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INVESTIGATING POTENTIAL APPLICATIONS OF THE MALE MATING PHEROMONE IN SEA LAMPREY MANAGEMENT

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INVESTIGATING POTENTIAL APPLICATIONS OF THE MALE MATING PHEROMONE IN SEA LAMPREY MANAGEMENT

By

Nicholas S. Johnson

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ABSTRACT

INVESTIGATING POTENTIAL APPLICATIONS OF THE MALE MATING PHEROMONE IN SEA LAMPREY MANAGEMENT

By

Nicholas S. Johnson

Spermiating male sea lampreys (*Petromyzon marinus*) release mating pheromones that are highly attractive to ovulating females and influence their locomotion in spawning streams. It has been hypothesized that pheromone-baited traps might be used to directly remove females from spawning grounds and aid sea lamprey management in the Great Lakes. However, no field studies have been conducted to determine the efficacy of using male mating pheromones to lure females into traps and no studies have described female behaviors around pheromone-baited traps. This thesis describes in-stream trapping experiments, which demonstrate that more than 50% of ovulating females are captured in traps baited with spermiating males and in traps baited with water conditioned by spermiating males (spermiating male washings), whereas unbaited traps do not capture females. Additionally, the behavior of females near traps baited with pulsed spermiating male washings was characterized by more downstream and side-stream movements than females near traps with continuous washings. Furthermore, results demonstrate that ovulating females with occluded olfactory organs are unable to locate males in spawning streams. This thesis conclusively shows that traps baited with spermiating males and spermiating male washings capture significant numbers of females, that olfaction is used to detect pheromones, and that females may use pheromone plume structure to locate the exact source of pheromones. These results support the utility of mating pheromones to manage sea lamprey in the Great Lakes.

I dedicate this research in loving memory of my older brother Todd Johnson who spurred my interest in hunting and fishing. Todd was tragically killed while this thesis was being drafted.

ACKNOWLEDGMENTS

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CHAPTER 1

INTRODUCTION

Sea lamprey invasion and control in the Great Lakes

Sea lamprey *(Petromyzon marinus)* invaded the upper Laurentian Great Lakes during the first half of the 20th century and were a primary contributor to catastrophic ecological and economical damage to the Great Lakes fisheries (Smith and Tibbles 1980). Sea lamprey entered the upper Great Lakes via the Welland Canal, built to allow the passage of ships around Niagara Falls (Applegate 1950). Prior to sea lamprey establishment (1940s), the upper Great Lakes produced an annual lake trout (*Salvelinus namaycush*) harvest of 7,000 tons. However, in the 1950s and 1960s, sea lamprey predation and overfishing resulted in a 95% reduction in lake trout harvest. Lake trout were not the only species targeted by sea lamprey; high lamprey scarring rates and drastic reductions in harvest were also observed in lake whitefish (*Coregonus clupeaformis*), catostomids (*Catostomus* spp. and *Moxostoma* spp.), walleye (*Sander vitreus*), and rainbow trout (*Oncorhynchus mykiss*) (Smith and Tibbles 1980).

The first attempt to control sea lamprey occurred in the 1940s and 1950s when mechanical and electrical barriers were used to block access to spawning grounds (Applegate 1950). Barriers constructed at that time were typically expensive to build and maintain, and lampreys commonly escaped upstream (Applegate 1951; Smith and Tibbles 1980). Advances in barrier design and construction in the 1970s and 1980s resulted in the use of smaller, less expensive structures that successfully blocked lamprey migrations (reviewed in Lavis et al. 2003). Since the 1980s, barriers have become a primary component of integrated sea lamprey management in the Great Lakes. Currently, more than 60 barriers are used to block access to lamprey spawning habitat,

trap migrating adults, and provide males for the sterile male release program (Lavis et al. 2003).

In the late 1950s and early 1960s, the search for a larval lampricide proved productive when two compounds selectively toxic to lamprey were identified: 3-trifluoromethyl-4-nitrophenol (TFM) and 5, 2'-dichloro-4`-nitrosalicylanilide (Bayer 73) (Applegate et al. 1961; Howell et al. 1964). Application of TFM and Bayer 73 in the 1960s and 1970s significantly reduced lamprey populations. In Lake Superior tributaries, lamprey spawning runs dropped by 86% after chemical treatments and similar trends were reported throughout the Great Lakes (Smith and Tibbles 1980; Pearce et al. 1980). However, lampricide application only constitutes a single, temporary, and expensive method of lamprey control (Smith and Tibbles 1980). Growing concern about the social acceptance of chemical lampricide treatments and the untreatable nature of some streams, require that additional control techniques be developed (Christie and Goddard 2003).

In response to this concern, the sterile male release technique was developed as part of integrated sea lamprey management (Hanson and Manion 1980). Since 1997, approximately 30,000 adult male sea lampreys have been sterilized and released annually into the St. Mary's River (connecting Lake Superior and Lake Huron) resulting in a theoretical reduction in reproduction of 86% when combined with trapping (Twohey et al. 2003). All available sterilized males are released into the St. Mary's River because it is too large to be completely treated with lampricide and, therefore, has been the source of more than 90% of the parasitic sea lampreys in Lake Huron (Twohey et al. 2003). Thus, the remaining 430 lamprey-producing tributaries of the Great Lakes can not be stocked with sterile males and can only be managed with lampricides and barriers.

Additional sea lamprey control techniques would improve integrated sea lamprey management in the Great Lakes (Christie and Goddard 2003).

Sea lamprey life history

The sea lamprey (*Petromyzon marinus*) is a jawless anadromous fish native to the north Atlantic Ocean with a complex life cycle consisting of larval, parasitic, and spawning phases (Applegate 1950; Hardisty and Potter 1971). Sea lamprey begin their life as sedentary filter feeding larvae in freshwater streams. Upon reaching a critical length of approximately 150 mm, larvae metamorphose (develop eyes, teeth, and a sucker mouth) into the parasitic phase and migrate downstream to an ocean or lake. Parasitic sea lamprey are efficient ectoparasites of large fishes and extract blood and lymph from their host. After spending 12 to 18 months as parasites, sea lamprey stop feeding and enter the spawning phase. Spawning-phase sea lamprey migrate up suitable spawning streams in the spring and early summer. Selection of spawning streams is influenced by migratory pheromones released by larval lamprey (Polkinghorne 2001). Sea lamprey require gravel substrate with unidirectional water flow at speeds of 0.5 to 1.5 m/sec for successful spawning. Spermiating males typically arrive on the spawning grounds before females, construct several nests, and release pheromones to attract females (Appelgate 1950; Li et al. 2002). Males are joined by one or more ovulating females and generally spawn for 1 to 3 days. Spent lampreys die shortly after spawning. Fertilized eggs hatch into larvae and the life cycle repeats itself.

Sexually mature sea lampreys are highly congregated at specific spawning habitats in streams and, therefore, may be highly vulnerable to control. Spawning success could be reduced if sea lampreys could be directly removed from spawning grounds in traps. It has been hypothesized that traps baited with male mating pheromones might be used to directly remove females from spawning grounds (Li et al. 2003).

Current understanding of sea lamprey pheromones

Sea lamprey rely on a highly developed olfactory organ throughout their life cycle to find prey (Kleerekoper 1972) and to locate suitable spawning streams (Polkinghorne 2001; Teeter 1980) and potential mates (Li et al. 2002; Teeter 1980). Pheromones, chemical cues that elicit a specific behavioral or physiological response in conspecifics (Wyatt 2003), coordinate sea lamprey migration and spawning (Li et al. 2003; Sorensen and Vrieze 2003). Larval sea lampreys release migratory pheromones that attract adult sea lampreys to streams with suitable spawning habitat (Polkinghorne 2001). Four components of the migratory pheromone have been identified as petromyoamine disulfate, petromyzosterol disulfate, petromyzonol sulfate, and allocholic acid (Sorensen et al. 2005). Reception of migratory pheromones by adult lampreys is a critical component of sea lamprey life history. For example, it has been shown that anosmic lampreys are unable to locate spawning streams (Vrieze and Sorensen 2001).

Spermiating male sea lampreys release mating pheromones into the water via the gills, which are highly attractive to ovulating females (Li et al. 2002; Siefkes et al. 2003).

Two components of the mating pheromone have been identified as 3-keto petromyzonol sulfate and 3-keto allocholic acid (Li et al. 2002; Yun et al. 2003). Mating pheromones influence the locomotive responses of ovulating females. For example, it has been demonstrated that synthesized 3-keto petromyzonal sulfate can attract ovulating females 70 m upstream to the exact point of release (Siefkes et al. 2005). This thesis investigates the function of sea lamprey mating pheromones because they are a useful model to investigate how a fish orients to pheromone plumes, how pheromonal communication influences spawning success, and how pheromonal communication may be exploited to manage sea lamprey in the Great Lakes.

For 25 years it has been hypothesized that sea lamprey mating pheromones might be of use in an integrated sea lamprey management program (Smith 1980; Teeter 1980; Li et al. 2003). In its strategic vision statement, the Great Lakes Fishery Commission states that one alternative control technique needs be developed by 2010, the most promising of which is pheromone based (Great Lakes Fishery Commission 2005). Invasive insect species have been successfully controlled by using pheromone-baited traps to mass-trap individuals (Howse et al. 1998). Similarly, sea lamprey mating pheromones could be used to mass-trap ovulating females, redistribute ovulating females into streams with poor spawning habitat, or disrupt pheromone communication among spawning lampreys, ultimately reducing their reproductive success (Teeter 1980; Li et al. 2003). However, no field studies have tested the efficacy of using mating pheromones to trap, redistribute, or disrupt reproduction in ovulating females.

Questions critical to the application of mating pheromones in sea lamprey management

Although it has been clearly demonstrated that mating pheromones strongly influence locomotive responses of females in a spawning stream (Li et al. 2002; Siefkes et al. 2005), research should now focus on how to exploit mating pheromones for use in sea lamprey management. Many questions must be addressed before field application of mating-pheromone-based techniques in sea lamprey management. The central question addresses the feasibility of using pheromones to reduce the reproductive potential of sea lamprey populations. To answer the central question it must be determined whether sea lamprey mating pheromones can be used to 1) increase the capture rate of females in traps, 2) attract females to streams not suitable for spawning, or 3) disrupt pheromone communication in spawning sea lampreys.

Questions addressed in this thesis

This thesis investigates potential applications of the sea lamprey male mating pheromone in sea lamprey management by addressing questions pertinent to removing females from spawning grounds, redistributing females to streams with poor spawning habitat, and disrupting pheromone communication in spawning sea lampreys. Chapter two, "Mating Pheromone Reception and Induced Behavior in Ovulating Female Sea Lampreys", describes experiments that address the following questions:

1) What is the capture rate of females in traps baited with spermiating male washings?

2) How do females navigate continuous and pulsed pheromone plumes and can a pulsed plume be used to capture or redistribute females?

3) Is olfaction the only means by which females detect mating pheromones and locate spermiating males in spawning streams?

Questions 1 and 2 are critical to understanding the ability of mating pheromones to lure females into traps or to redistribute females. Question 3 is critical to understanding the mechanism of pheromone reception in females and the importance of pheromone reception for mate-finding in spawning streams.

Appendix A, "Behavior of Naris-plugged Ovulating Female Sea Lampreys Among Spawning Conspecifics", further investigates the importance of pheromone reception for female mate-finding on sea lamprey spawning grounds. Appendix B, "Capture of Ovulating Female Sea Lampreys in Traps Baited with Spermiating Male Sea Lampreys", describes the first study to demonstrate that spermiating male odors can lure females into traps and reports the capture rate of females in traps baited with spermiating males.

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CHAPTER 2

MATING PHEROMONE RECEPTION AND INDUCED BEHAVIOR IN OVULATING FEMALE SEA LAMPREYS

Johnson, N. S., Luehring, M. A., Siefkes, M. J., and Li, W. 2005. Mating pheromone reception and induced behavior in ovulating female sea lampreys. North American Journal of Fisheries Management, in press.

ABSTRACT

This study was conducted to determine how ovulating female sea lampreys 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 females near traps with continuous washings. We occluded the olfactory organ of ovulating females and tested if they were attracted to spermiating male washings in a two-choice maze and if 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.

INTRODUCTION

Sea lamprey *Petromyzon marinus* invaded the upper Laurentian Great Lakes during the first half of the 20th century and inflicted catastrophic ecological and economical 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 about the social acceptance of chemical lampricide treatments, increasing cost of lampricides, and the untreatable nature of some streams requires 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 lamprey control programs.

Sea lamprey mating pheromones have potential in controlling sea lampreys in the Great Lakes (Teeter 1980; Li et al. 2002; Li et al. 2003). Spermiating male sea lampreys release the mating pheromone, 3-keto petromyzonol sulfate, at high rates that 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 if 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 et al. 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).

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 Sorenson 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 if ovulating female sea lampreys could be lured into traps baited with water conditioned with spermiating males, 2)

describe behaviors of ovulating females near male pheromone-baited traps, 3) determine if 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 and 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 to 22 °C. Non-spermiating males and pre-ovulating females were stored in separate 1000 L flow through tanks at temperatures ranging from 4 to 14 °C. Non-spermiating males and pre-ovulating females were checked weekly for spermiation and ovulation. To induce female ovulation, several pre-ovulating 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 to 25 °C. Additionally, several pre-ovulating females were stored with spermiating males in a 1000 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. A 65 m section of the river was enclosed using two block nets. At the upstream block net the river is divided into two distinct channels with nearly equal discharge of 0.5 m^3/sec. 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/sec at the downstream barrier. Stream flow was measured weekly or after significant precipitation events using a Marsh-McBirney (Marsh-McBirney Incorporated, Fredrick, Maryland) flow meter.

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/sec. 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

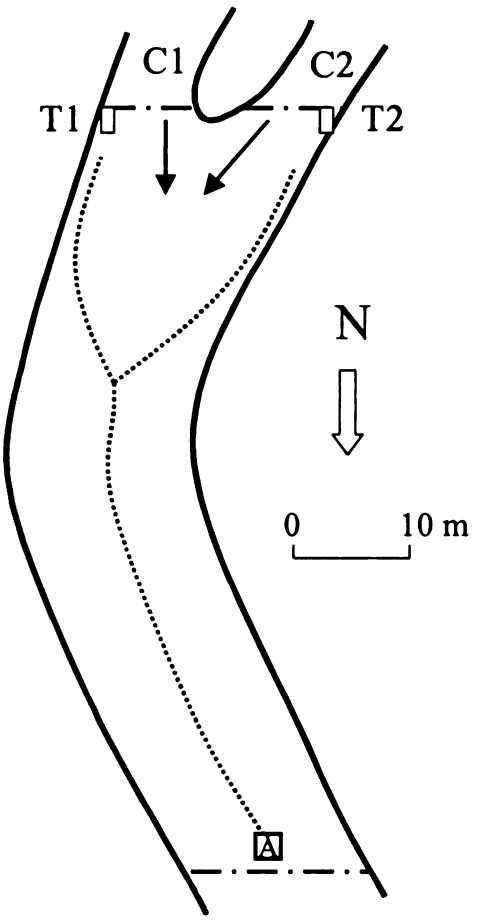

Figure 1. The 65-m section of the Ocqueoc River, Presque Isle County, Michigan, USA, used for sea lamprey trapping experiments between 18 June and 8 July 2004. The section of river was enclosed with upstream and downstream block nets (dashed horizontal lines). An island naturally divides the river into 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.

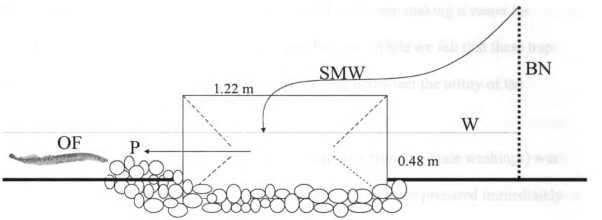

Figure 2. Sea lamprey trap dimensions and trap set used to capture ovulating female sea lampreys on the Ocqueoc River, Presque Isle County, Michigan, USA, between 18 June and 8 July 2004. Sea lamprey traps were set in 0.3 m of water (W), 1 m downstream of a block net (BN), and were placed in a hand constructed depression in the stream bottom. Rocks approximately 5 cm in diameter were placed around the traps 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.

because spermiating males initiate nest building (Applegate 1950) and release pheromones that 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 five spermiating males (spermiating male washings) was used to bait sea lamprey traps. Spermiating male washings were prepared immediately prior to experimentation by placing five 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 five spermiating males in 2.5 hours. More specifically, based on the estimated release rate of 3-keto petromyzonol sulfate (3kPZS) by a spermiating male, approximately 500 $ug \cdot h^{1} \cdot animal^{-1}$ (Yun et al. 2002), and the average streamflow, 1.01 m^3/sec, the average in stream concentration of 3kPZS when spermiating male washings were applied to a trap was 1.5 X 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. in press) 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 to 20 °C. Ovulating females were fitted with external radio tags (Advanced Telemetry System, Isanti, Minnesota) according to Siefkes et al. (2003) 15 h prior to experimentation. Females were transported to the Ocqueoc River and placed in the acclimation cage 12 h prior to experimentation. Spermiating male washings were applied to a randomly chosen (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 prior to 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 two ovulating females were released due to 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. (in press) 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 Incorporated, 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 downsteam and side-stream movements, were recorded. A downstream movement was defined as a continuous 2 m or greater movement 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 two was conducted at the same location using the same equipment and design as experiment one. 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 in-stream 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 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 Stern Vantage Quick Light Body (Sterngold, Boston, Massachusetts), a dental impression adhesive, was injected into the olfactory cavity to occlude the nasopharyngeal opening. Next, a drop of Vetbond (Minnesota Mining and Manufacture, St Paul, Minnesota) was applied in the naris to adhere to the Stern Vantage and completely block the movement of water through the naris. The Stern Vantage 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 the nose-plugging procedure stress. Control ovulating females were removed from water and a 5 mm tip of a 1-200 uL volume pipet-tip (Dot Scientific Incorporated, Lippincott Burton, Michigan) was inserted into the nasopharyngeal opening. Immediately following, 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 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/sec. 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 one 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 time 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 signed rank test (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 if they could locate five spermiating males 10 m or 65 m upstream. This experiment was conducted at the same location as experiments one and two (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 to 24 °C. Naris-plugged and control ovulating females were fitted with external radio tags (Advanced Telemetry Systems, Isanti, Minnesota) according to Siefkes et al. (2003) at least 12 h prior to experimentation.

Ovulating females were held in the acclimation cage prior to experimentation. Five spermiating males were randomly placed 10 m or 65 m upstream of the ovulating females 30 min prior to 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 two naris-plugged and three control ovulating females were released or three naris-plugged and two 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 was recorded. If females were not visible, they were tracked with a directional radio antenna and receiver (Lotek Engineering Incorporated, Newmarket, Ontario, Canada). 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 was used to compare the number of naris-plugged and control ovulating females that located five

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. (in press) 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%; $z = 2.11$, $n = 27$ and 25 respectively, $P = 0.035$). Traps baited with continuous washings and pulsed washings lured approximately equal proportions of ovulating females to within 5 m of the baited trap ($z = 0.739$, $n = 27$ and 25 respectively, $P = 0.460$; 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 experiment ended at 2 h. The average time to capture in a trap baited with continuous washings (40.5 min, SD 27.2) was not different from the average time to capture in a trap baited with pulsed washings (41.7 min, SD 34.9) (Table 1) ($t = 0.803$; $df = 19$; $P = 0.432$). 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. Two of the seven females that were captured in traps baited with pulsed washings immediately

Table 1. Number of ovulating female sea lampreys that entered within 5 m (Within 5 m) and were captured (Captured) in traps baited with continuous spermiating male washings (Constant) and pulsed spermiating male washings (Pulsed) within 2 h after release, and the average time to capture (Time capture) an ovulating female and the average number of downstream (DS movements) and sidestream (SS movements) movements of ovulating females within 5 m of traps baited with continuous and pulsed spermiating male washings. Lower case letters "z", "y" and "x" indicate significant differences between continuous and pulsed treatments. Experiments were conducted on the Ocqueoc River, Presque Isle County, Michigan, USA, between 18 June and 8 July 2004.

Observation	Constant	Pulsed
n	27	25
Within 5 m	16	14
Captured z	14	7
Time capture	41	42
DS movements y	0.38	6.08
SS movements x	0.19	1.77

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, downstream movements, and side-stream movements. 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 ($t = 2.49$; $df = 13$; $P = 0.027$) movements 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 towards 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 for spermiating male washings in a two-choice maze (Wilcoxon Signed Ranks Test; $n = 11$; $P > 0.250$; Table 2). Control ovulating females showed a significant preference for spermiating male washings in a two choice maze (Wilcoxon Signed Ranks Test; $n = 12$; $P < 0.010$; Table 2).

Fourteen individual naris-plugged ovulating females and 14 individual control ovulating females were released when spermiating males were placed 10 m upstream and 65 m upstream of ovulating females. Naris-plugged ovulating females were unable to locate five 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 five 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 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

Table 2. Number of naris-plugged ovulating female sea lampreys (NPOF) and control ovulating female sea lampreys (COF) that showed a preference response to spermiating male sea lamprey washings (SMW) in a two-choice maze. Statistical significance (P-value) was determined with a two-tailed Wilcoxon Signed Ranks Test (W–Value) based on the test statistic and the number of animals tested (n).

Test Subject	n	SMW	W-value	P-value
NPOF	11	3	27	NS
COF	12	10	73	<0.010

Table 3. Number of control ovulating female sea lampreys (COF) and naris-plugged ovulating female sea lampreys (NPOF) that were released (n) and located five spermiating male sea lampreys (Males) placed 65 m upstream (65) and 10 m upstream (10) in the Ocqueoc River, Presque Isle County, Michigan, USA, between 22 July and 10 August, 2004.

Animal	Distance	n	Males
COF	65	14	7
NPOF	65	14	0
COF	10	14	10
NPOF	10	14	0

not significantly different when males were placed at 65 m (z-test, $P = 0.106$). 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 five spermiating males significantly more often than naris-plugged ovulating females when males were placed 10 m and 65 m upstream (Fisher's Exact Test, $P=0.003$; $P < 0.001$).

DISCUSSION

Our results demonstrate that spermiating male washings are able to lure ovulating female sea lampreys into traps. At 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 which 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 motivate 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, 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 burst 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. Down 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.

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 with 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, and showed little ability to locate spawning streams.

In-stream olfactory occlusion experiments demonstrate that pheromone reception in sexually mature sea lamprey is essential for locating mates. Locating mates without pheromonal communication would likely be inefficient because sexually mature sea lampreys have poor vision (Manion and Hanson 1980) and electroreception is limited to a few centimeters (Bodznick and Nortcutt 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; Li et al. 2003). Some insect control programs have exploited the dependency on pheromonal 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, more than 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 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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APPENDIX A

BEHAVIOR OF NARIS-PLUGGED OVULATING FEMALE SEA LAMPREYS AMONG SPAWNING CONSPECIFICS.

Data described in chapter 2 demonstrated that naris-plugged ovulating females are not attracted to spermiating male washings and do not interact with caged spermiating males in a spawning stream. However, it was not determined whether naris-plugged females released among spawning sea lampreys would interact and spawn with spermiating males. Here I describe an experiment in which 12 naris-plugged females and 21 control females were released directly below a lamprey spawning area in the Ocqueoc River, Presque Isle County, Michigan. Naris-plugging and control treatment procedures were conducted as described in chapter 2. From June 6th to June 11th, 2005, in a 115 m spawning ground, the average number of spermiating males observed on nests during experimentation was 17 (SE = 6) and the average number of females observed on nests was 22 (SE = 11). Three naris-plugged females were released each day from June 8th to June 11th, 2005. Five control females were released each day from June 6th to June 8th, 2005, and 6 control females were released on June 9th, 2005. Females were observed for 2 hours after release and the percentage of females that swam within 1 m of a spermiating male on a spawning nest, interacted (as defined in chapter 2) with a spermiating male, and spawned with a spermiating male were recorded.

The percentage of females that swam within 1 m of a spermiating male did not differ significantly between control and naris-plugged females (Fisher's Exact Test; $p = 0.095$). However, naris-plugged ovulating females never interacted or spawned with spermiating males, while 67 % of control females interacted with a male(s) and 57 % of control females were observed spawning. Naris-plugged females interacted with significantly fewer males and spawned significantly less than control females (Fisher's Exact Test; $p < 0.001$; Table 1).

Table 1. The number of control ovulating females and naris-plugged ovulating females that swam within 1 m of a spermiating male on a spawning nest (One Meter), interacted with a spermiating male (Interact), and spawned with a spermiating male (Spawn) in the Ocqueoc River, Presque Isle County, Michigan, 2005. (n = sample size)

Test Female	n	One Meter	Interact	Spawn
Control	21	15	14	12
Naris-plugged	12	5	0	0

Results show that mating pheromone reception is critical for mate finding and reproductive success in female sea lampreys. Chapter 2 demonstrated that ovulating females incapable of olfaction were not attracted to spermiating male washings and were unable to locate caged males in a spawning stream. This study shows that naris-plugged ovulating females do not interact or spawn with free-ranging spermiating males in a spawning stream and furthermore, spermiating males do not search out naris-plugged ovulating females to reproduce. These results support the hypothesis that females actively search for spermiating males by navigating pheromone plumes originating from spermiating males (Li et al. 2002) and indicate that disrupting pheromonal communication with a pheromone antagonist or supernormal concentrations of pheromones may reduce the reproductive success of spawning lampreys. (Twohey et al. 2003).

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APPENDIX B

CAPTURE OF OVULATING FEMALE SEA LAMPREYS IN TRAPS BAITED WITH SPERMIATING MALE SEA LAMPREYS

Johnson, N.S., Siefkes, M.J., Li W. 2005. Capture of ovulating female sea lampreys in traps baited with spermiating male sea lampreys. North American Journal of Fisheries Management 25: 67-72.

ABSTRACT

This study was conducted as an initial step in the development of a trapping technique for sexually mature female sea lampreys *Petromyzon marinus*. Recent research has demonstrated that spermiating male sea lampreys release a sex pheromone that attracts ovulating females. This discovery prompted us to hypothesize that traps baited with spermiating males would capture more ovulating females than empty traps or traps baited with non-spermiating males. We found that traps baited with spermiating males captured nearly 74% of the ovulating females released, whereas empty traps and traps baited with non-spermiating males did not capture any ovulating females. We conclude that pheromone-baited traps may compliment current sea lamprey management through direct removal of ripe females from spawning grounds.

INTRODUCTION

The sea lamprey *Petromyzon marinus* invaded the Laurentian Great Lakes and caused the collapse of numerous economically valuable fish populations (Smith and Tibbles 1980). Integrated management of sea lamprey is essential to maintain and restore the Great Lakes ecosystem (Great Lakes Fisheries Commission 2003). Lampricides, barriers, trapping, and sterile-male releases are used to control sea lamprey populations (Klar and Young 2002), yet sea lampreys continue to be a significant source of fish mortality in the Great Lakes (Bergstedt and Scheider 1988; Kitchell 1990). Further, these techniques can be costly and some have uncertain environmental consequences (Lamsa et al. 1980; Smith and Tibbles 1980). Additional control techniques will improve sea lamprey management in the Great Lakes (Hanson and Manion 1980; Smith and Tibbles 1980).

Trapping currently targets sexually immature sea lampreys as they migrate upstream to their spawning grounds (Great Lakes Fisheries Commission 2003). Captured females are killed and captured males are used in the sterile-male release program. Trapping reduces the population of migratory sea lampreys in rivers by an average of 39% in the whole Great Lakes basin and up to 60-80% in some rivers (Klar and Young 2002). We reasoned that if a trapping technique could be developed to further reduce the abundance of ovulating females, the reproductive potential of sea lamprey populations would be further reduced. This may be logistically challenging because mature sea lampreys do not appear to move great distances within a stream when compared to immature sea lampreys en route to spawning grounds (personal observation).

Nevertheless, recent advances in our understanding of sea lamprey sex pheromone communication indicated that baited trapping may provide a solution to this technical difficulty (Li et al. 2002; Siefkes et al. 2003).

Pheromones play an important role in mate searching and courtship behavior in sea lamprey (Teeter 1980). Recent research indicates that spermiating males release at least one sex pheromone that induces a strong preference and searching behavior in ovulating females (Li et al. 2002; Siefkes et al. 2003). Insect traps baited with specific female pheromones have successfully captured sexually mature males of the same species (Beroza and Knipling 1972; Oehlschlager et al. 2003). In principle, lamprey traps baited with a male pheromone or spermiating males could also be used to capture ovulating female sea lampreys to reduce the reproductive potential of sea lamprey populations (Teeter 1980, Li et al. 2002). Our objectives were to determine whether baiting traps with spermiating males could significantly increase the capture rate of ovulating females and if trapping rates varied significantly between day and night.

METHODS

Experimental animals

Sea lampreys were captured by hand or in traps from Lake Michigan and Huron tributaries from May until July 2003. Males and females were identified and separated according to the protocol established by Vladykov (1949). Each sex was further assigned to one of two maturity classes according to the protocol established by Siefkes et al. (2003). Males were classified as nonspermiating or spermiating. Females were classified as preovulating or ovulating. Spermiating males and ovulating females were held in 150-L flow-through tanks at ambient temperatures ranging from 7^0C to 20^0C for immediate experimentation. Nonspermiating males and nonovulating females were held in 1,000-L, flow-through tanks at temperatures ranging from 4-8 °C for future experimentation. Several nonspermiating males and nonovulating females were held together with spermiating males and ovulating females in an artificial spawning stream to induce maturation.

Experimental test site and equipment

Trapping experiments were conducted above the lamprey barrier in a 65-m section of the Ocqueoc River (a Lake Huron tributary, Presque Isle County, Michigan; Figure 1). The Ocqueoc River is historically known for its large population of spawning

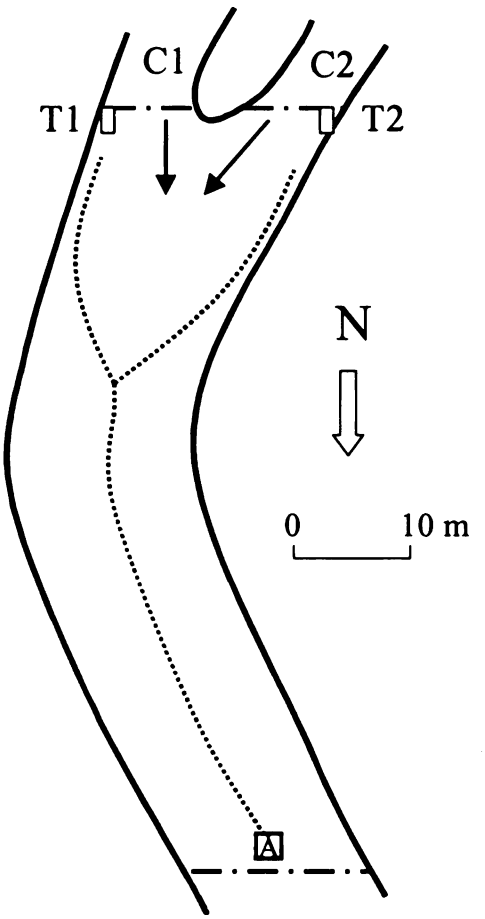

Figure 1. The 65-m enclosed section of the Ocqueoc River, Presque Isle County, Michigan used for trapping experiments between 27 June and 12 August 2003. At the upstream block net, an island naturally divides the river into two channels (C1 and C2). The arrows represent the flow of water coming from each channel. A sea lamprey trap was placed in each channel of the river approximately 0.2 m from the block net and 1.0 m from the shore (T1 and T2). Ovulating females were released from an acclimation cage (A) at the downstream block net and observed until they entered a trap or the end of the experiment (12 h from the time of release).

sea lampreys (Applegate 1950; Heinrich et al. 1980; Coble et al. 1990; Houston and Kelso 1991). However, above the barrier, no sea lampreys were known to be present (personal observation) and the stream contains suitable physical qualities and habitat for spawning (Applegate 1950). The 65-m section was enclosed using two block nets. At the upstream block net, an island naturally divides the river into two distinct channels with nearly equal discharge (1.6 m^3/s). The two channels converge and mix in the middle of the enclosed river section. Sea lampreys swimming upstream during an experiment must choose which channel to enter.

Two identical traps (0.359 m^3) were used to capture ovulating females in the enclosed section of the river (Figure 2). A trap was placed in each channel of the river along the upstream barrier. Each trap was approximately 1 m from the nearest shore and 0.2 m from the upstream block net. The traps were set parallel to the current to create a pheromone plume originating from the downstream funnel of the trap (Figure 2). Water flow through each trap was approximately 0.30 m^3/s. Ovulating females were held in an acclimation cage along the downstream barrier (Figure 1). Males were held in cages placed inside the trap (Figure 2) to prevent males from escaping or physically interacting with ovulating females.

Experimental design and procedures

The study was conducted between 27 June and 12 August, 2003. Ovulating females were fitted with external radio tags (Advanced Telemetry System, Isanti, Minnesota) according to Siefkes et al. (2003) 24 h prior to experimentation. Five

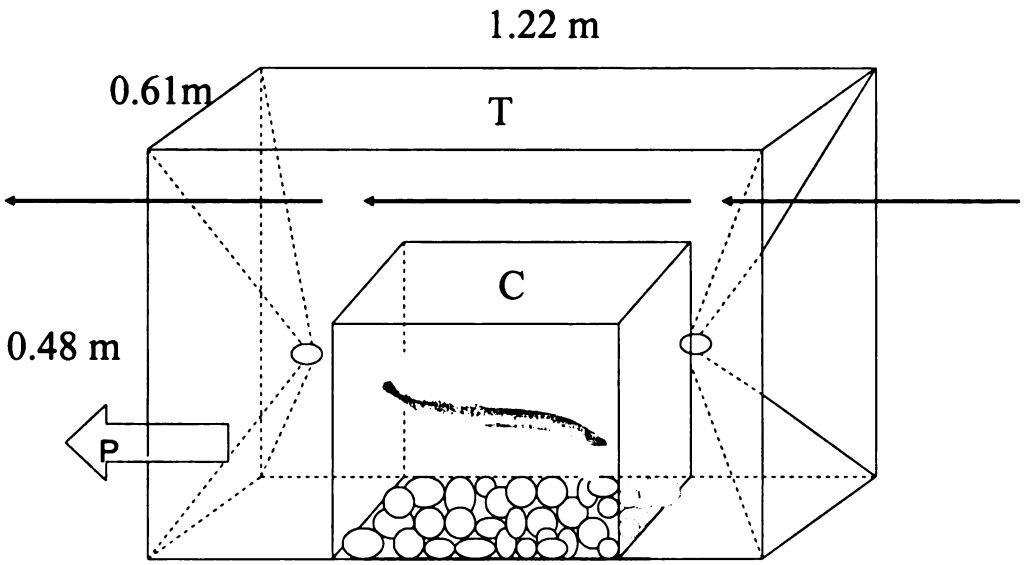

Figure 2. The design and dimensions of sea lamprey traps used on the Ocqueoc River, Presque Isle County, Michigan between 27 June and 12 August 2003. The dark arrows represent the current flowing through the trap. This created a pheromone plume (P) originating directly from the downstream funnel of the trap. A cage (C) with rocky substrate was placed inside the trap to hold spermiating male sea lampreys.

spermiating and five non-spermiating males were randomly assigned to a trap by flipping a coin. Males in traps and ovulating females were allowed to acclimate in the river for a minimum of 30 min before experimentation.

The study consisted of two experiments. In the first experiment, a single female was released in each trial to estimate the trapping rate of individual females. In the second experiment, five females were released in each trial to estimate the trapping rate of a group of females. Each experiment further consisted of treatment and control trials. Treatment trials were conducted by randomly placing five spermiating males in one trap and five nonspermiating males in the other. Control trials were conducted with no males in either trap and estimated the trapping rate of ovulating females using current trapping techniques. Treatment and control trials were conducted both day and night to investigate changes in trapping rates under specific lighting conditions (Teeter 1980). Day trials started at 0900 hours and ended at 2100 hours with ambient water temperatures of 15.2-29.2°C. Night trials and multiple female releases started at 2100 hours and ended at 0900 hours with ambient water temperatures of 14.3-29.3°C.

Thirty-nine experimental trials (16 during the day and 23 during the night) and twenty-four control trials (11 during day and 13 during night) were conducted when individual females were released. Four treatment trials and one control trial was conducted during the night when five females were simultaneously released. In all trials, females were released at the downstream barrier and visually observed. If females were not visible, they were tracked with a directional radio antenna and receiver (Advanced Telemetry Systems, Isanti, Minnesota).

Ovulating females were allowed 12 h to enter a trap. Females that entered a trap before 12 h were removed and the experiment was terminated. If a female escaped from the enclosed river section the trial was counted because the female failed to respond to the pheromone cue. If a female died, the trial was not counted because we believed the female was physically unable to respond to the pheromone cue. Ovulating females were either captured in the trap containing spermiating males, captured in the trap containing nonspermiating males, captured in the empty trap, or not trapped. Finally, the behaviors of ovulating females around the traps were observed and described, but not quantified.

Capture rates of ovulating females were calculated by dividing the total number of females released by the number captured in each trap. A Fisher's Exact Test was used to compare night and day trials, and to compare single female releases and five female releases. If no significant differences were observed between day and night trials and single- and five- female release trials, the data were combined.

RESULTS

Six ovulating females died during treatment trials and one female died during control trials. These trials were not included in our data analyses. Eight ovulating females escaped the enclosed river section during treatment trials and three escaped during control trials. These trials were included in our data analyses.

Trapping rates did not differ significantly between day and night trials (Fisher's Exact Test, $P = 1.00$) or between single- and five-female releases (Fisher's Exact Test, $P = 0.86$; Table 1). Ovulating females were captured in traps containing spermiating males but not in those with nonspermiating males. During the 53 countable treatment trials, traps with spermiating males caught nearly 74% of ovulating females released, traps with nonspermiating males did not catch any ovulating females released, and approximately 26% of ovulating females were not captured in either trap (Table 1).

Furthermore, ovulating females were quickly captured in the spermiating male trap. Twenty-one of the 39 females captured entered the trap within 30 min after their release. The mean time a female was observed entering the trap was 27 min (range, 7-75 min). Observations of 17 of the 39 females captured revealed that 9 swam around the trap (swimming up and down one or both sides of the trap often touching the trap) for up to several minutes before entering the trap; where as the other eight swam directly into the trap.

During the 27 countable control trials, none of the ovulating females were captured in empty sea lamprey traps (Table 1). Additionally, most ovulating females did not move upstream when the traps were empty. Of 25 countable control trials, 19

Table 1. Results of experiments using traps baited with spermiating male sea lampreys to attract ovulating females in the Ocqueoc River, Presque Isle County, Michigan, USA, between June 27 and August 12, 2003. Three types of traps were used in the experiments: traps containing spermiating males, traps containing nonspermiating males, and traps containing no males (control). Trials involved groups of five females, single females released during the day, and single females released at night. The following abbreviations are used: N = is the number of trials for each experiment, S = the number of females captured in traps with spermiating males, NS = the number of females captured in traps with nonspermiating males, NC = the number of females not captured in traps, and E = the number of females captured in empty traps.

	Experimental				Control		
Trial	N	S	NS	NC	N	E	NC
Five releases	19	14	0	5	4	0	4
Single day	16	13	0	3	12	0	12
Single night	18	12	0	6	11	0	11
Total	53	39	0	14	27	0	27

females did not move upstream, 3 swam halfway up the enclosed river section, and 3 swam to the upstream block net.

DISCUSSION

Our results imply that traps baited with spermiating male sea lampreys may be used to remove ovulating female sea lampreys from spawning grounds. In our experimental site, the capture rate of ovulating females was increased from zero to more than 70% by baiting the traps with spermiating males. These results are consistent with previous studies conducted in mazes and in the field. In a two-choice maze, male sex pheromones have been shown to induce a strong preference and searching response in ovulating females (Li et al. 2002; Siefkes et al. 2003). In field conditions, spermiating males have been shown to attract ovulating females upstream (Li et al. 2002; Siefkes et al. 2003); therefore, it is likely that sex pheromones released by spermiating males attracted ovulating females into the traps. Our study also confirms the "infallible" practice of French fishermen who used male lampreys to bait traps to capture females (Fontaine 1938).

An interesting observation in this study was that some females swam around the spermiating male trap before entering. This behavior may be due to some of the pheromone plume exiting the side of the trap rather than the funnel. Therefore, females may have followed the plume to the sides of the trap before finding the funnel and entering the trap. This pheromone-induced behavior could also be an important mechanism mediating the attraction of females to males and important to consider when designing a trapping strategy. More research is needed to find the exact cause of this behavior and to further quantify the behavior of ovulating females around pheromone-baited traps.

Our experiments were conducted under ideal conditions; large-scale studies in actual spawning situations are needed. First, male sex pheromones have been shown to attract ovulating females from up to 65 m away (Li et al. 2002; Siefkes et al. 2003; and data from this study). However, the maximum or effective distance ovulating females are attracted to pheromone-baited traps has yet to be determined. Second, resident spermiating males in the field may reduce the capture rate of ovulating females in pheromone-baited traps. Under the experimental conditions of this study, when no other spermiating males were present in the stream, traps with spermiating males yielded high capture rates of ovulating females. However, in actual spawning situations, background pheromones released by conspecific spermiating males may reduce the effectiveness of pheromone trapping.

Sex pheromone trapping techniques may be improved by additional research. First, specially designed traps may increase the capture rate of ovulating females (Teeter 1980). Traps designed to deflect the pheromone plume of spermiating males through the funnel(s) may result in higher capture rates. Second, if synthetic pheromones can be created that are as potent as spermiating males, they would eliminate the difficulties of using live spermiating males to bait traps and allow more males to be used in the sterile-male program. Currently, components of the pheromone mixture, 3-keto petromyzonol sulfate and 3-keto allocholic acid, are being chemically synthesized and tested in a two-choice maze to determine if they elicit a searching response similar to that of water conditioned with spermiating males (unpublished data).

Management implications

Sex pheromones have appealing qualities that may benefit sea lamprey management in the Great Lakes. Although the behavior of ovulating females appears to be difficult to exploit using current trapping practice (personal observation), our results suggest that with the use of spermiating males, ovulating females might be efficiently trapped. In our study, the capture rate of ovulating females was high, pheromones were naturally released from spermiating males with no apparent environmental damage, and experiments were conducted at a low cost. Our study supports claims that sex pheromones are likely to be potent, environmentally benign, species-specific, easy to apply, and have a low cost of development. (Teeter 1980; Li et al 2002).

Our results indicate that pheromone-baited traps may compliment integrated sea lamprey management in the Great Lakes (Smith and Tibbles 1980). Pheromone-baited traps may reduce the reproductive potential of sea lamprey populations through direct removal of females from spawning grounds. Additionally, by reducing female abundance, sterile-male releases may become more effective (Hanson and Manion 1980). Furthermore, in this study, females were commonly observed swimming directly upstream into the trap without interacting with the upstream barrier. Therefore, pheromone-trapping strategies may be conducted on streams that do not have barriers, while traditional trapping techniques are often conducted in streams with barriers. Finally, our initial results showed no significant differences between day and night trials; hence, pheromone trapping may be effective 24 h a day. This may be another advantage

to pheromone-based trapping, because traditional trapping techniques are only effective at night.

Pheromone-baited traps may have broad applications in fisheries management (Young et al. 2003) because sex pheromones are used by a variety of fish species (Sveinsson and Hara 1995; Vermeirssen and Scott 2001; Young et al. 2003). Numerous undesirable fish species are present in North America (Courtenay et al. 1986). Therefore, sex pheromones may provide an inexpensive and environmentally benign method of fish control or population estimation (Young et al. 2003).

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APENDIX C

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