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SPERMIATING MALE SEA LAMPREYS
RELEASE A SEX PHEROMONE THAT ATTRACTS
POST-OVULATORY FEMALE SEA LAMPREYS

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Michael J. Siefkes

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By

Michael J. Siefkes

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ABSTRACT

SPERMIATING MALE SEA LAMPREYS RELEASE A SEX PHEROMONE THAT ATTRACTS POST-OVULATORY FEMALE SEA LAMPREYS

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Behavioral observations suggested that a jawless fish, the sea lamprey, *Petromyzon marinus* releases sex pheromones that influence the behavior of conspecifics. Further, electrophysiological studies showed that male sea lampreys release a potent odorant during spermiation. I hypothesized that this odorant is a sex pheromone and examined its effects on the behavior of adult sea lampreys. My results show that this odorant specifically influences the behavior of post-ovulatory females. When placed in a two-choice maze, post-ovulatory females spent more time and showed increased searching or swimming activity in the side containing a spermiating male odorant. Similar behavioral responses were not observed in male and pre-ovulatory female sea lampreys. Also, in a natural spawning stream, post-ovulatory females located and swam to cages containing spermiating males, but pre-ovulatory females did not. I conclude that this odorant is a sex pheromone that specifically attracts post-ovulatory female sea lampreys through increased searching behavior and further speculate that it ultimately functions to coordinate spawning.

I would like to dedicate this thesis to my family, friends, and Rebecca Otto. Their continuous and enthusiastic support is essential in my quest to further my education.

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INTRODUCTION

In many species of fish, pheromones produced during final maturation have been shown to function as both behavioral and physiological agents in the synchronization of mating (Partridge et al. 1976; Liley 1982; Stacey & Sorensen 1991; Stacey et al. 1994; Scott & Vermeirssen 1994). Specifically, sex pheromones have been shown to stimulate vitellogenesis (Van Weerd and Kamen 1998) and ovulation (Dmitrieva & Ostroumov 1986), induce courtship behavior (Ostroumov & Dmitrieva 1990) and synchronize the release of sperm and eggs (Stacey & Hoursten 1982). The sex pheromones of goldfish, *Carassius auratus,* currently are the most extensively studied among those found in fish. Female goldfish release 17,20 β-dihydroxy-4-pregnen-3-one and 17,20β-dihydroxy-4-pregnen-3-one sulfate into water just before ovulation (Stacey et al. 1989; Sorensen et al. 1995). Both substances are highly stimulatory to the olfactory organ of conspecifics. Also, male goldfish show elevated levels of plasma gonadotropin II (Dulka et al. 1987; Sorensen et al. 1995) and sperm (Stacey et al. 1989) in response to these substances. In addition, post-ovulatory female goldfish release prostaglandin F2α and its metabolites into water, which also induce neuro-endocrine and behavioral responses in male goldfish (Sorensen et al. 1988, 1989). Male sex pheromones, although less extensively studied in fish, have been reported in numerous species such as *Ictalurus* catfish (Todd et al. 1967; Richards 1974); rainbow trout, *Oncorhynchus mykiss*

(Newcombe & Hartman 1973); blenny, *Blennius pavo* (Laumen et al. 1974); five belontiid species (Lee & Ingersoll 1979); black goby, *Gobius jozo* (Colombo et al. 1980, 1982); and Pacific herring, *Clupea harengus pallasi* (Stacey & Hourston 1982; Sherwood et al. 1991; Carolsfeld et al. 1997). These olfactory signals have been found to attract mature females to mature males or to induce physiological changes such as ovulation in female conspecifics.

It has been suggested that sea lampreys, one of the lone survivors of the most ancient vertebrate classes Agnatha, also use male pheromones to coordinate spawning (Teeter 1980; Li 1994). Behavioral observations of sea lampreys suggest that odorants released by adult males attract adult females and repel other adult males (Teeter 1980; Li 1994). These findings are consistent with the reproductive biology of the sea lamprey where males usually initiate nest building and are later joined by one or more females (Applegate 1950). Also, electro-olfactograms made using female sea lampreys showed that washings from spermiating males were a thousand times more potent than those from non-spermiating males (Li 1994; Bjerselius et al. 1996). However, there has been no direct link between this potent spermiating male odorant and the attraction/repulsion behavioral responses previously observed.

I formulated the hypothesis that spermiating male sea lampreys release a potent and specific sex pheromone. Because there has been no direct evidence of the significance of spermiation to male pheromone release, experimental exploration of my hypothesis should be focused on the timing of release and specific behavioral mechanism of this pheromone. To test this hypothesis I examined the following specific questions; 1) when placed in a two-choice maze, whether post-ovulatory female sea lampreys will be attracted or spend more time in the side of the maze containing a spermiating male odorant, 2) when in the two-choice maze, whether post-ovulatory female sea lampreys will show increased searching behavior in the side of the maze containing a spermiating male odorant, and 3) when observed in a natural spawning stream exposed to odorants from both spermiating and non-spermiating males, whether post-ovulatory females will be attracted to the spermiating male odorant.

METHODS

Collection and Maintenance of Animals

Adult sea lampreys were trapped or collected by hand from tributaries to Lakes Huron and Michigan of North America by the staff of the United States Fish and Wildlife Service, Marquette Biological Station, Marquette, Michigan USA. All adult sea lampreys were transferred to the United States Geological Survey, Hammond Bay Biological Station (HBBS), Presque Isle County, Michigan USA. The sea lampreys were held in flow-through tanks (1000L) with Lake Huron water at temperatures ranging from of 7°C to 20°C.

Adult males were induced to spermiate by holding them at 18 °C and then injecting them intra-peritoneally with [D-Ala^{6}]-LH-RH(pGlu-His-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-Gly-NH_2, Sigma Chemical Co., St Louis, MO USA) at a dose of 100 µg/Kg body weight (Li 1994). This injection was repeated in two days and then again in five days. Males were checked each day until spermiation occurred. Females were similarly treated to induce ovulation and were checked each day until ovulation occurred.

Adult males and females were used as both test subjects and odor sources for behavioral tests. Males were identified by a raised dorsal ridge and females by an enlarged and soft abdomen along with no dorsal ridge (Vladykov 1949). Each sex was further assigned one of two maturity classifications. For males, individuals that did not emit sperm after gentle pressure was applied to the abdomen were classified as non-spermiating males (NSM); males that did emit sperm were classified as spermiating males (SM). For females, individuals that did not release eggs after gentle pressure was applied to the abdomen were classified as pre-ovulatory females (NOF); females that did release eggs were classified as post-ovulatory females. These four classifications formed the test subject and odor source combinations used in experiments.

Behavioral Preference Tests

Preference tests were used to determine the attraction or repulsion of adult sea lampreys within each sex and maturity class to odorants produced by conspecific adults of each sex and maturity class. These tests were carried out using a two-choice (Y-maze) design (Figure 1). Water was able to flow through these mazes, but sea lampreys were blocked from escaping by sealing the maze with plastic mesh. Odor chambers are located at the head of each side of the mazes. Two mazes were assembled in the raceways at HBBS (referred to as indoor mazes). Another maze was constructed at a field site located on the Ocqueoc River, Presque Isle County, Michigan USA, a Lake Huron tributary (referred to as outdoor maze, Figure 2) to independently confirm responses to odorants in a different setting using river water. The Ocqueoc River is a historic sea lamprey spawning stream (Applegate 1950)

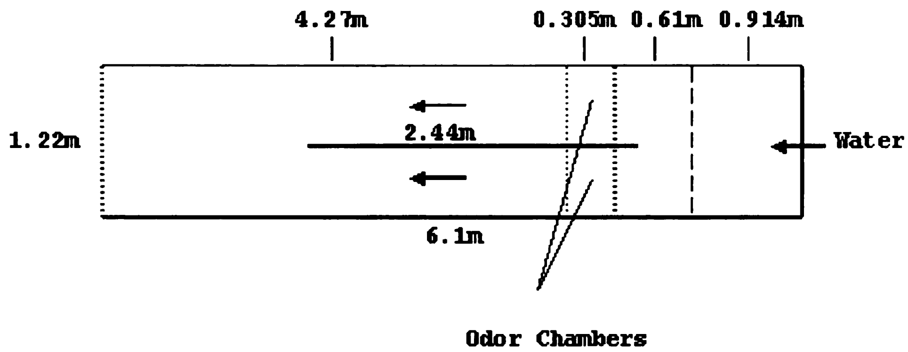

Figure 1. Design of indoor and outdoor two-choice mazes used in preference and searching behavior tests. Arrows indicate water flow. Dotted lines indicate flow through plastic mesh. Dashed line indicates low-head laminar flow device.

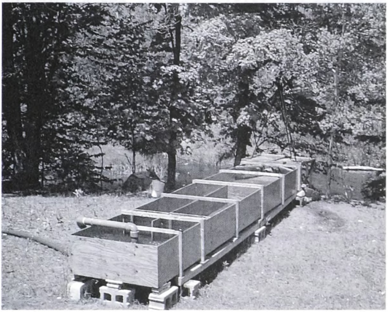

Figure 2. Two-choice maze used in outdoor experiments. Water was pumped into the head of the maze and allowed to flow through back into the river. A single sea lamprey was introduced and its behavior in the maze was monitored both before and after an odorant from sea lampreys of a specific sex and maturity was introduced.

in which a sea lamprey barrier was recently constructed to stop adults from migrating upstream to spawn. The absence of animals from the study area assured that there were low background levels of odorants from other sea lampreys to interfere with behavioral tests and yet offered water temperature and quality known to be suitable for sea lamprey reproduction. The dimension of each arm of the maze (see Figure 1) and the water flow (0.05 m/s indoor and 0.07 m/s outdoor) were identical.

In all, numerous tests were conducted representing all combinations of sex and maturity classes as test subjects and odor sources. A single sea lamprey of a specific sex and maturity class was chosen and introduced into the maze. The sea lamprey was allowed to acclimate for 10 minutes and then its behavior was video-recorded for 20 minutes. Then, sea lampreys or washings from sea lampreys of a specific sex and maturity class were introduced into the odor chamber of one side of the maze chosen randomly by the toss of a coin. Actual fish (five individuals used per test) or water in which spermiating males were held (referred to as washings) were used for odor sources. The odorant was allowed to perfuse through the maze for 5 minutes, and the behavior of the sea lamprey was recorded again in the presence of the odor source for 20 minutes. The maze was allowed to flush free of odorants for 10 minutes before another test was started. Each test cycle lasted about 65 minutes from acclimation to the end. Tests were conducted between 0700 and 1700 hours in water temperatures that ranged from 7°C to 20°C for indoor tests and 12°C to 24°C for outdoor tests. Videotapes were viewed and the time (seconds) spent in the treatment (odorant) side and the control (no odorant) side before odorant introduction (Be and Bc, where e is treatment and c is control) and the time spent in each side after odorant introduction (Ae and Ac, where e is treatment and c is control) were recorded. Proportions of time spent in each side of the maze before and after the odorant introduction (Ae/Be & Ac/Bc) were used to calculate an index of preference (I)for each individual test subject:

$$I = (Ae/Be) - (Ac/Bc)$$

This index standardizes results by comparing test subject behavior in each arm before and after odorant introduction. A positive I value indicates an overall attraction and a negative value an overall repulsion for a single test (see Appendices A and B).

Tests in which sea lampreys did not explore both sides of the maze in the 20 minutes before odorant introduction, and those that did not explore either side in the 20 minutes after odorant introduction, were not used in analysis. This was necessary because proportions could not be generated from these situations which occurred in approximately 25% of all tests. We attempted to collect a sample size of 12 valid tests for indoor tests and eight for outdoor tests for each combination of test subject and odor source class. The I values (differences in the proportion of times spent in the treatment and control sides of the maze) were analyzed with a two-tailed Wilcoxon Signed Ranks Test (Rao 1998) to determine if there was significant attraction or repulsion in each combination of test subject and odor source class.

Searching Behavior Tests

The same two-choice mazes were used to assess the effect of male odorants on searching behavior of adult sea lampreys in each sex and maturity class. In the initial preference tests I observed individual test subjects often displaying increased swimming activity toward the mesh that was preventing them from reaching the odor source at the head of the maze. This activity is characterized by pacing back and forth across the upstream block, increased swimming intensity and the rapid beating or vibration of the tail as the sea lamprey tries to overcome the upstream block. These activities were interpreted as searching behavior. I reviewed the videotapes of preference tests where spermiating males were the odor source (including spermiating male washings) and also those where non-spermiating males were the odor source and post-ovulatory females were the test subjects. I recorded the time (seconds) spent searching in both sides of the maze before and after the odorant introduction and described the results similarly to those of the preference experiments. Again, tests in which sea lampreys did not display searching behavior in both sides of the maze in the 20 minutes before odorant introduction, and those that did not search in either side in the 20 minutes after odorant introduction, were not used in analysis. To promote objectivity in estimating the time spent searching, the identity the treatment side was withheld until after viewing. The indices of searching (see Appendices C and D) were calculated similarly to the indices of preference and analyzed with a Wilcoxon Signed Ranks Test to determine whether the spermiating male odorant induces an increase in searching activity.

Behavior in a Spawning Stream

To assess whether females exhibit similar preference and activity behavior in a natural setting, I monitored pre and post-ovulatory female behavior in response to male odorants in a 65 meter section of the Ocqueoc River, which was chosen for the reasons stated earlier. At the upstream portion of the study section, an island naturally divides the river (Figure 3). Cages (1 m^3) for odor sources were constructed with a wood frame encased in plastic mesh (~1.5 cm gap) and were placed in the channels on each side of the island. A block net (~1.5 cm gap mesh) was placed at the downstream end of the section. An acclimation cage (0.5 m^3) for test subjects constructed with the

Figure 3. The upstream portion of the study section including an island that divides the river and the odor cages used in spawning stream behavior experiments. Post-ovulatory and pre-ovulated female sea lampreys were fitted with radio tags and their behavior monitored in response to spermiating and non-spermiating male odorants.

same material was placed at the downstream end just above the block net.

Tests were conducted between 0700 and 1700 hours in water temperatures ranging from 12°C to 24°C. A day in advance of testing a female sea lamprey (post-ovulatory or pre-ovulatory) was fitted with a radio tag designed for external mount (Advanced Telemetry System, Isanti, Minnesota USA). The following morning sea lampreys being used for odor sources (spermiating males and non-spermiating males) and the tagged test subject were transported to the study site. The odor sources (spermiating and non-spermiating males) were randomly assigned to the two cages on the side of the island. The test subject could choose to swim to spermiating males, non-spermiating males or to none at all. The tagged female was then placed in the acclimation cage and allowed to acclimate, exposed to the two odor sources, for 2 hours.

In each test the subject was released and its location observed visually or by a radio receiver (Lotek Engineering Inc., Newmarket, Ontario, Canada) and recorded on a map grid of the site every 5 minutes. If test subjects failed to move from the release site within an hour they were removed. If tests subjects did move from the release site they were observed until (1) they reached an odor source and stayed there for an hour, (2) they swam past the odor sources, or (3) it was the end of the day (which usually was 4 hours from the start of the test).

A contingency table was used to tally the behavior of female test subjects of different maturation. Categories of response consisted of swimming to the spermiating male odor source, swimming to the non-spermiating male odor source, or not choosing an odor source (staying at an intermediate position within the stream section or not leaving the acclimation cage). A Fisher's Exact Test was used to compare the choice of females in a natural environment (proc freq, 1998 SAS Institute Inc., Cary, North Carolina USA). The times in which females swam to the odor sources were also recorded (see Appendix E).

RESULTS

Behavioral Preference Tests

The behavior of sea lampreys observed in the two-choice maze preference experiments varied greatly. Most sea lampreys alternated between attachment to the side of the mazes and swimming throughout the mazes. Sea lampreys showed no bias to either side of the mazes.

The indoor two-choice mazes were used to characterize the preference responses of individuals in all four sex and maturity classes to all four sex and maturity classes of odor sources. Post-ovulatory female preference responses to spermiating male washings were also characterized. These experiments resulted in 17 combinations in all (Table 1). In most combinations, there were not significant preference or avoidance responses (Wilcoxon Signed Ranks Test, see Table 1 for sample sizes, $P>0.10$). The most consistent preference observed was in post-ovulatory females in response to a spermiating male odorant; 14 of 14 post-ovulatory females spent significantly more time in the treatment side when actual sea lampreys were used as the odor source (Wilcoxon Signed Ranks Test, $N=14$, $P<0.0001$). In addition, 10 of 11 post-ovulatory females tested spent significantly more time in the control side of the maze (no odorant) when exposed to a non-spermiating male odorant (Wilcoxon Signed Ranks Test, $N=11$,

Table 1. Number of adult sea lampreys of specific sex and maturity choosing the treatment (odorant) or control (no odorant) side of an indoor two-choice maze. The treatment side used spermiating male (SM), non-spermiating male (NSM), post-ovulatory female (OF), and pre-ovulatory female (NOF) sea lampreys and also water collected from spermiating males (SMW) as odor sources. Test subjects were spermiating males (SM), non-spermiating males (NSM), post-ovulatory females (OF), and pre-ovulatory females (NOF). Statistical significance was determined with a Wilcoxon Signed Ranks Test (2-tailed) using indices of preference (see Appendix A).

Odor	Test		Side		Test	
Source	Subject	n	Treatment	Control	Statistic	p value
SM	OF	14	14	0	105	<0.0001
SM	NOF	16	4	12	34	NS
SM	SM	12	6	6	49	NS
SM	NSM	11	7	4	36	NS
SMW	OF	8	6	2	32	NS
NSM	OF	11	1	10	1	0.002
NSM	NOF	29	12	17	173	NS
NSM	SM	18	8	10	76	NS
NSM	NSM	11	4	7	31	NS
OF	OF	14	8	6	84	NS
OF	NOF	11	7	4	37	NS
OF	SM	18	15	3	135	NS
OF	NSM	11	5	6	41	NS
NOF	OF	13	6	7	35	NS

Table 1 (cont'd).

Odor Source	Test Subject	n	Side: Treatment	Side: Control	Test Statistic	p value
NOF	NOF	11	5	6	37	NS
NOF	SM	13	8	5	49	NS
NOF	NSM	11	4	7	24	NS

P=0.002). Further, 6 of 8 post-ovulatory females spent more time in the side of the maze with a spermiating male odorant when washings were used as an odor source even though the statistical outcome was not significant.

The outdoor two-choice maze was used to further explore the preference responses to a spermiating male odorant observed in the inside mazes (Table 2). These tests showed that 8 of 8 post-ovulatory female sea lampreys spent significantly more time in the treatment side of the maze when actual spermiating males were the odor source (Wilcoxon Signed Ranks Test, N=8, P=0.004). Also, 7 of 8 post-ovulatory females tested spent more time in the side of the maze containing a spermiating male odorant when washings were used as the odor source even though the outcome was not significant. Individuals in all other sex and maturity classes did not show this same behavior towards a spermiating male odorant. Indices of preference for indoor and outdoor experiments are displayed in Appendices A and B.

Searching Behavior Tests

Post-ovulatory female sea lampreys in both indoor and outdoor experiments increased their searching activity when exposed to a spermiating male odorant (Table 3 and Table 4). When actual spermiating males were used as an odor source, 11 of 11 (indoor mazes, Wilcoxon Signed Ranks Test, N=11, P<0.0001) and

Table 2. Number of adult sea lampreys of specific sex and maturity choosing the treatment (odorant) or control (no odorant) side of an outdoor two-choice maze. The treatment side used spermiating male (SM) and non-spermiating male (NSM) sea lampreys and also water collected from spermiating males (SMW) as odor sources. Test subjects were spermiating males (SM), non-spermiating males (NSM), post-ovulatory females (OF), and pre-ovulatory females (NOF). Statistical significance was determined with a Wilcoxon Signed Ranks Test (2-tailed) using indices of preference (see Appendix B).

Odor	Test		Side		Test	
Source	Subject	n	Treatment	Control	Statistic	p value
SM	OF	8	8	0	36	0.004
SM	NOF	8	5	3	17	NS
SM	SM	10	6	4	30	NS
SM	NSM	13	6	7	36	NS
SMW	OF	8	7	1	33	NS
NSM	OF	10	7	3	41	NS

Table 3. Number of adult sea lampreys of specific sex and maturity spending more time searching in the treatment (odorant) or control (no odorant) side of an indoor two-choice maze. The treatment side used spermiating male (SM) and non-spermiating male (NSM) sea lampreys and also water collected from spermiating males (SMW) as odor sources. Test subjects were spermiating males (SM), non-spermiating males (NSM), post-ovulatory females (OF), and pre-ovulatory females (NOF). Statistical significance was determined with a Wilcoxon Signed Ranks Test (2-tailed) using indices of searching (see Appendix C).

Odor	Test		Side		Test	
Source	Subject	n	Treatment	Control	Statistic	P-value
SM	OF	11	11	0	66	<0.0001
SM	NOF	13	7	6	46	NS
SM	SM	9	4	5	21	NS
SM	NSM	10	6	4	33	NS
SMW	OF	7	7	0	29	0.008
NSM	OF	9	4	5	19	NS

Table 4. Number of adult sea lampreys of specific sex and maturity spending more time searching in the treatment (odorant) or control (no odorant) side of an outdoor two-choice maze. The treatment side used spermiating male (SM) and non-spermiating male (NSM) sea lampreys and also water collected from spermiating males (SMW) as odor sources. Test subjects were spermiating males (SM), non-spermiating males (NSM), post-ovulatory females (OF), and pre-ovulatory females (NOF). Statistical significance was determined with a Wilcoxon Signed Ranks Test (2-tailed) using indices of searching (see Appendix D).

Odor Source	Test Subject	n	Side: Treatment	Side: Control	Test Statistic	P-value
SM	OF	8	8	0	36	0.004
SM	NOF	6	3	3	13	NS
SM	SM	10	6	4	39	NS
SM	NSM	8	2	6	13	NS
SMW	OF	7	7	0	29	0.008
NSM	OF	7	4	3	22	NS

8 of 8 (outdoor mazes, Wilcoxon Signed Ranks Test, N=8, P=0.004) post-ovulatory females tested showed a significant increase in searching behavior in the side of the maze with this odor source. Also, when washings from spermiating males were used as an odor source 7 of 7 post-ovulatory females tested (both inside and outside mazes, Wilcoxon Signed Ranks Test, N=7, P=0.008) showed a significant increase in searching behavior in the side containing the odorant. Individuals in all other sex and maturity classes did not show significant increases in searching behavior in response to a spermiating male odorant. Indices of searching for indoor and outdoor experiments are displayed in Appendices C and D.

Behavior in a Spawning Stream

The odorant released by spermiating males appeared to influence the behavior of post-ovulatory females within the spawning stream section (Table 5). The behavior of adult female sea lampreys in the Ocqueoc River varied greatly, ranging from fast movement upstream towards one of the odor cages to no movement from the acclimation cage. Females were not biased to either side of the stream. Of 13 post-ovulatory females tested, nine swam to and then stayed at the cage containing spermiating males, two stayed at an intermediate position in the study area, and two did not move upstream from the acclimation cage. No post-ovulatory females swam to the cage containing

Table 5. Number of post-ovulatory (OF) and pre-ovulatory (NOF) female sea lampreys swimming to spermiating males (SM), non-spermiating males (NSM), or staying at an intermediate (Int.) position within a section of a known sea lamprey spawning stream. The distribution of choices were significantly different between post-ovulatory and pre-ovulatory females (Fisher's Exact Test, 2-Tail, $P = 0.024$).

	Behavioral Choice		
Maturation	SM	NSM	Int.
OF	9	0	4
NOF	1	2	4

non-spermiating males. Among the seven pre-ovulatory females tested, one swam to the cage containing spermiating males, two swam to the cage containing non-spermiating males, three stayed at an intermediate position in the study area, and one did not move upstream from the acclimation cage. The distribution of choices was significantly different between pre and post-ovulatory females suggesting the attraction of post-ovulatory females to spermiating males (Fisher's Exact Test P=0.024). The actual times in which females reached the odor sources are displayed in Appendix E.

DISCUSSION

All three experiments support my hypothesis that the odorant released by spermiating male sea lampreys is a sex pheromone. In the first experiment where preference/avoidance responses were observed, post-ovulatory females spent more time in the side of the maze with a spermiating male odorant. In contrast, post-ovulatory females did not show a similar preference for the odorants of either non-spermiating males or other female sea lampreys (pre and post-ovulatory). This strict specificity in the sex and maturity of the releaser and receiver of the signal suggests it does not function in general aggregation. Also, this signal seems to function through chemoreception, most likely olfaction because a similar preference response was observed from post-ovulatory females when using washings from spermiating males, which eliminated detection by certain other sensory modes such as sight, sound and electricity. The most likely function of an odorant with this specificity is as a sex pheromone coordinating mating activity.

The attraction response of post-ovulatory female sea lampreys appears to be mediated through an increase in searching behavior. In the second experiment post-ovulatory females swam more actively upstream in the side of the maze with a spermiating male odorant. This behavior makes sense because it would bring them to the odor source (ripe males in a spawning stream). This potential is confirmed in the third experiment in which post-ovulatory females are found to localize spermiating males in an actual spawning stream. This also confirms that the odorant is potent enough to function in their natural habitat. Taken together, my three lines of behavioral experiments demonstrate that this odorant is a specific and potent sex pheromone.

The hypothesis that spermiating male sea lampreys release a sex pheromone is also supported by previous observations. French fishermen have been known to bait their traps with adult male sea lampreys in hopes of increasing their catch rates (Hardisty & Potter 1971). Past behavioral studies suggested that adult male sea lampreys produce an odorant that not only attracts adult females, but also repels other adult males (Teeter 1980; Li 1994). My results showed that males release a pheromonal odorant only during spermiation and that it specifically attracts post-ovulatory females. These varying results could be explained by differences in classification of sexual maturity in both males and females. What is notable, is that in all previous studies and in my results, an attraction of some degree to a male odorant was observed in females. My study extends this knowledge by showing the significance of maturation (spermiation and ovulation) in the function of this sex pheromone system to attract only post-ovulatory females to spermiating males.

Reproductive biology of the sea lamprey also suggests that a male sex pheromone system would be critical to coordination of spawning behavior. Mating begins with nest construction that is typically initiated by a male who is then joined by females

(Applegate 1950). Sea lampreys die shortly after ovulation and spermiation (Applegate & Smith 1951; Larsen 1980), so they only have a limited period of time to reproduce. An attractant would be effective in serving as a final link in synchronization of spawning allowing males to draw ripe females to their nests and also for females to locate males that are ready to spawn.

It has been demonstrated that a wide variety of post-larval sea lamprey behaviors are regulated by odorants. Studies show that the olfactory system is well-developed (Kleerekoper 1972) and important in prey searching and migration. Anatomical studies show that the olfactory organ is relatively large and its sensory epithelium is further enlarged through longitudinal folding (Kleerekoper & van Erkel 1960; Thornhill 1967). Post-larval lampreys have the largest proportion of the brain dedicated to the olfactory sense among vertebrates (Stoddart 1990). Prey searching experiments have shown that parasitic sea lampreys, when placed in a two choice maze, show greater swimming activity and are attracted to rinses from brook trout, *Salvelinus fontinalis*, (Kleerekoper & Mogensen 1959; 1963) which are potential prey. These responses are eliminated by blocking the nose (Kleerekoper 1972). Migratory pheromone studies have also shown that adults are attracted to conspecific larvae (Teeter 1980; Li 1994). Adults are found to be sensitive to a unique bile acid, petromyzonol sulfate, released by larval sea lampreys (Li et al. 1995). It appears that the post-larval life stage of the sea lamprey relies on olfaction for prey searching and migration. My study now also documents the use of olfaction for mating.

In summary, it is evident that spermiating male sea lampreys release a potent sex pheromone that specifically induces an increase in searching behavior, which ultimately brings post-ovulatory females to spermiating males. My results, combined with previous electrophysiology studies, clearly demonstrate the significance of spermiation and ovulation in the release of and response to this pheromone. More research is needed to clarify the exact pattern of release and the physiological mechanism for the production of this pheromone.

APPENDICES

APPENDIX A

Indoor Preference Test Raw Data

Table 6. Time spent (seconds) in each side of the indoor mazes before and after odorant introduction and indices of preference (I) for post-ovulatory female test subjects exposed to a spermiating male odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be)-(Ac/Bc)
1	58	4	463	209	0.3824
2	204	145	504	561	0.4023
3	476	333	362	464	0.5822
4	130	69	188	229	0.6873
5	149	57	139	204	1.0851
6	227	71	475	808	1.3883
7	230	63	254	561	1.9347
8	260	74	136	328	2.1271
9	356	0	104	341	3.2788
10	226	0	289	1200	4.1522
11	429	0	95	433	4.5579
12	85	0	250	1200	4.8000
13	127	79	141	951	6.1226
14	61	38	62	600	9.0545

Table 7. Time spent (seconds) in each side of the indoor mazes before and after odorant introduction and indices of preference (I) for pre-ovulatory female test subjects exposed to a spermiating male odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be)-(Ac/Bc)
1	72	9	132	15	-0.0114
2	360	379	465	479	-0.0227
3	365	418	350	301	-0.2852
4	174	40	84	47	0.3296
5	191	130	307	30	-0.5829
6	347	582	269	121	-1.2274
7	260	0	180	239	1.3278
8	232	564	357	252	-1.7252
9	282	580	491	104	-1.8449
10	143	88	185	484	2.0008
11	242	535	491	50	-2.1089
12	169	484	573	228	-2.4660
13	373	162	174	544	2.6921
14	86	241	117	0	-2.8023
15	123	508	571	113	-3.9322
16	93	586	576	77	-6.1674

Table 8. Time spent (seconds) in each side of the indoor mazes before and after odorant introduction and indices of preference (I) for spermiating male test subjects exposed to a spermiating male odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be)-(Ac/Bc)
1	269	89	308	92	-0.0322
2	287	390	438	496	-0.2265
3	29	47	215	276	-0.3370
4	449	112	114	123	0.8295
5	80	0	870	957	1.1000
6	365	438	314	15	-1.1522
7	84	0	891	1096	1.2301
8	220	390	343	167	-1.2858
9	280	1026	80	152	-1.7643
10	421	0	448	1000	2.2321
11	401	82	52	382	7.1417
12	120	63	46	685	14.3663

Table 9. Time spent (seconds) in each side of the indoor mazes before and after odorant introduction and indices of preference (I) for non-spermiating male test subjects exposed to a spermiating male odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be)-(Ac/Bc)
1	97	123	512	575	-0.1450
2	222	191	454	563	0.3797
3	199	293	461	432	-0.5353
4	249	209	396	546	0.5394
5	393	126	377	523	1.0667
6	215	362	351	156	-1.2393
7	550	41	377	548	1.3790
8	76	69	205	547	1.7604
9	318	582	321	0	1.8302
10	310	97	54	140	-2.2797
11	56	407	338	454	5.9247

Table 10. Time spent (seconds) in each side of the indoor mazes before and after odorant introduction and indices of preference (I) for post-ovulatory female test subjects exposed to a spermiating male washing odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be)-(Ac/Bc)
1	272	220	150	37	-0.5622
2	128	39	311	319	0.7210
3	296	427	539	7	-1.4296
4	326	47	162	371	2.1460
5	131	0	555	1200	2.1622
6	337	62	385	983	2.3693
7	195	31	104	279	2.5237
8	293	9	182	886	4.8374

Table 11. Time spent (seconds) in each side of the indoor mazes before and after odorant introduction and indices of preference (I) for post-ovulatory female test subjects exposed to a non-spermiating male odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be)-(Ac/Bc)
1	447	71	24	6	0.0912
2	253	74	404	26	-0.2281
3	47	47	260	194	-0.2538
4	183	86	56	0	-0.4699
5	127	224	295	332	-0.6384
6	151	110	43	0	-0.7285
7	373	462	583	286	-0.7480
8	164	271	349	257	-0.9160
9	123	305	175	216	-1.2454
10	67	390	156	74	-5.3465
11	18	195	622	606	-9.8591

Table 12. Time spent (seconds) in each side of the indoor mazes before and after odorant introduction and indices of preference (I) for pre-ovulatory female test subjects exposed to a non-spermiating male odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial	Before	After	Before	After	Preference (I)
Number	(Bc)	(Ac)	(Be)	(Ae)	(Ae/Be)-(Ac/Bc)
1	408	335	360	283	-0.0350
2	257	281	195	184	-0.1498
3	441	446	265	333	0.2453
4	174	175	545	713	0.3025
5	360	226	493	473	0.3317
6	238	136	209	49	-0.3370
7	297	274	392	502	0.3581
8	154	219	286	574	0.5849
9	387	425	251	122	-0.6121
10	278	226	385	63	-0.6493
11	358	493	411	220	-0.8418
12	238	192	346	606	0.9447
13	307	445	255	118	-0.9868
14	509	559	40	1	-1.0732
15	156	311	469	403	-1.1343
16	182	0	467	533	1.1413

Table 12 (cont'd).

Trial Number	Control Before (Bc)	Control After (Ac)	Treatment Before (Be)	Treatment After (Ae)	Index of Preference (I) (Ae/Be) - (Ac/Bc)
17	411	659	266	115	-1.1711
18	559	167	261	410	1.2721
19	481	337	168	355	1.4125
20	197	444	250	207	-1.4258
21	174	409	507	464	-1.4354
22	373	649	389	0	-1.7399
23	116	295	503	305	-1.9367
24	128	348	551	419	-1.9583
25	111	323	240	207	-2.0474
26	221	55	82	249	2.7877
27	128	0	394	1200	3.0457
28	606	0	260	977	3.7577
29	22	217	462	339	-9.1299

Table 13. Time spent (seconds) in each side of the indoor mazes before and after odorant introduction and indices of preference (I) for spermiating male test subjects exposed to a non-spermiating male odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed using a Wilcoxon Signed Ranks Test (two-tailed)to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be)-(Ac/Bc)
1	224	187	187	153	-0.0166
2	889	30	140	0	-0.0337
3	42	69	75	116	-0.0962
4	304	30	138	27	0.0970
5	236	165	387	312	0.1070
6	216	0	152	29	0.1908
7	228	220	214	250	0.2033
8	210	92	63	4	-0.3746
9	215	560	88	187	-0.4797
10	294	337	166	85	-0.6342
11	319	26	103	82	0.7146
12	146	47	259	282	0.7669
13	28	73	31	44	-1.1878
14	33	67	682	495	-1.3045
15	334	0	625	1200	1.9200
16	515	361	121	333	2.0511

Table 13 (cont'd).

Trial Number	Control Before (Bc)	Control After (Ac)	Treatment Before (Be)	Treatment After (Ae)	Index of Preference (I) (Ae/Be) - (Ac/Bc)
17	532	1200	123	0	-2.2556
18	24	76	338	137	-2.7613

Table 14. Time spent (seconds) in each side of the indoor mazes before and after odorant introduction and indices of preference (I) for non-spermiating male test subjects exposed to a non-spermiating male odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

Trial Number	Control		Treatment		Index of Preference (I)
	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	(Ae/Be)-(Ac/Bc)
1	125	101	689	468	-0.1288
2	269	166	417	188	-0.1663
3	175	48	468	268	0.2984
4	384	316	320	136	-0.3979
5	438	351	158	59	-0.4280
6	362	368	492	274	-0.4597
7	297	348	216	398	0.6709
8	83	172	716	558	-1.2930
9	81	206	509	575	-1.4135
10	326	154	118	348	2.4768
11	560	437	22	133	5.2651

Table 15. Time spent (seconds) in each side of the indoor mazes before and after odorant introduction and indices of preference (I) for post-ovulatory female test subjects exposed to a post-ovulatory female odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be)-(Ac/Bc)
1	800	412	282	144	-0.0044
2	37	57	359	549	-0.0113
3	165	136	365	268	-0.0900
4	328	246	359	183	-0.2403
5	244	378	162	183	-0.4196
6	43	72	768	934	-0.4583
7	678	148	205	145	0.4890
8	216	51	361	299	0.5921
9	419	182	751	958	0.8413
10	566	442	216	630	2.1357
11	267	13	93	374	3.9728
12	303	38	43	246	5.5955
13	372	26	104	600	-5.6993
14	133	0	72	1200	-16.6667

Table 16. Time spent (seconds) in each side of the indoor mazes before and after odorant introduction and indices of preference (I) for pre-ovulatory female test subjects exposed to a post-ovulatory female odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be) - (Ac/Bc)
1	113	116	613	706	0.1252
2	272	216	188	232	0.4399
3	434	444	88	41	-0.5571
4	434	205	392	430	0.6246
5	512	677	229	147	-0.6803
6	75	114	214	493	0.7837
7	510	345	193	319	0.9764
8	432	46	456	755	1.5492
9	122	229	120	416	1.5896
10	42	126	316	217	-2.3133
11	26	118	97	210	-2.3735

Table 17. Time spent (seconds) in each side of the indoor mazes before and after odorant introduction and indices of preference (I) for spermiating male test subjects exposed to a post-ovulatory female odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be)-(Ac/Bc)
1	150	93	230	196	0.2322
2	277	0	137	35	0.2555
3	347	152	345	368	0.6286
4	265	98	260	272	0.6763
5	340	330	275	509	0.8803
6	381	129	395	500	0.9272
7	136	192	119	51	-0.9832
8	18	55	10	41	1.0444
9	115	116	136	288	1.1090
10	250	235	94	223	1.4323
11	65	0	169	280	1.6568
12	558	170	248	527	1.8203
13	329	148	205	472	1.8526
14	22	70	97	112	-2.0272
15	428	1200	124	0	-2.8037
16	109	100	56	225	3.1004

Table 17 (cont'd).

Trial Number	Control Before (Bc)	Control After (Ac)	Treatment Before (Be)	Treatment After (Ae)	Index of Preference (I) (Ae/Be) - (Ac/Bc)
17	50	0	193	748	3.8756
18	69	74	28	271	8.6061

Table 18. Time spent (seconds) in each side of the indoor mazes before and after odorant introduction and indices of preference (I) for non-spermiating male test subjects exposed to a post-ovulatory female odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be)-(Ac/Bc)
1	188	254	288	383	-0.0212
2	189	195	428	419	-0.0528
3	319	436	290	155	-0.8323
4	259	235	298	10	-0.8738
5	474	588	240	85	-0.8863
6	172	0	364	402	1.1044
7	323	152	412	667	1.1483
8	468	100	320	488	1.3113
9	71	0	419	666	1.5895
10	325	596	318	30	-1.7395
11	381	179	16	266	16.1552

Table 19. Time spent (seconds) in each side of the indoor mazes before and after odorant introduction and indices of preference (I) for post-ovulatory female test subjects exposed to a pre-ovulatory female odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be)-(Ac/Bc)
1	271	232	231	205	0.0314
2	152	238	267	427	0.0335
3	166	25	135	0	-0.1506
4	233	221	198	225	0.1879
5	433	494	313	290	-0.2144
6	138	54	378	345	0.5214
7	299	377	722	352	-0.7733
8	309	577	258	227	-0.9875
9	151	357	279	364	-1.0596
10	271	132	56	261	4.1736
11	29	195	685	898	-5.4132
12	95	35	7	144	20.2030
13	13	589	454	219	-44.8253

Table 20. Time spent (seconds) in each side of the indoor mazes before and after odorant introduction and indices of preference (I) for pre-ovulatory female test subjects exposed to a pre-ovulatory female odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be) - (Ac/Bc)
1	236	29	47	0	-0.1229
2	202	152	90	45	-0.2525
3	167	50	54	0	-0.2994
4	79	72	273	332	0.3047
5	2	0	25	12	0.4800
6	249	272	435	237	-0.5475
7	71	105	279	219	-0.6939
8	84	41	243	309	0.7835
9	364	409	41	98	1.2666
10	102	255	328	243	-1.7591
11	182	31	170	448	2.4650

Table 21. Time spent (seconds) in each side of the indoor mazes before and after odorant introduction and indices of preference (I) for spermiating male test subjects exposed to a pre-ovulatory female odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be)-(Ac/Bc)
1	248	279	489	616	0.1347
2	454	426	319	252	-0.1484
3	207	143	358	317	0.1947
4	235	34	121	57	0.3264
5	154	75	330	289	0.3887
6	396	217	246	244	0.4439
7	109	50	123	134	0.6307
8	218	326	522	365	-0.7962
9	337	444	326	165	-0.8114
10	320	688	162	151	-1.2179
11	508	0	148	202	1.3649
12	92	0	658	1113	1.6915
13	303	814	550	220	-2.2865

Table 22. Time spent (seconds) in each side of the indoor mazes before and after odorant introduction and indices of preference (I) for non-spermiating male test subjects exposed to a pre-ovulatory female odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial	Before	After	Before	After	Preference (I)
Number	(Bc)	(Ac)	(Be)	(Ae)	(Ae/Be) - (Ac/Bc)
1	282	226	544	369	-0.1231
2	529	460	237	166	-0.1691
3	388	433	139	71	-0.6052
4	483	369	272	380	0.6331
5	553	427	115	197	0.9409
6	243	399	421	191	-1.1883
7	299	3	716	877	1.2148
8	198	316	298	838	1.2161
9	83	187	126	117	-1.3244
10	292	472	392	110	-1.3358
11	235	410	351	135	-1.3601

APPENDIX B

Outdoor Preference Test Raw Data

Table 23. Time spent (seconds) in each side of the outdoor maze before and after odorant introduction and indices of preference (I) for post-ovulatory female test subjects exposed to a spermiating male odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial	Before	After	Before	After	Preference (I)
Number	(Bc)	(Ac)	(Be)	(Ae)	(Ae/Be) - (Ac/Bc)
1	472	358	370	343	0.1686
2	353	493	155	445	1.4744
3	641	361	263	551	1.5319
4	307	186	203	617	2.4335
5	179	22	229	1115	4.7461
6	369	53	144	835	5.6550
7	158	52	28	838	29.5995
8	549	79	1	1013	1012.8561

Table 24. Time spent (seconds) in each side of the outdoor maze before and after odorant introduction and indices of preference (I) for pre-ovulatory female test subjects exposed to a spermiating male odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be)-(Ac/Bc)
1	321	117	150	65	0.0688
2	216	251	162	237	0.3009
3	180	48	797	877	0.8337
4	612	868	41	121	1.5329
5	253	556	208	85	-1.7890
6	132	369	475	325	-2.1112
7	114	0	281	959	3.4128
8	54	298	210	413	-3.5519

Table 25. Time spent (seconds) in each side of the outdoor maze before and after odorant introduction and indices of preference (I) for spermiating male test subjects exposed to a spermiating male odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be) - (Ac/Bc)
1	476	149	313	55	-0.1373
2	188	148	169	216	0.4909
3	271	154	580	676	0.5973
4	341	399	275	502	0.6554
5	573	236	100	127	0.8581
6	171	0	457	413	0.9037
7	332	639	174	39	-1.7006
8	215	569	748	426	-2.0770
9	339	855	472	184	-2.1323
10	229	0	247	767	3.1053

Table 26. Time spent (seconds) in each side of the outdoor maze before and after odorant introduction and indices of preference (I) for non-spermiating male test subjects exposed to a spermiating male odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be)-(Ac/Bc)
1	431	478	552	595	-0.0312
2	504	407	321	271	0.0367
3	377	441	39	49	0.0866
4	188	190	120	147	0.2144
5	226	343	490	514	-0.4687
6	553	585	253	144	-0.4887
7	364	228	321	403	0.6291
8	25	0	1033	930	0.9003
9	229	501	411	329	-1.3873
10	130	246	339	87	-1.6357
11	211	472	457	141	-1.9284
12	472	490	76	410	4.3566
13	42	307	658	225	-6.9676

Table 27. Time spent (seconds) in each side of the outdoor maze before and after odorant introduction and indices of preference (I) for post-ovulatory female test subjects exposed to a spermiating male washing odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be) - (Ac/Bc)
1	708	902	157	216	0.1018
2	621	548	76	102	0.4597
3	160	154	450	162	-0.6025
4	351	0	663	692	1.0437
5	225	0	540	921	1.7056
6	247	60	299	806	2.4527
7	260	3	186	573	3.0691
8	602	656	52	424	7.0641

Table 28. Time spent (seconds) in each side of the outdoor maze before and after odorant introduction and indices of preference (I) for post-ovulatory female test subjects exposed to a non-spermiating male odorant. Positive I values indicate an attraction and negative I values indicate a repulsion. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Preference (I) (Ae/Be)-(Ac/Bc)
1	414	368	381	385	0.1216
2	327	251	613	244	-0.3695
3	439	177	138	0	-0.4032
4	56	35	339	501	0.8529
5	268	22	241	249	0.9511
6	486	0	638	1200	1.8809
7	855	241	341	776	1.9938
8	17	0	128	312	2.4375
9	219	711	612	217	-2.8920
10	396	145	40	723	17.7088

APPENDIX C

Indoor Searching Behavior Raw Data

Table 29. Time spent (seconds) searching in each side of the indoor mazes before and after odorant introduction and indices of searching (I) for post-ovulatory female test subjects exposed to a spermiating male odorant. Positive I values indicate more searching in treatment side and negative I values indicate more searching in control side. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall searching behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Searching (I) (Ae/Be)-(Ac/Bc)
1	129	85	151	200	0.6656
2	15	20	150	300	0.6667
3	44	12	67	86	1.0109
4	52	11	85	110	1.0826
5	325	200	110	215	1.3392
6	135	15	135	240	1.6667
7	75	30	180	465	2.1833
8	25	25	76	258	2.3947
9	450	45	30	75	2.4000
10	240	360	15	60	2.5000
11	24	17	15	480	31.2917

Table 30. Time spent (seconds) searching in each side of the indoor mazes before and after odorant introduction and indices of searching (I) for pre-ovulatory female test subjects exposed to a spermiating male odorant. Positive I values indicate more searching in treatment side and negative I values indicate more searching in control side. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall searching behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Searching (I) (Ae/Be) - (Ac/Bc)
1	120	120	188	195	0.0372
2	68	15	30	15	0.2794
3	68	30	120	15	-0.3162
4	225	173	143	45	-0.4542
5	113	0	75	45	0.6000
6	98	165	135	120	-0.7948
7	90	38	105	135	0.8635
8	105	68	120	203	1.0440
9	105	255	135	98	-1.7026
10	113	210	158	15	-1.7635
11	173	90	60	195	2.7298
12	143	120	38	210	4.6872
13	38	218	225	38	-5.5680

Table 31. Time spent (seconds) searching in each side of the indoor mazes before and after odorant introduction and indices of searching (I) for spermiating male test subjects exposed to a spermiating male odorant. Positive I values indicate more searching in treatment side and negative I values indicate more searching in control side. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall searching behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Searching (I) (Ae/Be)-(Ac/Bc)
1	83	15	83	0	-0.1807
2	45	75	128	180	-0.2604
3	195	83	113	135	0.7690
4	30	0	195	165	0.8462
5	105	90	83	0	-0.8571
6	15	0	113	128	1.1327
7	60	90	195	38	-1.3051
8	45	23	38	143	3.2520
9	30	210	23	0	-7.0000

Table 32. Time spent (seconds) searching in each side of the indoor mazes before and after odorant introduction and indices of searching (I) for non-spermiating male test subjects exposed to a spermiating male odorant. Positive I values indicate more searching in treatment side and negative I values indicate more searching in control side. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall searching behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Searching (I) (Ae/Be)-(Ac/Bc)
1	90	53	90	45	-0.0889
2	30	23	173	173	0.2333
3	60	0	90	38	0.4222
4	68	98	165	165	-0.4412
5	98	68	150	173	0.4595
6	75	60	173	218	0.4601
7	45	30	90	105	0.5000
8	68	113	195	165	-0.8156
9	158	218	120	30	-1.1297
10	158	15	128	210	1.5457

Table 33. Time spent (seconds) searching in each side of the indoor mazes before and after odorant introduction and indices of searching (I) for post-ovulatory female test subjects exposed to a spermiating male washing odorant. Positive I values indicate more searching in treatment side and negative I values indicate more searching in control side. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall searching behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Searching (I) (Ae/Be)-(Ac/Bc)
1	115	100	250	255	0.1504
2	200	175	150	180	0.3250
3	90	70	45	65	0.6667
4	300	150	275	400	0.9545
5	35	25	55	105	1.1948
6	135	100	35	95	1.9735
7	25	15	15	50	2.7333

Table 34. Time spent (seconds) searching in each side of the indoor mazes before and after odorant introduction and indices of searching (I) for post-ovulatory female test subjects exposed to a non-spermiating male odorant. Positive I values indicate more searching in treatment side and negative I values indicate more searching in control side. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall searching behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Searching (I) (Ae/Be)-(Ac/Bc)
1	15	15	45	30	-0.3333
2	60	15	38	23	0.3553
3	38	38	60	38	-0.3667
4	60	45	105	120	0.3929
5	23	15	105	120	0.4907
6	60	30	38	0	-0.5000
7	203	180	315	90	-0.6010
8	15	0	68	120	1.7647
9	68	195	150	23	-2.7143

APPENDIX D

Outdoor Searching Behavior Raw Data

Table 35. Time spent (seconds) searching in each side of the outdoor mazes before and after odorant introduction and indices of searching (I) for post-ovulatory female test subjects exposed to a spermiating male odorant. Positive I values indicate more searching in treatment side and negative I values indicate more searching in control side. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall searching behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Searching (I) (Ae/Be)-(Ac/Bc)
1	170	146	146	212	0.5932
2	67	25	62	86	1.0140
3	110	89	56	148	1.8338
4	159	180	37	174	3.5706
5	104	0	72	405	5.6250
6	49	30	23	190	7.6486
7	25	26	3	527	174.6267

Table 36. Time spent (seconds) searching in each side of the outdoor mazes before and after odorant introduction and indices of searching (I) for pre-ovulatory female test subjects exposed to a spermiating male odorant. Positive I values indicate more searching in treatment side and negative I values indicate more searching in control side. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall searching behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Searching (I) (Ae/Be)-(Ac/Bc)
1	127	211	48	45	-0.7239
2	78	181	253	146	-1.7434
3	103	23	223	537	2.1848
4	16	72	35	273	3.3000
5	18	75	414	287	-3.4734
6	17	0	31	808	26.0645

Table 37. Time spent (seconds) searching in each side of the outdoor mazes before and after odorant introduction and indices of searching (I) for spermiating male test subjects exposed to a spermiating male odorant. Positive I values indicate more searching in treatment side and negative I values indicate more searching in control side. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall searching behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Searching (I) (Ae/Be)-(Ac/Bc)
1	122	86	132	87	-0.0458
2	166	50	88	14	-0.1421
3	89	42	41	27	0.1866
4	60	31	52	67	0.7718
5	157	122	94	146	0.7761
6	82	146	24	19	-0.9888
7	85	209	228	158	-1.7658
8	29	0	44	80	1.8182
9	36	0	35	77	2.2000
10	213	49	69	169	2.2192

Table 38. Time spent (seconds) searching in each side of the outdoor mazes before and after odorant introduction and indices of searching (I) for non-spermiating male test subjects exposed to a spermiating male odorant. Positive I values indicate more searching in treatment side and negative I values indicate more searching in control side. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Searching (I) (Ae/Be)-(Ac/Bc)
1	254	185	176	125	-0.0181
2	109	116	87	37	-0.6389
3	97	160	263	214	-0.8358
4	74	88	157	21	-1.0554
5	54	120	65	52	-1.4222
6	40	62	7	29	2.5929
7	128	78	35	131	3.1335
8	20	138	159	81	-6.3906

Table 39. Time spent (seconds) searching in each side of the outdoor mazes before and after odorant introduction and indices of searching (I) for post-ovulatory female test subjects exposed to a spermiating male washing odorant. Positive I values indicate more searching in treatment side and negative I values indicate more searching in control side. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall searching behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Searching (I) (Ae/Be)-(Ac/Bc)
1	145	210	170	350	0.6105
2	25	14	15	55	3.1067
3	110	90	50	150	2.1818
4	200	150	195	300	0.7885
5	40	45	45	165	2.5417
6	15	10	7	45	5.7619
7	175	97	67	127	1.3412

Table 40. Time spent (seconds) searching in each side of the outdoor mazes before and after odorant introduction and indices of searching (I) for post-ovulatory female test subjects exposed to a non-spermiating male odorant. Positive I values indicate more searching in treatment side and negative I values indicate more searching in control side. The absolute values of the I values were analyzed with a Wilcoxon Signed Ranks Test (two-tailed) to determine overall preference behavior.

	Control		Treatment		Index of
Trial Number	Before (Bc)	After (Ac)	Before (Be)	After (Ae)	Searching (I) (Ae/Be) - (Ac/Bc)
1	133	49	32	0	-0.3684
2	98	88	206	55	-0.6310
3	100	184	190	215	-0.7084
4	31	0	69	60	0.8696
5	40	25	38	57	0.8750
6	295	122	70	215	2.6579
7	25	20	36	184	4.3111

APPENDIX E

Spawning Stream Behavior Raw Data

Table 41. Post-ovulatory females choosing spermiating males (SM), non-spermiating males (NSM) or staying at an intermediate position (Int.) within a spawning stream study section. Times in which females reached either spermiating or non-spermiating males are recorded in minutes. Time is not applicable (NA) to those females choosing an intermediate position.

Trial Number	Choice	Time (minutes)
1	SM	25
2	SM	45
3	SM	10
4	SM	15
5	SM	15
6	SM	10
7	SM	45
8	SM	10
9	SM	15
10	Int.	NA
11	Int.	NA
12	Int.	NA
13	Int.	NA

Table 41. Pre-ovulatory females choosing spermiating males (SM), non-spermiating males (NSM) or staying at an intermediate position (Int.) within a spawning stream study section. Times in which females reached either spermiating or non-spermiating males are recorded in minutes. Time is not applicable (NA) to those females choosing an intermediate position.

Trial Number	Choice	Time (minutes)
1	NSM	10
2	NSM	15
3	SM	85
4	Int.	NA
5	Int.	NA
6	Int.	NA
7	Int.	NA

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