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[Article]

Mating Pheromone Reception and Induced Behavior in Ovulating Female Sea Lampreys

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Abstract.—This study was conducted to determine how ovulating female sea lampreys *Petromyzon marinus* respond to water conditioned with spermiating males (spermiating male washings) and how trap efficiency can be improved through their use. The capture rate of ovulating female sea lampreys was observed in traps baited with continuous or pulsed spermiating male washings. The behavior of ovulating females around baited traps was quantified. Within 2 h, traps baited with continuous spermiating male washings captured 52% of ovulating females ($n = 27$) and traps baited with pulsed washings captured 28% ($n = 25$) of ovulating females. Unbaited traps did not capture ovulating females. The behavior of females near traps baited with pulsed spermiating male washings was characterized by significantly more downstream and side-stream movements than that of females near traps with continuous washings. We occluded the olfactory organ of ovulating females and tested whether they were attracted to spermiating male washings in a two-choice maze and whether they could locate spermiating males in a spawning stream. Ovulating females with occluded olfactory organs were unable to locate spermiating males in a spawning stream. Furthermore, anosmic females were not attracted to spermiating male washings in a two-choice maze. We conclude that traps baited with spermiating male washings are able to capture females and that females may use the structure of the pheromone plume to locate the exact source of pheromones. It is likely that olfaction is the only means for ovulating females to detect a pheromone that is released by spermiating males.

Sea lampreys *Petromyzon marinus* invaded the Laurentian Great Lakes during the first half of the 20th century and inflicted catastrophic ecological and economic damage to the Great Lakes fisheries (Smith and Tibbles 1980). The destruction caused by the sea lamprey prompted one of the most extensive efforts to control an exotic vertebrate species in North America (Smith and Tibbles 1980). Currently, sea lamprey populations in the Great Lakes are controlled with lampricide treatments, sea lamprey barriers, trapping, and sterile male releases (Christie and Goddard 2003). The integration of these control techniques has reduced sea lamprey populations to levels that allow the Great Lakes ecosystem to support productive salmonid fisheries (Heinrich et al. 2003; Lavis et al. 2003; Morse et al. 2003). Sea lamprey control remains highly dependent on lampricide treatments that kill filter-feeding ammocoete larvae in natal streams (Christie et al. 2003). Growing concern regarding the social acceptance of chemical lampricide treatments, increasing cost of lampricides, and the untreatable nature of some streams require that new control techniques be developed and integrated into the Great Lakes sea lamprey management program (Christie and Goddard 2003). Mating pheromones, commonly integrated into insect control programs, have been successfully used to monitor, mass trap, and disrupt reproduction in pest populations (Howse et al. 1998) and may be useful in sea lamprey control programs.

Sea lamprey mating pheromones have potential in controlling sea lampreys in the Great Lakes (Teeter 1980; Li et al. 2002, 2003). Spermiating male sea lampreys release the mating pheromone 3-keto petromyzonol sulfate (3kPZS) at high rates to attract ovulating females (Li et al. 2002). Johnson et al. (2005) baited traps with spermiating males, found that over 70% of ovulating females were captured in baited traps, and concluded that traps baited with spermiating males may be used to remove females from spawning grounds. It is not known whether females used other sensory modalities to locate males placed in traps. Furthermore, pheromone-baited traps could be designed more efficiently (Carde et al. 1998) if pheromone-induced behaviors are described, and schemes to disrupt pheromone communication may become apparent if pheromone reception in ovulating females is understood (Carde 1990; Sanders 1996). Currently, the behavior of ovulating females near pheromone-baited traps is poorly described (Johnson et al. 2005) and the physiological mechanisms of pheromone reception are not fully identified in ovulating female sea lampreys (Li et al. 2003).

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Sea lampreys are believed to detect pheromones through the olfactory organ via a single dorsal nasopharyngeal opening (Li et al. 1995; Siefkes and Li 2004). The olfactory epithelium of adult sea lampreys has been shown to have highly independent receptor sites for mating pheromones, migratory pheromones, and other bile acids (Li and Sorensen 1997; Siefkes and Li 2004). Vrieze and Sorensen (2001) showed that migratory sea lampreys with impaired olfactory systems were not attracted to sea lamprey migratory pheromones and showed little ability to locate spawning streams. It is not known to what extent olfaction mediates mating pheromone reception and mate finding in ovulating female sea lampreys (Li et al. 2003).

The objectives of this study were to (1) determine whether ovulating female sea lampreys could be lured into traps baited with water conditioned with spermiating males, (2) describe the behaviors of ovulating females near male-pheromone-baited traps, and (3) determine whether ovulating females use their olfactory organs to locate spermiating males.

Methods

Experimental Animals

Sea lampreys were captured by hand or in mechanical traps from Lake Michigan and Lake Huron tributaries from May through July 2004. Females were identified by their soft abdomen and were separated from males identified by their dorsal ridge (Vladykov 1949). Furthermore, adults were classified as spermiating males and ovulating females if milt or eggs, respectively, were expressed by manual pressure (Siefkes et al. 2003). Spermiating males and ovulating females were used for experimental purposes and were stored in separate 150-L flow-through tanks at temperatures ranging from 15°C to 22°C. Nonspermiating males and preovulating females were stored in separate 1,000-L flow-through tanks at temperatures ranging from 4°C to 14°C. Nonspermiating males and preovulating females were checked weekly for spermiation and ovulation. To induce female ovulation, several preovulating females were placed in cages with spermiating males in the Ocqueoc River, a Lake Huron tributary in Presque Isle County, Michigan, at temperatures ranging from 14°C to 25°C. Additionally, several preovulating females were stored with spermiating males in a 1,000-L flow-through tank at 16°C to induce maturation.

Experiment 1. Do Traps Baited with Water Conditioned by Spermiating Males Capture Ovulating Female Sea Lampreys?

Test site and equipment.—Experiments were conducted above the lamprey barrier on the Ocqueoc River (Figure 1). Historically, the Ocqueoc River produced significant spawning runs of sea lampreys (Applegate 1950); however, above the barrier, no sea lampreys have been observed and the stream contains suitable physical characteristics for spawning. Using two block nets, we enclosed a 65-m section of the river. At the

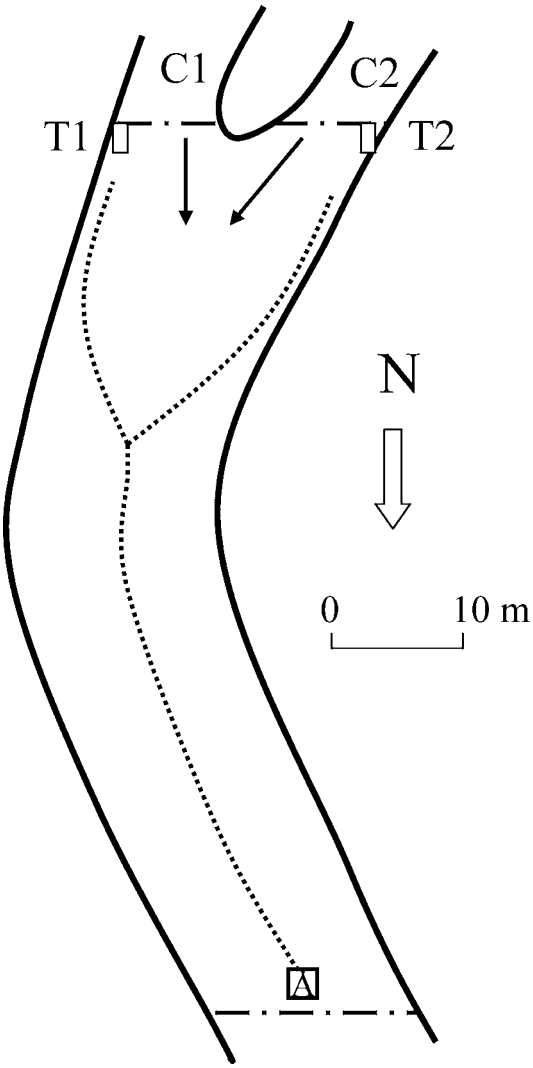

FIGURE 1.—The 65-m section of the Ocqueoc River, Presque Isle County, Michigan, used for sea lamprey trapping experiments between 18 June and 8 July 2004. The section of river was enclosed with upstream and downstream block nets (dashed horizontal lines). An island naturally divides the river into channel one (C1) and channel two (C2). A sea lamprey trap was placed in each channel of the river approximately 1 m from the block net and 0.5 m from the shore (T1 and T2). The arrows represent the flow of water. Females were released from an acclimation cage (A) at the downstream block net. As females move upstream (dotted line), they must enter C1 or C2.

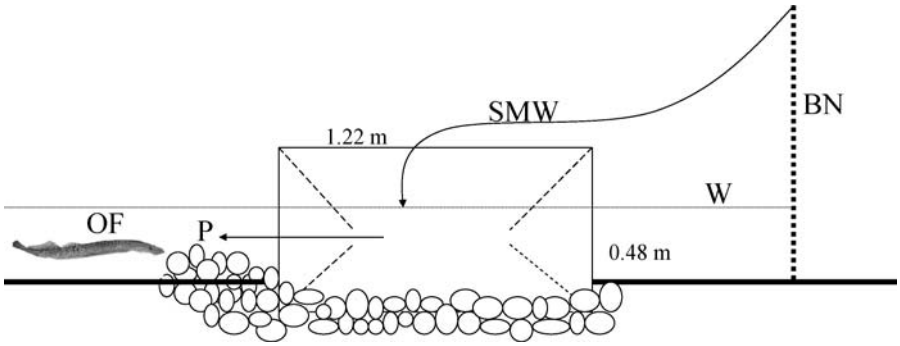

FIGURE 2.—Sea lamprey trap dimensions and trap set used to capture ovulating female sea lampreys on the Ocqueoc River, Presque Isle County, Michigan, between 18 June and 8 July 2004. Sea lamprey traps were set in 0.3 m of water (W) 1 m downstream of a block net (BN) and were placed in hand-constructed depressions in the stream bottom. Rocks approximately 5 cm in diameter were placed around and in front of the traps to imitate a sea lamprey nest. Water conditioned with spermiating males was pumped in the traps (SMW) and created a pheromone plume (P) exiting the downstream funnel of the trap. Ovulating females (OF) downstream of the trap would follow the pheromone plume to the trap.

upstream block net, the river is divided into two distinct channels with nearly equal flows of 0.5 m^3/s. The two channels converge and mix in the middle of the enclosed stream. The stream is a single channel with an average discharge of 1.01 m^3/s at the downstream barrier. Streamflow was measured weekly or after significant precipitation events with a Marsh-McBirney (Frederick, Maryland) flowmeter.

Two identical sea lamprey traps (0.359 m^3) were used to capture ovulating females (Figure 2). A trap was placed in each channel of the stream in approximately 0.3 m of water. Traps were placed 1 m below the upstream barrier and 0.5 m away from the near shore. The long axes of the traps were positioned parallel to the current to create a pheromone plume exiting the downstream funnel of the trap. The average velocity of water flowing through the downstream funnel was 0.24 m/s. Traps were placed in a depression in the stream bottom approximately 0.1 m deep, and rocks approximately 5 cm in diameter were placed in front of the trap to imitate a sea lamprey nest. Setting pheromone-baited traps in hand-constructed spawning nests makes sense biologically because spermiating males initiate nest building (Applegate 1950) and release pheromones to guide females to their nest (Teeter 1980). Additionally, placing traps in hand-constructed spawning nests lowers the funnel of the trap, making it easier for females to enter as they search along the stream bottom. While we felt that these trap modifications aided capture efficiency, we did not explicitly test the utility of the modifications.

Water conditioned by 5 spermiating males (spermiating male washings) was used to bait sea lamprey traps. Spermiating male washings were prepared immediately before experimentation by placing 5 spermiating males in a 25-L bucket of water for 2.5 h. A peristaltic pump was used to apply washings to a trap at a rate of 167 mL/min (25 L/2.5 h) for 2.5 h. Therefore, the amount of pheromones pumped into a trap over 2.5 h was equal to the amount of pheromones released by 5 spermiating males in 2.5 h. More specifically, based on the estimated release rate of 3kPZS by a spermiating male, approximately 500 $\mu g \cdot h^{-1} \cdot animal^{-1}$ (Yun et al. 2002), and the average streamflow, 1.01 m^3/s, the average instream concentration of 3kPZS when spermiating male washings were applied to a trap was 1.5×10^{-12} M. This concentration is very close to the detection threshold of 3kPZS as determined by both electrophysiological (Siefkes and Li 2004) and behavioral (Siefkes et al. 2005) assays. Ovulating females were released from an acclimation cage (1 m^3) placed at the downstream barrier. The acclimation cage was constructed of 1-cm plastic mesh stapled to a wood frame.

Experimental design.—Studies were conducted from 18 June to 8 July 2004 between 0900 hours and 1300 hours in water temperatures ranging from 17°C to 20°C. Ovulating females were fitted with external radio tags (Advanced Telemetry Systems, Inc., Isanti, Minnesota) according to Siefkes et al. (2003) 15 h before experimentation. Females were transported to the Ocqueoc River and placed in the acclimation cage 12 h before experimentation.

Spermiating male washings were applied to a randomly chosen (determined by flipping a coin) trap and river water was applied to the other trap. Spermiating male washings and river water were introduced to the traps 30 min before female release. After 30 min, the acclimation cage was opened and ovulating females were allowed to swim out. Five ovulating females were

simultaneously released in all trials but one, in which only 2 ovulating females were released because of a shortage of experimental animals. It was assumed that the behavior of each ovulating female released was independent from other ovulating females because Johnson et al. (2005) found no significant difference in the capture rate of individually released ovulating females and simultaneously released females. Furthermore, Siefkes et al. (2005) found that ovulating females released in groups do not move in synchrony. Ovulating females were visually observed for 2 h. If females were not visible, they were tracked with a directional radio antenna and receiver (Lotek Engineering, Inc., Newmarket, Ontario, Canada). The 5-m radius of each trap was marked on the stream bottom with flagging stakes. The time a female entered within a 5-m radius of the trap and the time of capture were recorded. When a female entered within 5 m of the baited trap, their behaviors, including downstream and side-stream movements, were recorded. A downstream movement was defined as a continuous movement 2 m or greater downstream with less than 2-m side-stream progress. A side-stream movement was defined as a continuous movement perpendicular to streamflow greater than 2 m with less than 2-m downstream or upstream progress. The distance a female traversed downstream and side-stream was visually estimated. The capture rate of ovulating females in traps baited with spermiating male washings was determined.

Experiment 2. Is a Pulsed Pheromone Plume as Effective as a Continuous Pheromone Plume?

Experiment 2 was conducted at the same location with the same equipment and design as experiment 1. In this experiment, spermiating male washings were pulsed into a trap in a pattern of on for 1 min and off for 1 min. When the odor was applied to a trap, the instream concentration of 3kPZS was approximately 1.5×10^{-12} M. Only 12.5 L of the 25 L of washings were applied to the trap during an experiment because the washings were pulsed.

Trials were conducted from 22 June to 7 July 2004 between 0900 hours and 1300 hours in water temperatures ranging from 17°C to 20°C. A *z*-test for two proportions was used to compare the proportion of ovulating females that left the acclimation cage, entered within a 5-m radius of the baited trap, and were captured when washings were continuously applied to a trap and when they were pulsed into a trap. A two-way *t*-test assuming equal variance was used to compare the average time of capture for females in traps baited with continuous washings and pulsed washings. A two-way *t*-test assuming unequal variance was used to compare the average number of downstream movements and side-stream movements of females that entered within 5 m of traps baited with continuous washings and pulsed washings.

Experiment 3. Are Naris-Plugged Ovulating Females Attracted to Water Conditioned by Spermiating Males in a Two-Choice Maze?

Naris-plugging and control treatment procedures.—Ovulating female sea lampreys were treated with a naris plug or a control treatment 12 h before experimentation. Naris-plugged animals were removed from water and SternVantage Quick Light Body (Sterngold Dental, LLC, Attleboro, Massachusetts), a dental impression adhesive, was injected into the olfactory cavity to occlude the nasopharyngeal opening. Next, a drop of Vetbond (Minnesota Mining and Manufacturing Co., St. Paul, Minnesota) was applied in the naris to adhere to the SternVantage and completely block the movement of water through the naris. The SternVantage and Vetbond were allowed to air dry for 10 s before females were returned to water. Naris-plugging procedures took approximately 1 min.

A control treatment was applied to non-naris-plugged females to control for olfactory irritation and nose-plugging procedure stress. Control ovulating females were removed from water and a 5-mm tip of a 1–200-μL-volume pipette (DOT Scientific, Inc., Burton, Michigan) was inserted into the nasopharyngeal opening. Immediately after, Vetbond was applied around the naris, but not in the naris. Vetbond was allowed to air dry for 10 s before females were returned to water. Control treatment procedures took approximately 1 min.

Two-choice maze procedures.—Experiments were conducted from 20 July to 29 July 2004 between 0700 hours and 1300 hours in water temperatures ranging from 16°C to 23°C. Sea lamprey preference response in a two-choice maze can be used to assess attraction to odors (Li et al. 2002; Siefkes et al. 2003). Therefore, a two-choice maze was used to assess the attraction of naris-plugged and control ovulating females to water conditioned by spermiating males. A two-choice maze was constructed on the bank of the Ocqueoc River and was of exact dimensions of the maze used by Li et al. (2002) and Siefkes et al. (2003). River water was pumped into the maze to create a flow of approximately 0.07 m^3/s. In each test, the preference behavior of either a naris-plugged ovulating female or a control ovulating female was recorded before and after the introduction of 10 L of water conditioned with 1 spermiating male for 1 h (spermiating male washings). Spermiating male washings were applied after a 20-min control period to a random arm of the maze at

a rate of 400 mL/min for 25 min. Data were collected and analyzed according to the procedure described in Li et al. (2002) and Siefkes et al. (2003). Briefly, the times spent (preference) in the treatment and the control arm of the maze before and after the introduction of spermiating male washings were summed by a naive observer. A two-tailed Wilcoxon's signed rank test (W; Rao 1998) was used to determine significant differences in preference between naris-plugged and control ovulating females.

Experiment 4. Can Naris-Plugged Ovulating Females Locate Spermiating Males in a Spawning Stream?

Experimental site and equipment.—Naris-plugged and control ovulating females were released to determine whether they could locate 5 spermiating males 10 m or 65 m upstream. This experiment was conducted at the same location as experiments 1 and 2 (Figure 1). Ovulating females were released from an acclimation cage (1 m^3). A block net (1-cm plastic mesh) was placed 5 m downstream of the acclimation cage to prevent females from moving downstream. No block net was placed upstream of the acclimation cage to obstruct upstream movement. Spermiating males were held in a cage (0.002 m^3) constructed of 1-cm plastic mesh stapled to a wood frame and were placed 10 m or 65 m upstream of the acclimation cage in a hand-constructed depression in the stream bottom 0.1 m deep to imitate a sea lamprey nest.

Experimental design.—Experiments were conducted from 22 July to 10 August 2004 between 0700 hours and 1800 hours in water temperatures ranging from 18°C to 24°C. Naris-plugged and control ovulating females were fitted with external radio tags (Advanced Telemetry Systems) according to Siefkes et al. (2003) at least 12 h before experimentation.

Ovulating females were held in the acclimation cage before experimentation. Five spermiating males were randomly placed 10 m or 65 m upstream of the ovulating females 30 min before release. Spermiating males placed 10 m upstream of ovulating females were positioned so that the pheromone plume passed directly through the acclimation cage. Spermiating males positioned 65 m upstream of ovulating females were randomly placed in one of two channels of the river by flipping a coin. Naris-plugged and control ovulating females were released together to control for environmental variability between tests. Either 2 naris-plugged and 3 control ovulating females were released or 3 naris-plugged and 2 control ovulating females were released during each trial. When the males had been in the stream for 30 min, the acclimation cage was opened and females were allowed to swim out. Females were visually observed for 2 h and the location and behavior of each female were recorded. If females were not visible, they were tracked with a directional radio antenna and receiver (Lotek Engineering Inc.). Females that came within 0.5 m of the spermiating males and searched around the cage for longer than 1 min were deemed to have located the spermiating males. Females that searched around the cage for longer than 30 min were removed from the stream. Females that swam more than 20 m upstream of the spermiating males were removed from the stream.

A z-test for two proportions was used to compare the proportion of naris-plugged and control ovulating females that moved upstream when males were at 65 m. A Fisher's exact test was used to compare the proportion of naris-plugged and control ovulating females that moved upstream when males were at 10 m. A Fisher's exact test also was used to compare the number of naris-plugged and control ovulating females that located 5 spermiating males at 10 m and 65 m. It was assumed that the behavior of each ovulating female released was independent from other ovulating females because Siefkes et al. (2005) found that females released simultaneously do not interact or move in synchrony.

Results

Twenty-seven individual ovulating females were released when spermiating male washings were applied continuously to a trap and 25 individual ovulating females were released when spermiating male washings were pulsed into a trap. Ovulating females were captured in traps baited with continuous and pulsed spermiating male washings but not in traps baited with river water (Table 1). When washings were continuously applied to a trap, significantly more ovulating females were captured within 2 h (capture rate = 52%) than in traps baited with pulsed washings (capture rate = 28%; $z = 2.11$; $P = 0.035$). Traps baited with continuous washings and pulsed washings lured approximately equal proportions of ovulating females to within 5 m of the baited trap ($z = 0.739$; $P = 0.460$; Table 1).

During the 2-h test period, two-thirds of the ovulating females moved upstream toward the channel with the trap baited with continuous or pulsed spermiating male washings. One-third of the ovulating females did not move upstream during experiments. The time of capture ranged from 15 min to 2 h after release. Eight percent of the females released were still progressing upstream toward the baited trap when the 2-h experiment ended. The average time of capture in a trap baited with continuous washings (40.5 min; SD = 27.2) was not different from the average time of capture in a trap baited with pulsed washings (41.7 min; SD = 34.9; Table 1; $t = 0.803$; df = 19; $P =$

TABLE 1.—Number of ovulating female sea lampreys that came within 5 m of traps baited with continuous (constant) and pulsed spermiating male washings within 2 h after release, number that were captured in traps, average time to capture, and average number of downstream and side-stream movements of fish. Values in bold italics were significantly different between treatments. Experiments were conducted on the Ocqueoc River, Michigan, between 18 June and 8 July 2004.

Observation	Washings	
	Constant	Pulsed
n	27	25
Lampreys that came within 5 m of trap	16	14
Lampreys captured in trap	***14***	***7***
Average time to capture (min)	41	42
Downstream movements	***0.38***	***6.08***
Side-stream movements	***0.19***	***1.77***

0.432). Four of the 14 females that were captured in traps baited with continuous washings immediately entered the trap without resting or searching around the trap, and 2 of the 7 females that were captured in traps baited with pulsed washings immediately entered the trap without resting or searching around the trap. However, most ovulating females spent several minutes searching around the trap before being captured. Common behaviors near baited traps included resting in front of the trap, rubbing on the sides of the trap, swimming under the trap, passing in front of the funnel, moving rocks, moving downstream, and moving side-stream. The average time an ovulating female spent within 5 m of a trap baited with continuous washings before being captured was 8.3 min (SD = 9.6), and the average time spent within 5 m of a trap with pulsed washings before being captured was 8.7 min (SD = 6.3). Ovulating females always entered the trap through the downstream funnel and never entered the trap as a result of an interaction with the upstream barrier.

The behavior of ovulating females near traps was strongly influenced by pulsing spermiating male washings. First, ovulating females only entered traps baited with pulsed washings during the periods when the washings were applied to the trap. Secondly, ovulating females near traps with pulsed washings had significantly more downstream movements ($t = 3.55$; df = 12; $P = 0.004$) and side-stream movements ($t = 2.49$; df = 13; $P = 0.027$) than females near traps with continuous washings (Table 1). Eighty-six percent of females that entered within 5 m of a trap baited with pulsed washings moved upstream when washings were applied to the trap and moved downstream or side-stream when the washings were not applied to the trap. The culmination of successive upstream and downstream movements resulted in cyclic movement patterns away from the trap and toward the trap. Fifty-four percent of the females that exhibited cyclic movement patterns were not captured in traps baited with pulsed washings.

Eleven individual naris-plugged ovulating females and 12 individual control ovulating females were tested in a two-choice maze for attraction to spermiating male washings. Naris-plugged ovulating females did not show a preference to spermiating male washings in a two-choice maze ($W = 27$; $n = 11$; $P > 0.250$; Table 2). Control ovulating females showed a significant preference to spermiating male washings in a two-choice maze ($W = 73$; $n = 12$; $P < 0.010$; Table 2).

Fourteen individual naris-plugged ovulating females and 14 individual control ovulating females were released when spermiating males were placed 10 and 65 m upstream of ovulating females. Naris-plugged ovulating females were unable to locate 5 spermiating males 65 m or 10 m upstream of females (Table 3). When males were placed 65 m upstream, 36% of naris-plugged females swam past the males, 21% swam upstream but did not make it to the males, and 43% remained in the acclimation cage or swam to the downstream barrier. When males were placed 10 m upstream, 42% of naris-plugged females swam past the males and 58% remained in the acclimation cage or swam to the downstream barrier. Control ovulating females were able to locate 5 spermiating males 65 m and 10 m upstream of females. When males were placed 65 m upstream, 50% of control ovulating females located spermiating males, 14% swam past the males, 14% swam upstream but did not make it to the

TABLE 2.—Number of naris-plugged and control ovulating female sea lampreys that showed a preference response to spermiating male sea lamprey washings in a two-choice maze constructed on the Ocqueoc River, Michigan. Statistical significance was determined with a two-tailed Wilcoxon's signed rank test (*W*-value) based on the test statistic and the number of animals tested (*n*).

Test subject	*n*	Spermiating male washings	*W*-value	*P*-value
Naris-plugged females	11	3	27	[a]
Control females	12	10	73	<0.010

[a]Not significant.

TABLE 3.—Number of control and naris-plugged ovulating female sea lampreys that were released (n) and that located 5 spermiating male sea lampreys placed 65 and 10 m upstream in the Ocqueoc River, Michigan, between 22 July and 10 August 2004.

Females	Distance (m)	n	Number locating males
Control	65	14	7
Naris-plugged	65	14	0
Control	10	14	10
Naris-plugged	10	14	0

males, and 21% remained in the acclimation cage or swam to the downstream barrier. When males were placed 10 m upstream, 71% of control ovulating females located spermiating males, 21% swam past the males, and 7% remained in the acclimation cage or swam to the downstream barrier (Table 3). The proportion of naris-plugged and control females that moved upstream was not significantly different when males were placed at 65 m ($z = 1.62$; $P = 0.106$). The proportion of control females that moved upstream was significantly greater than the proportion of naris-plugged females that moved upstream when males were placed at 10 m (Fisher's exact test: $P = 0.016$). Control ovulating females located 5 spermiating males significantly more often than naris-plugged ovulating females when males were placed 10 m upstream (Fisher's exact test: $P = 0.003$) and 65 m upstream (Fisher's exact test: $P < 0.001$).

Discussion

Our results demonstrate that spermiating male washings are able to lure ovulating female sea lampreys into traps. In our experimental site, traps baited with continuous spermiating male washings captured 52% of ovulating females within 2 h. The capture rate of females in traps baited with continuous spermiating male washings is similar to the capture rate of females in traps baited with spermiating males, where 40% of ovulating females were captured within 30 min and 70% of ovulating females were captured within 12 h (Johnson et al. 2005). Therefore, our study suggests that pheromones are a powerful trap bait that could be used instead of baiting traps with spermiating males. Future studies should investigate differences in capture rates in traps baited with spermiating males, spermiating male washings, extracted pheromones, and synthetic pheromones.

We hypothesize that the detection of male pheromones by ovulating females motivates their upstream movement. Pheromone plumes are described as turbulent, unpredictable filaments, which become widely spaced as they are carried away from the source (Keller et al. 2001; Sherman and Moore 2001; Wyatt 2003). In many insect species, fluctuating pheromone plumes are required for sustained upwind flight (Carde and Elkinton 1984; Baker and Haynes 1989). In the aquatic environment, virile crayfish *Orconectes virilis* have been shown to approach an odor source more quickly when the odor plume is turbulent (Moore and Grills 1999; Keller et al. 2001). Similarly, in our experiment, a pulsed pheromone plume lured ovulating females upstream, and equal proportions of females 65 m downstream of continuous and pulsed pheromones were lured to within 5 m of the baited trap. This may have occurred because the pheromone plume of both continuous and pulsed pheromone sources may have only consisted of random filaments of pheromones 65 m downstream from the source. It is possible, but has not yet been unequivocally demonstrated, that random, widely spaced filaments of pheromones may trigger upstream movement in ovulating females.

It is likely that ovulating females rely on pheromone plume structure to locate the exact source of pheromones. Near a pheromone source, the plume is described as a continuous burst of pheromones (Baker and Haynes 1989; Zimmer-Faust et al. 1995; Keller et al. 2001). In many insect species, continuous bursts of pheromones cause the arrestment of upwind progress (Carde and Elkinton 1984; Baker and Haynes 1989). Similarly, in our experiment, ovulating females typically spent several minutes below the baited trap before entering and never swam past a pheromone-baited trap. It is possible that ovulating females slowed upstream movement near baited traps because they encountered continuous bursts of pheromones, indicating they were near the source. Further evidence to support this hypothesis is that when washings were pulsed into a trap, ovulating females moved downstream and side-stream when the odor was not applied. Downstream and side-stream movement may have occurred because, when washings were suddenly discontinued, the instinctual interpretation was that the odor source moved downstream or side-stream, but not upstream, because the pheromone bursts did not become less frequent but, instead, stopped completely. Therefore, the female may have drifted downstream and moved side-stream in an attempt to reencounter the pheromone plume.

The two-choice maze results demonstrate that ovulating females incapable of olfaction are not attracted to mating pheromones. Our results are consistent with Siefkes and Li (2004) who hypothesized that olfaction is the primary means of pheromone detection and characterized pheromone receptor sites in the olfactory epithelium of female sea lampreys. Our

results also parallel those of Vrieze and Sorensen (2001) who showed that migratory sea lampreys with occluded olfactory systems were not attracted to larval sea lamprey washings in a two-choice maze or showed little ability to locate spawning streams.

Instream olfactory occlusion experiments demonstrate that pheromone reception in sexually mature sea lampreys is essential for locating mates. Locating mates without pheromone communication would probably be inefficient because sexually mature sea lampreys have poor vision (Manion and Hanson 1980) and electroreception is limited to a few centimeters (Bodznick and Northcutt 1981). It is also unlikely that males actively search for females, since males arrive at the spawning grounds before females, initiate nest building, and actively signal females with pheromones (Applegate 1950; Li et al. 2002, 2003). Some insect control programs have exploited the dependency on pheromone communication for mate finding by using high concentrations of synthetic pheromones to disrupt orientation to natural pheromone sources (Carde et al. 1998). Insect control programs have also developed pheromone antagonists that completely block pheromone reception and stop pheromone-induced behavior (Millar and Rice 1996; Evenden et al. 1999).

Management Implications

Pheromone-baited traps are able to capture ovulating females, even when spermiating males are not placed inside. In this study, over 50% of ovulating females were captured within 2 h in traps baited with spermiating male washings. The capture rate of females in traps baited with spermiating male washings is similar to the capture rate in traps baited with spermiating males (Johnson et al. 2005). Additionally, females captured in this experiment never interacted with the upstream barrier. Therefore, pheromone-baited traps may be used to remove ripe females from spawning grounds without the use of a barrier. Mating pheromones may be applied to traps in three different manners. First, spermiating male washings could be directly pumped into traps; for example, excess water from a flow-through tank stocked with spermiating males could be pumped into a trap at relatively low cost. Secondly, mating pheromones could be extracted from spermiating male washings and metered into traps. Lastly, synthetic pheromones, if developed, may be pumped into traps (Li et al. 2003). Future research should focus on which pheromone application method is most cost-effective.

Pulsed mating pheromones may be used to redistribute ovulating female sea lampreys into tributaries not suitable for spawning. Our results showed that pulsed washings applied at a rate of on for 1 min and off for 1 min lured equal numbers of females to within 5 m of the pheromone source. Therefore, if management goals are to redistribute ovulating female sea lampreys with synthetic mating pheromones into tributaries or areas not suitable for spawning (Li et al. 2003; Twohey et al. 2003), a pulsed source may be equally as effective and cost half as much as a continuous pheromone source.

A mating pheromone antagonist, if developed, may reduce the reproductive success of sea lamprey populations by inhibiting pheromone reception in ovulating females. In our study, females without the ability to use olfactory pheromone receptor sites did not exhibit pheromone-induced behavior and were unable to locate spermiating males in a spawning stream.

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