm upon gentle squeezing of the abdomen) according to the procedures described by Siefkes et al. (2003a). Mature lampreys were returned to HBBS and stored for 1-2 days in tanks at temperatures of 15-20 °C until needed for experimentation.

In-stream experiment

I conducted trials from June 8 to July 18, 2006, in a 170 m section of the Ocqueoc River, MI, USA, upstream of the U.S. Fish and Wildlife Service electro-mechanical lamprey barrier to eliminate uncontrolled pheromone sources. The Ocqueoc River is a tributary to Lake Huron with a history of sea lamprey spawning runs. Nylon block nets with 1 cm mesh were placed across the channel at the upstream and downstream ends of the section. To facilitate visual tracking of females I established transects every 10 m by placing stakes into the bank. An acclimation cage for females was placed at the downstream block net. I spaced two traps across the channel 115 m upstream of the female acclimation cage, and two male holding cages 165 m upstream of the female acclimation cage. Passive integrated transponder (PIT) (Oregon RFID, Portland, Oregon, USA) antennas were arrayed as shown in Figure 1. I wired the PIT antennas to a multiplexer (Oregon RFID, Portland, Oregon, USA) that recorded the date, time, and identification number of each tagged lamprey swimming through the electromagnetic plane. The PIT antenna setup was designed to measure swimming speed (time between antennas 1 or 2, and 3), initial channel choice (antennas 1 and 2), time spent in the vicinity of the traps (time between antennas 3 and 4), and trap approaches (antenna 5 or 6) (Figure 1).

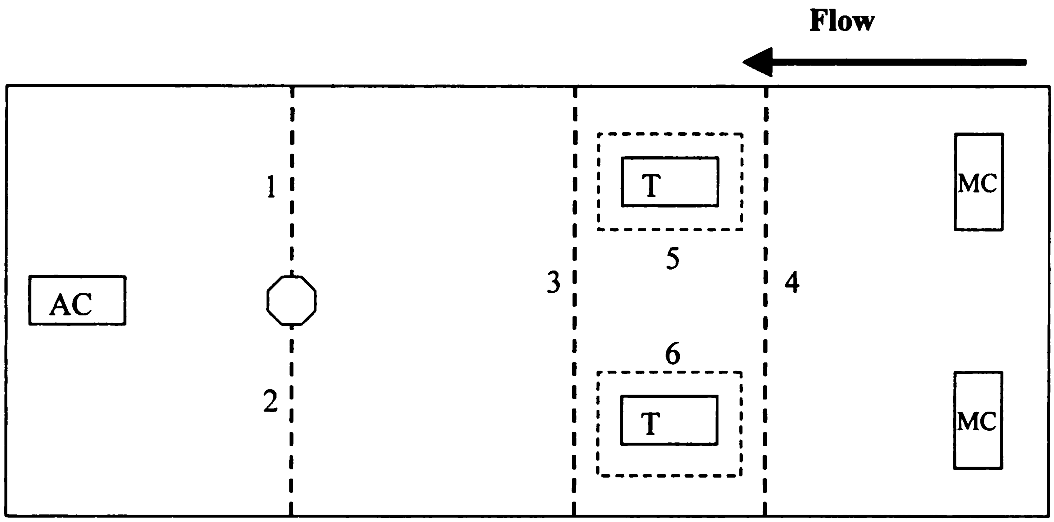

Figure 1. Field site set-up for field experiment. Outside border indicates the barriers and river bank. PIT antennae are denoted by dashed lines. Traps (T), the female acclimation cage (AC), and the male holding cages (MC) were arrayed as shown. The space between antenna 1 and antenna 2 represents a dead zone for PIT tag detection. This zone was blocked off by 1 cm mesh.

Bridge Organics (Kalamazoo, MI) synthesized the two pheromone components in January-March 2006. I stored both components in salt form (3kACA: $C_{24}H_{38}O_5$, MW 406.56; 3kPZS: $C_{24}H_{39}O_7S\text{-}NH_4$, MW 492.00) at -80 °C until needed. Stock solutions (20 mg 3kPZS/mL methanol, 10 mg 3kACA/mL methanol) were prepared for each compound. For each trial, pheromone amounts mimicked the estimated amount of pheromone released by five males (500 µg/h/male 3kPZS, 50 µg/h/male 3kACA; Yun et al. 2002) over a 4 h time period. I chose to mimic five males because the amount of pheromone produced by five males is highly attractive to females, and increasing the odor beyond those levels has shown diminishing returns in female attraction (Chapter 1; Wagner et al. 2006). For trap odors including 3kPZS, 0.518 mL of stock solution was pipetted into a micro-centrifuge tube; for treatments including 3kACA, 0.104 mL of stock solution was pipetted into a micro-centrifuge tube; and for treatments including both, 0.518mL of 3kPZS stock solution and 0.104 mL 3kACA stock solution were added to a microcentrifuge tube. The pheromone from the tubes was mixed into buckets containing 6.25 L of Lake Huron water immediately before being pumped into the traps for the trial.

I directly compared each single pheromone component to the pheromone mixture with and without a natural background pheromone plume provided by 10 live spermiating males 50 m upstream of the traps (five on each side). In each trial, a single odor (3kPZS or 3kACA) was pumped into the trap on one side of the stream, and a mixture of the two compounds (3kPZS+3kACA) was pumped into the trap on the other side of the stream. The four treatments were 3kPZS+3kACA vs. 3kPZS (no background), 3kPZS+3kACA vs. 3kPZS (plus background), 3kPZS+3kACA vs. 3kACA (no background), and

3kPZS+3kACA vs. 3kACA (plus background). I conducted eight trials for each of the four treatments and alternated sides between replicates for each treatment.

One day prior to the start of each trial, 10-12 ovulating females were measured, weighed, and tagged externally with fin tags and PIT tags. Eight hours before the trial, I chose the 10 tagged females that appeared to be in the best condition, transported them to the field site, and placed in the acclimation cage. One hour before the trial, I placed five healthy spermiating males in each upstream cage to produce the natural background odors (plus background treatments) and dispensed synthesized pheromone into each trap using peristaltic pumps. Within each treatment, the pheromone was released four times each from the right- and left-hand traps for a total of eight replicates. I pumped pheromone at the rate equivalent to the production of five males (according to Yun et al. 2002) in each trap. After 1 h of odor introduction, females were released and monitored by PIT antennas for 3 h. I also visually tracked females for at least the first 2 h of each trial, and recorded in which half of the stream they were moving at each transect. At the end of each trial, the males and captured females were removed from the traps or cages, and the fin tag numbers were recorded for the females. Water temperature was recorded every hour by a HOBO water temperature data logger (Onset Computer Corporation, Borne, MA).

Habitat and Dye Study

I surveyed the stream habitat on May 31, 2006. At each 10 m transect, I measured depth, velocity, and classified substrate type at 1 m intervals across the stream. Substrate was visually classified according to the procedures in Murphy and Willis

(1996). These data were then averaged for the right and left side of the stream for each transect and are displayed in Table 1.

I conducted a rhodamine dye study on June 5, 2006 to determine how the pheromone is carried from the traps by the current. I measured the discharge of the stream using the method described by Murphy and Willis (2006) and calculated the pumping rate necessary for a fully mixed within stream dye concentration of 5 ppm. Before sampling, dye was pumped into each trap for 10 min to establish an equilibrium plume. The plumes from each trap were sampled separately, to determine the part of the pheromone plume specifically attributable to each trap. Five evenly spaced (across the channel) samples were taken at each 10 m transect, starting 20 m below the traps and continuing downstream to where the acclimation cage was present. Samples were taken in 5 mL cuvettes from the middle of the water column, and were placed in a 17 °C water bath until the dye concentration could be measured using a field fluorimeter. I waited 30 min between the sampling of each plume to ensure that the dye from the previous sampling period was discharged downstream of the study site.

Data Analysis

The number of females approaching each trap was the main response variable for data analyses. I used two-by-two factorial ANOVA with background and trap bait as the fixed factors in the 3kACA treatments and the 3kPZS treatments. I used an arcsin square-root transformation to compare the proportion of responding females that actually approached a trap between background and no background treatments (the transformed data I compared using a t-test). I used ANCOVA to compare swimming speed (measured as time between the first antennas and the trap antennas) between treatments with water temperature as the covariate. Similarly, I tested treatment effects in terms of time spent in the vicinity of the traps, with stream temperature as the covariate. Finally, ANCOVA (temperature was the covariate) was used to test for differences in time spent near 3kPZS traps vs. time spent near the 3kPZS+3kACA traps in the treatments that compared 3kPZS to 3kPZS+3kACA. Not enough females approached the 3kACA traps for the same test to be conducted on those traps. Time data (in seconds) were square-root converted, and then natural log-transformed so that the residuals were approximately normal.

RESULTS

The background of males did not increase the total number of females that swam upstream in any case [3kPZS vs. mixture treatments: background (mean 5.0 ± SE 0.98) no background (mean 5.62 ± SE 1.02) ($F_{[1,28]}$=0.01; P=0.93); 3kACA vs. mixture treatments: background (mean 5.62 ± SE 1.03) no background (mean 6.13 ± SE 0.85) ($F_{[1,28]}$=1.19; P=0.28)]. In all cases, at least 50% of the tagged females approached the traps. Of the females that swam upstream, 88% approached a pheromone-baited trap in the no background treatments, while 75% approached a pheromone-baited trap in the plus background treatments. This difference was significant (t_{30}=2.04, P=0.03).

The mixed odor trap was not more attractive than the 3kPZS trap (Figure 2; $F_{[1,28]}$=1.18; P=0.29), and did not retain females in the vicinity longer than the 3kPZS trap (3kPZS trap mean 59.1s; 3kPZS+3kACA trap mean 53.5 s; $F_{[1,53]}$=0.34; P=0.56). However, females did prefer the mixed odor over 3kACA (Figure 3; $F_{[1,28]}$=43.00; P<0.0001). Not enough females approached the 3kACA trap to test for differences in time spent near it vs. time spent near the 3kPZS+3kACA trap. These preferences did not differ between no background and plus background treatments (Figures 2-3).

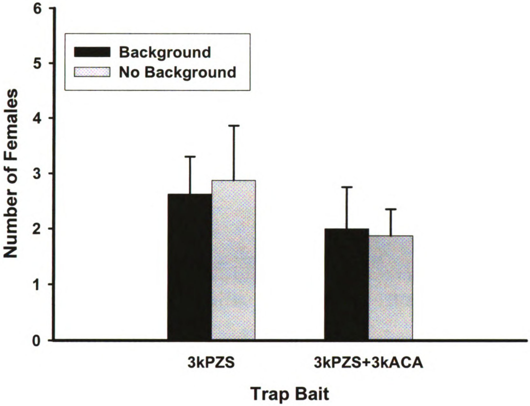

Figure 2. Mean (±SE) number of females approaching each trap for the 3kPZS vs. 3kPZS+3kACA treatments (N=8 for each bar). None of the means differed significantly.

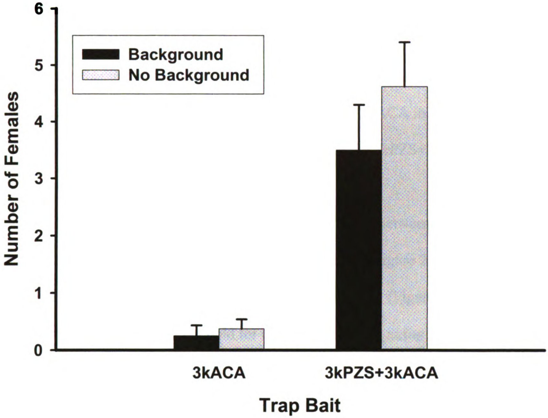

Figure 3. Mean (±SE) number of females that approached each trap for the 3kACA vs. 3kPZS+3kACA treatments (N=8 for each bar). 3kPZS+3kACA was significantly more attractive than 3 kACA alone in background and no background treatments.

Ground speed decreased as water temperature increased ($F_{[1,165]}=5.42$; $P=0.02$), and did not differ between treatments with or without background ($F_{[4,165]}=1.98$; $P=0.10$). Temperature was negatively associated with the time females spent near the traps ($F_{[1,150]}=5.11$; $P=0.025$). Females spent twice as much time near the traps in the no background treatments (134 s vs. 67 s; $F_{[1,150]}=21.13$; $P<0.0001$). There was no difference in the time females spent near the traps between 3kPZS and 3kACA treatments ($F_{[1,150]}$=0.33;P=0.57).

I only captured 3 total females out of the 320 released overall. None of the females were captured in the plus background trials. One female was captured in a trap baited with 3kPZS and one in a trap baited with 3kPZS+3kACA in the 3kPZS vs. 3kPZS+3kACA trials, and one female was captured in the 3kPZS+3kACA trap in the 3kACA vs. 3kPZS+3kACA trials.

While there was no difference in female preference between 3kPZS and the mixture in the background and no background treatments (Figure 2), females generally preferred whichever odor was on the right side of the stream (Figure 4; $F_{[1,28]}$=31.11; P<0.0001). This pattern did not hold for the treatments comparing 3kACA to the mixed odor; females preferred the mixed odor no matter which side of the stream it was in (Figure 3).

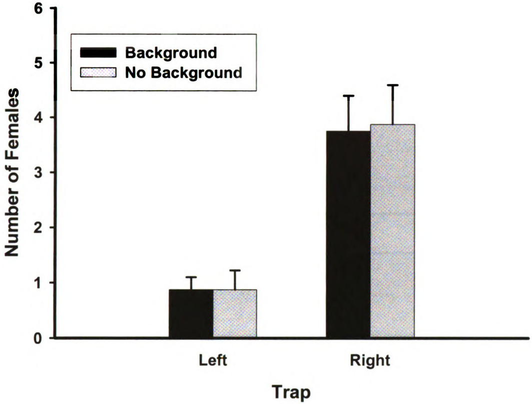

Figure 4. Mean (±SE) number of females that approached each trap for the 3kPZS vs. 3kPZS+3kACA treatments (N=8 for each bar). Because treatments were alternated so that each odor was on each side an equal number of times, these data show that the females usually chose the right side of the stream in these treatments regardless of whether 3kPZS or 3kPZS+3kACA was on the right side. Right side means were significantly greater than left side means for background and no background treatments.

I found seven different types of substrate in the habitat survey. The most common substrate in the stream reach was clay (40%) followed by sand (35%), silt (11%), woody debris (7%), cobble (4%), gravel (less than 1%), and vegetation (less than 1%). Depths ranged from 0.08-1.2 m, and velocities ranged from 0.00-0.65 m/s. The right side of the stream had lower average velocity, sandier substrate, and more woody debris (Table 1); whereas the left side of the stream had higher average velocity and substrate dominated by clay.

Table 1. Physical stream features comparing the right and left side of the stream as measured in transects.

Factor	Data Type	Left Side	Right Side
Woody Debris	Proportion of Habitat	0.00	0.14
Clay	Proportion of Habitat	0.79	0.02
Sand	Proportion of Habitat	0.06	0.65
Velocity	Mean m/s	0.32	0.21
Pheromone Concentration	Mean dye concentration relative to highest measured	1.61	1.55

DISCUSSION

My results support the main hypothesis: that 3kPZS is sufficient to attract ovulating females on spawning grounds. Females successfully located 3kPZS point sources even in the plus background treatments (75% of those that swam upstream approached a pheromone-baited trap), and 3kPZS was as attractive as the mixed odor both with and without a background (Figure 2). I also postulated that 3kACA would increase female retention near a 3kPZS source and/or serve as a short range attractant (important when 3kPZS was already present). My data do not support either of these hypotheses: 3kACA was not attractive in the no background or plus background treatments (Figure 3), and retention near the synthesized pheromone was not increased by the addition of 3kACA to 3kPZS. I found that a background odor produced by 10 males did not change female preferences for the compounds (Figures 2-3), but did reduce the amount of time females spent near the pheromone sources. Temperature was an important covariate for female swimming speed and the time females spent near the traps. Finally, I found a right side stream bias when 3kPZS was applied to both sides.

3kPZS is clearly the main component necessary to attract ovulating females on spawning grounds. 3kPZS and the mixture of 3kPZS+3kACA were able to attract females upstream even without a natural male background, and to a specific area in the stream even when a diffuse pheromone background was present. Female sea lampreys are capable of tracking to a point source of 3kPZS in a stream system even when males are releasing pheromone upstream. Therefore, ovulating females must be able to respond to 3kPZS gradients in the stream because presence vs. absence is not informative in a diffuse background. Initial studies of spermiating male extract showed 3kPZS to be the compound exclusively released by spermiating males with the highest electro-olfactogram potency (Li et al. 2002), and that the strength of the EOG response increased exponentially from initial detection (10^{-12} M) to the highest concentration measure (10^{-8} M) (Siefkes and Li 2004). My previous work showed that females preferred high concentration signals well downstream of their source (Chapter 1). Therefore, females are likely highly sensitive to 3kPZS concentrations in a stream, and could use chemotaxis to take advantage of the transverse gradient known to be present in pheromone plumes (Vickers 2000) to track to the highest 3kPZS concentration. The fact that this gradient becomes even more defined close to the pheromone source also could assist females in location of the point source.

While 3kPZS is important for mate-finding, other sensory input probably augments 3kPZS gradients for females. In the current study, 75% of the females that swam upstream investigated the synthesized pheromone before swimming to the background signal when both traps contained 3kPZS. However, traps baited with spermiating males attracted 95% of the females that swam upstream before they moved on to investigate a similar concentration background signal (Luehring Chapter 1). Visual signals and alternate olfactory signals could both be important in the final few feet of mate-finding for a female. In the prior study (Chapter 1), spermiating males were placed in metal mesh holding cages behind the traps. While the mesh was somewhat transparent, the sight of the several males in a mesh holding cage is very different from an unimpeded view of a male on a nest, and vision is known to deteriorate in spawning phase lampreys (Manion and Hanson 1980). Therefore, the most likely explanation is that additional odorants in spermiating male washings assist females in fine-scale mate-finding. Females detect 3kPZS at 10^{-12}M concentrations in electro-olfactogram tests, but only detect 3kACA at 10^{-10}M concentrations (Siefkes and Li 2004). 3kPZS is also released at the highest rate of the identified compounds in the spermiating male washings (Siefkes and Li 2004). Although 3kPZS is a sufficient signal alone to locate a point source, minor pheromone components released at a lower rate and detected at a higher concentration could help direct females in a spawning stream with background pheromone present.

At least one unidentified compound is responsible for retaining the females on the nest. Synthesized compounds were not adequate for retention with or without a background. Despite the effectiveness of synthesized 3kPZS in attracting females within 1 ft of a trap, I found short retention (about 134 s) near 3kPZS even in the absence of background pheromone. Observations of traps containing spermiating males showed that females investigate a natural pheromone source for well over 2 h (Luehring Chapter 1), and Siefkes et al. (2005) also observed a marked reduction in retention near 3kPZS as compared with spermiating male washings. I postulated that 3kACA would improve retention near the pheromone source, but I observed no difference in the time spent near 3kPZS traps and the time spent near 3kPZS+3kACA traps. Wittenberger (1983) suggested that females may use hierarchical cues to evaluate mates. Long range cues (in this case 3kPZS) could be important for females to eliminate lower quality areas for mate selection. Females prefer large signals as far as 90 m downstream of the pheromone source (Luehring Chapter 1). Then, short range cues assist in the final mate selection or selections. Obviously, a very short range attractant that retains females near the nest for mating opportunities would be critical for male mating success, and could be critical in female mate selection. If discovered, this compound or group of compounds may prove to be important in later stages of mate selection.

My data indicate that 3kACA does not attract or retain females on spawning grounds. Although similar in structure to 3kPZS and also only released by spermiating males (Yun et al. 2003), 3kACA does not appear to function as a redundant compound. EOG studies have shown that females have specialized receptors for 3kACA different from those for 3kPZS (Siefkes and Li 2004), and 3kACA is not attractive to females in any instance tested to date. 3kACA might only be important to female sea lampreys in the appropriate context. Females are not attracted to male washings or 3kPZS until fully mature, when they seek a mate (Siefkes et al. 2005). Similarly, 3kACA may not be relevant in the absence of other stimuli or prior to a necessary physiological stage. In other pheromone systems, specific pheromonal cues are augmented by secondary pheromone components or non-olfactory sensory modalities for successful mating (Rybak et al. 2002, Mclennan 2003, Thompson et al. 2004, Shine 2005, Papke et al. 2007). Multi-component pheromones in insects often display synergy between compounds. In early identifications of moth pheromones, single pheromone components were not attractive on their own, but regained full activity when the correct ratio of components was mixed (Silverstein 1977; Wyatt 2003). Although similar instances in sea lamprey could explain why 3kACA is not attractive in field tests, my data do not support the synergism hypothesis for the sea lamprey pheromone. 3kPZS is highly attractive on its own, and 3kACA did not prove attractive in a background of natural pheromone. For female sea lampreys, visual or tactile perception of a mature male, or the stage of nest interaction may be important for 3kACA function. Conceivably, 3kACA could be used for sex recognition in the species. Lamprey nests generally consist of oval-shaped depressions in gravel bars (Applegate 1950), which could potentially concentrate pheromone odors (i.e. 3kPZS). Late in the spawning season, females often predominate on nests (Applegate 1950). Thus, a low concentration odor such as 3kACA may serve to help females identify the male just before the spawning act. 3kACA could be important to sea lampreys for species recognition. No studies to date have determined whether mature adult males in other lamprey species release 3kPZS or 3kACA, but the general pattern of spawning behavior is preserved throughout the lamprey species studied (Hardisty and Potter 1971), and sea lamprey spawning times and ranges overlap with those of native lamprey species (*Ichthyomyzon unicuspis, I. fossor, I. castaneus,* and *Lampetra appendix*) (Becker 1983) within the Great Lakes basin. Therefore, sea lampreys must use some signal to distinguish potential mates from other lampreys. 3kACA itself is not important in species recognition for sea lamprey because it did not add to the attractiveness of 3kPZS, but it could combine with other pheromone components to produce a species specific mixture. In moth pheromone systems, minor pheromone components are especially important in species recognition because several related species include the same compounds in their pheromone signal (Wyatt 2003). However, large size differences between species suggest that visual or tactile stimuli are probably sufficient signals for sea lampreys to distinguish conspecifics. Finally, Li et al. (2005) suggested that 3kACA may function as a primer pheromone rather than a releaser pheromone. Primer pheromones combine with releaser pheromones to coordinate reproduction in other fish (Sorensen and Stacey 1999), and female sea lamprey mature at a faster rate in the presence of mature male odors (Siefkes unpublished).

Available pheromone upstream and water temperature both reduced female time spent near the synthesized compounds. Females averaged 57 s near the traps in background treatments and 134 s in the no background treatments. Previous studies have shown that a pheromone background reduces capture rate in traps baited with spermiating males (Luehring Chapter 1). The proposed mechanism for this was less time spent searching around the traps when other sources of pheromone were present to investigate (Luehring Chapter 1). Optimal choosiness theory suggests that decisions on choosing a mate are affected by the distribution of mate quality, costs of locating a mate, and the quality of the individual making the choice (Parker 1983; Real 1990; Real 1991). Upstream pheromone sources indicate additional options for females, and the full male signal may have suggested better mate quality upstream. Because warmer water temperatures generally occur near the end of the spawning season (Applegate 1950), they may represent reduced time available to spawn for females (a reduction in perceived quality for females). Also, males available for spawning become more limited later in the season (Applegate 1950). Because the synthesized signal is not complete, and the natural odor upstream is, females may move more quickly to the upstream signal (a more acceptable signal) at higher water temperatures to beat competitors to the viable males. Late in the spawning season, females occasionally begin nest construction on their own, and spent males often remain alone in nests for a time after spawning (Applegate 1950). Therefore, females may give up more quickly on pheromone sources without secondary sensory cues present (i.e. male movement, rock moving sounds, etc.) at higher water temperatures. Also, where measured, higher water temperatures result in increased respiration in lampreys (Hardisty and Potter 1972) in turn increasing the rate at which energy reserves become depleted. In theory, these conditions could make a female less choosy about the quality of mate, but more apt to give up on pheromone signal she deems incomplete in favor of a full signal upstream.

Higher water temperatures also reduced swimming speeds. Applegate (1950) describes sea lamprey spawning at 15-23°C. Temperatures during my trials ranged from 16-23°C (within the natural spawning range). Prior studies with parasitic and migratory phase sea lampreys showed better swimming performance with warmer water temperatures (Beamish 1974). However, these studies only ranged in temperature up to 15°C, and water temperatures above 18.5 °C actually inhibit migratory phase adults (Hardisty and Potter 1971). My data corroborate this finding in mating phase adults.

Females preferred the right side of the study stream when pheromone signals were essentially equal. Because 3kACA did not increase the attractiveness of 3kPZS, trials that compared 3kPZS to 3kPZS+3kACA essentially compared equivalent pheromone signals. The dye study data showed no average difference in overall dye concentration on the right vs. left side, so females were most likely choosing the right side based on physical stream features. Average velocity was lower on the right side of the stream (Table 1), and the habitat features on this side of the stream generally reflected that condition. Females may have chosen the right side of the stream because it is less energetically expensive to swim upstream against low velocity, and spawning phase sea lampreys must operate under a fixed energy budget (Hardisty and Potter 1971). Energy stores saved in locating males could result in more opportunities for the female to choose the ideal mate on the spawning grounds. The right side substrate was dominated by sand, silt, and woody debris, while the left side had more clay. These features make the right side of the stream advantageous for predator avoidance. Migratory and mature sea lamprey are preyed upon by gulls, hawks, owls, herons, bitterns, raccoons, muskrats, foxes, rats, weasels, minks, and large piscivorous fishes in Great Lakes and Atlantic Ocean tributaries (Applegate 1950). The substrate on the right side of the stream provided more overhead cover in the form of woody debris, and generally matched sea lamprey color patterns better than the left side.

MANAGEMENT IMPLICATIONS

Each female caught in a pheromone baited trap can be attributed to a combination of two general factors: Efficacy of the attractant and efficiency of the trap. My data show the importance of optimizing both factors to efficiently catch lampreys.

3kPZS increases the probability that an ovulating female will encounter a trap even in areas where background pheromone is present. Previous studies have shown that un-baited free-standing traps are not attractive to ovulating females (Johnson et al. 2005; Johnson et al. 2006). In my study, 3kPZS drew 45% of the females I released to the traps. Of those that actually swam upstream, 89% approached a pheromone baited trap with no background odors. Therefore, 3kPZS could be used to monitor areas believed to be lamprey free (such as upstream of barriers or streams with adult spawning runs that are believed to be minimal). Even with a pheromone background upstream of the traps, 3kPZS effectively drew 75% of the lampreys within 1 ft of the traps. Conceivably, 3kPZS could be effective in an intercept fishery (traps on the downstream edge of the spawning grounds) in a mass-trapping control strategy with a moderate number of males upstream.

I only captured 2% of the females released in my study; whereas, other studies using the mating pheromone full male signal had capture rates of 50% or more (Johnson et al. 2005; Johnson et al. 2006; Wagner et al. 2006). Wagner et al. (2006) used the full male signal as trap bait, and similar trap design. 3kPZS is an effective point source attractant, but does not retain females in the area of the point source nearly as well as the full male signal (Siefkes et al. 2005). 3kACA does not appear to offer any advantage to trapping sea lampreys. Female capture rate could be increased by identifying more pheromone components, and other important sensory cues for retaining females in the area of the traps.

Trap mechanics also play a large role in the capture rate of females. Other studies with improved trap designs displayed higher capture rates with synthesized 3kPZS (Johnson unpublished). In my study, several mechanical factors likely reduced sea lamprey capture rate. First, the traps were made out of aluminum mesh, and do not mimic natural substrate. Second, I pumped pheromone into the back of the trap in the middle of the water column. This allowed the plume to be wider at the trap opening, and higher in the column than the funnel opening. Other mechanical improvements (i.e. lead wings, more funnel openings, or trap placement) also would improve the capture rate.

IV. Concluding Remarks and Hypotheses

Females prefer large pheromone signals, and 3kPZS is most likely affecting this preference. In chapter 1, I demonstrated that preferences for multiple male signals were a result of more pheromone coming from the source rather than some preferred quality of the multiple male signal. Furthermore, I showed that female preference for larger signals was displayed well downstream from the pheromone source, and is likely the result of actual female preference as opposed to variable detection thresholds for females. Female preference for high concentration signals seemed to show diminishing returns with increasing levels of males above six. I believe this is evidence for a behavioral threshold, where females are satisfied with a reasonably large signal. In chapter 2, 3kPZS was a good point source attractant both with and without a background of pheromone. These data suggest that females can and do use 3kPZS gradients to locate a pheromone source. Thus, I hypothesize that 3kPZS concentration likely affects female preference.

I also found that 3kPZS has limitations as an attractant and a trap bait. In chapter 2, 75% of the females that swam upstream during background trials approached synthesized pheromone, while 95% approached spermiating male traps in background treatments (Chapter 1). I found very poor retention near the traps when we used synthesized pheromone, and good retention even when a pheromone background was present when use live males were used to bait the traps. 3kACA did not improve retention or attractiveness in any instance. Therefore, I believe that undiscovered pheromone components and potentially other sensory systems are useful in short range attraction and retention.

Diffuse pheromone backgrounds affect time spent searching around the traps more than they affect female ability to locate pheromone sources. In chapter 1, females located the high bait pheromone source with essentially the same precision as they did when there was no background, and 95% of the females that swam upstream approached a trap before investigating the background signal. Even with synthesized compounds, 75% of females investigated the traps before they moved upstream. Because we did not have an upstream antenna during the first treatment, we were not able to get good data on whether females swam upstream past the traps when there was no background, but my observations suggest that they did not. Other types of data suggested that females around the downstream traps left more quickly when a background signal was available upstream. In chapter 2, we had antennas to measure the amount of time females spent in the vicinity of the traps both in background and no background experiments. Although we did not have the full pheromone in the downstream traps, females still only spent about half as long near the traps when a background pheromone was present.

When pheromone signals are considered approximately equal, females respond to physical habitat features while tracking pheromone plumes. In chapter 2, females preferred the right side of the stream when we compared 3kPZS to 3kPZS+3kACA. In general, 3kACA had no effect on female preference, so these two trap baits were essentially equivalent. Females preferred the low velocity side of the stream with more sand, silt, vegetation, and woody debris.

My data demonstrate trapping capability for large pheromone signals and for 3kPZS. Pheromone signals of 6 or more males were very attractive to females. The fact that the 12 male signal was not a lot more attractive than the 6 male signal suggests that strategies involving several moderately high bait traps would be more effective on the spawning grounds than one trap with an extremely high pheromone output. 3kPZS has its limitations in retaining females near the source, but appears to be an adequate bait to attract females to traps. Improved trap designs which quickly capture females could significantly improved the capture rates in traps baited with 3kPZS.

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