THE ROLE OF CHEMICAL STIMULI IN THE PRE-SPAWNING BEHAVIOR OF THE STRIPED BLENNY, CHASMODES BOSQUIANUS

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APPROVAL SHEET

This thesis is submitted in partial fulfillment
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TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

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LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Ripe males of Chasmodes bosquianus release during courtship a sexual pheromone which attracts gravid females. Females tested 24 hours after spawning still significantly respond to the pheromone. Females detect the pheromone by olfaction. The "active substance" of the pheromone appears to be short-lived. Females showed no significant response to courtship water which had been stored in polypropylene bottles for 24 hours at the ambient water temperature.

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INTRODUCTION

Pheromones facilitate sex recognition in several groups of fishes (Kleerekoper 1969; Tavolga 1956). Losey (1969) reported a pheromone to be produced by males of three species of blennies, Hypsoblennius gentilis (Girard), H. robustus (Hildebrand), and H. jenkinsi (Jordan and Evermann) and suggested that the pheromone facilitated male courtship by releasing sexual appetitive behavior and by increasing sexual receptivity.

This report describes the agonistic and reproductive behavior of the striped blenny, Chasmodes bosquianus (Lacépède), and describes experiments demonstrating the presence of a pheromone which elicits and orients the prespawning behavior of C. bosquianus. The methods used in this investigation are similar to Losey's (1969) experimental methods.

REPRODUCTIVE AND AGONISTIC BEHAVIOR OF *CHASMODES BOSQUIANUS*

Introduction

The reproductive and agonistic behavior of *Chasmodes bosquianus* has not been previously described. Tavolga (1958) described the reproductive behavior of fishes which he listed as *C. bosquianus*, but which were in fact *C. saburrae* Jordan and Gilbert (Springer 1959).

Under natural conditions both males and females are territorial, but territoriality was not observed when groups of one sex were held in 20 gallon aquaria. However, when an adult, either male or female, was removed from a group and isolated in a smaller tank, the isolated animal soon established the entire bottom of the tank as its territory. A centrally placed clam shell was the territorial center. Reproductive and agonistic behavior were elicited by the introduction of a gravid female or a ripe male into a tank containing another fish which had been isolated for a week. The ensuing interaction was photographed with a Zeiss Ikon 16 mm camera using Kodak 4-X Reversal film. These films and observations are the basis of the agonistic and reproductive ethograms which follow.

Agonistic Ethogram

Male Resident - Male Intruder

When an adult male was placed in a tank occupied by another male, agonistic bouts usually occurred. As soon as one male became aware of the other, the alerted male changed color. The over-all coloration darkened and the characteristic dark blotches on the body were replaced by pale, narrow, stripes which extended most of the length of the body. The dorsal fin was tightly erected and the irridescent blue blotch on the anterior portion of the dorsal fin became prominent.

The males then approached each other and usually fought. Occasionally they assumed the threat posture before engaging in combat. The threat posture consisted of each male facing his opponent head-on, but displaced laterally several centimeters, rolling the dorsal surface toward the opponent, flaring the opercula, and gaping. The blue blotch of the dorsal fin probably is important in the threat display. From the threat posture, with a small amount of lateral displacement, the animals locked jaws. The threat display lasted only several seconds.

Combat consisted of biting and locking jaws. Often more than a minute elapsed before one of the males released

the opponent's jaw. They then bit each other's pectoral and dorsal fins, often tearing the dorsal. The anterior portion of the dorsal fin was most frequently attacked. Males often thrust the caudal peduncle laterally at one another.

Eventually the intruder fled to a far corner of the tank. The resident pursued and continually nipped the intruder until he assumed a nearly vertical swimming position with the head directed toward the surface and changed from dark to the normal color. The intruder's dorsal fin remained raised but not tightly erect as during combat. The intruder was rarely nipped while maintaining this submissive posture.

Occasionally during flight, the intruder entered the nest, where he was sometimes courted. After having given courtship displays, the resident entered the nest. Eventually the intruder was either accidentally or aggressively pushed out of the nest. The resident then attacked rather than courted the intruder which fled to a corner. The resident usually did not chase, but remained in the shell.

Male Resident - Non-gravid Female Intruder

Whenever a non-gravid or immature female was introduced into a tank which contained an isolated ripe male, the male immediately darkened his over-all coloration and erected his dorsal fin upon becoming aware of the female. The male then approached the female and either attacked or

courted her. In either event, the female responded by assuming a submissive posture identical to that exhibited by males. The female was rarely nipped if she maintained the submissive posture midway in the water column. However the male would often nip her as soon as she started to drift down to the bottom of the tank.

The male's propensity for attack did not appear to be related to the female's nearness to the territorial center, since the female was not attacked even directly in front of the shell as long as she maintained the submissive posture several centimeters above the tank bottom.

A resident male would sometimes court a non-gravid female after which she occasionally entered the shell. The non-gravid female was inattentive to the male's courtship display and never remained in the shell long. As soon as the female left the shell, the male nipped her. She then assumed the submissive posture.

The male's attack upon a female was never as vigorous as his attack upon males. Fin tearing was never observed in aggressive encounters between males and females.

Gravid Female Resident - Gravid Female Intruder

The agonistic behavior of females was similar to that exhibited by males except that females tended to give threat displays more frequently and attacks were less vigorous than those of males.

Whenever a female was placed in a tank already occupied

by another female, the resident animal dashed out of her shell and approached the intruder. Both females underwent conspicuous color changes. The over-all body color darkened. The normal checkered pattern disappeared and was replaced by distinct, dark vertical bands. The dorsal fin was tightly erected. The female threat display was similar to the male threat display and consisted of mouth gaping and the rolling of the dorsal surface toward the opponent. Locked jaw combat commonly occurred.

The intruder was always the first to stop combat by either fleeing to a corner of the tank or entering the shell. Occasionally the resident female courted the intruder while the intruder was in the shell. However, courtship changed to combat as soon as the resident entered the already occupied shell. The intruder then quickly fled to a corner of the tank. The resident female aggressively pursued and nipped at the fleeing intruder. As soon as the intruder assumed the submissive posture, the resident stopped her attack and returned to her shell. The intruder eventually drifted down to the bottom of the tank where she assumed a resting position. This usually did not provoke further attacks from the resident female.

Gravid Female Resident - Ripe Male Intruder

When a ripe male was introduced into a tank already occupied by a gravid female, she approached and threatened the male. The threat display was often mutual. Locked jaw

combat followed the threat display. Combat between males and females never involved fin tearing. After the locked jaw hold was broken, the female usually returned to her shell. The fish then appeared to ignore one another. If the male was left in the tank for an hour or so, he would eventually enter the shell which the female would relinquish without a fight.

A newly introduced male never courted the resident female. Perhaps the stress involved in the transfer from one tank to another and the foreign surroundings temporarily suppressed the male's sexual drive.

Reproductive Ethogram

Courtship Coloration

The courtship coloration of the male is darker than the normal coloration. The normal blotches disappear and pale, narrow, stripes become prominent. The pre-opercula become quite pale, while the chin and forehead remain dark. The pectoral fins darken. The white tips of the anal spines and the dark anal pads become conspicuous. The dorsal fin darkens except for a mid-anterior portion which becomes an amber color. The irridescent blue blotch on the anterior part of the dorsal fin darkens and becomes less conspicuous. During the early part of courtship, the iris of the eye changes from the normal dark color to an amber color.

Females do not change color during courtship or spawning.

Courtship Display

When a gravid female was placed in a tank which contained a ripe male, the following events usually occurred. As soon as the male became aware of the female, he assumed courtship coloration and erected his dorsal fin. The male then approached and with quick darting movements circled the female many times, all the while rapidly shaking his

head. A female sometimes initially responded to the male's actions by assuming a submissive posture, but she soon resumed the normal resting position. The male frequently returned to the nest, turned upside down and swam around on the underside of the upper valve of the clam shell. The male also performs this swimming action during actual spawning. He then turned upright and extended his head out of the shell toward the female and shook his head. If the female did not then enter the nest, the male again circled around her while shaking his head. The male sometimes herded the female toward the nest by reducing the size of his circles. If the female still did not enter the nest, the male then nipped her and butted her in the tail region with his snout. The male was often very violent in his attempts to persuade the female to enter the nest. Once the female had entered the nest, she positioned herself to face the front of the clam shell. The male then circled around the clam shell while vigorously shaking his head. The male swam faster around the back of the nest than in front of the nest, slowing as soon as he came within view of the female. The male did not always completely circle the shell, but sometimes swam around to a point where he was no longer readily visible to the female, then turned and swam the other way. The female was usually attentive to the male's displays and would continually position herself so that she would have optimum view of his display. The male eventually entered the nest.

Occasionally a newly introduced female entered the nest before the male had even approached her. When this occurred, the male would make a few circles around the shell, give a few head-shakes, and then enter the shell.

Certain elements of the male's courtship display were always observed i.e. the over-all color change, erection of the dorsal fin, and head-shakes. Other aspects of courtship such as circling the female, herding her, circling the nest, and nipping occurred frequently but not always.

Spawning

Spawning usually took place within 10 minutes of the introduction of a gravid female into the tank of a ripe male and often lasted for three hours or more. During spawning the male and female maintained close body contact in their trunk region. Both animals faced in the same direction and kept their bodies parallel to each other. The male laterally pushed the female around in the nest, occassionally and accidentally out of the nest. The female deposited eggs on both upper and lower valves of the clam shell, after which she either voluntarily left the shell or was pushed out by the male. The female did not return to the shell; the male remained with the eggs.

If another gravid female was placed in a tank containing an isolated, egg-guarding male, the male courted the female which then laid her eggs beside the eggs already present in the shell.

EXPERIMENTAL DESIGN AND RESULTS

To determine if a pheromone is produced, striped blennies were isolated in tanks equipped so that test water introduced at either end formed a concentration gradient. Different kinds of test water were introduced into either end of a tank and the resident fish's reaction was recorded.

Specimens of Chasmodes bosquianus were collected from oyster trays, shell bags, and empty beer cans in shallow water of the York River near Gloucester Point, Virginia.

The spawning period in the Chesapeake Bay region has been reported to extend from April through August (Hildebrand & Schroeder 1928). The present study was begun in late May and continued through September 3.

Twelve sexually ripe males were isolated in separate 10 gallon tanks which had three opaque sides to prevent the resident animal from viewing fishes in neighboring aquaria. A male was considered to be sexually ripe if he courted gravid females. One of the 12 males served as a control. Each tank contained an airstone and an empty clam shell which had the hinge secured with rubber cement to prevent collapse during

the experiment. The shell, centrally placed, served as a nest and shelter.

Aquaria were supplied with running estuarine water which entered both ends through a polyethylene funnel located several centimeters above the water surface. Both the estuarine water and the stimulus test water were introduced through the polyethylene funnel.

The stimulus test water was introduced from a separatory funnel at the rate of about 1 ml/sec (Fig. 1). During the testing of a water type, one of the separatory funnels contained the stimulus water while the other funnel contained estuarine water. Temperature differences between the test water, the control water, and the estuarine water did not exceed 0.5 C. The total inflow to each tank was maintained at 10 ml/sec or 5 ml/sec per line by Hoffman clamps. The total outflow was maintained. at 10 ml/sec by a constant-level siphon which kept the water level at 18 cm from the tank bottom (Breder 1969). The siphon over-flow was located about 5 cm from the bottom near the middle of the back wall of the tank. The flushing time was about 31 minutes for each tank. Preliminary tests with vegetable dyes demonstrated that concentration gradients were formed at each end of the tanks.

A trial for an individual male consisted of a 2.5 minute "control observation period" and a 2.5 minute "test observation period". The test water, which flowed for three minutes, was introduced into the tank at the end

of the "control observation period". The "test observation period" began 30 seconds after the test water started flowing into the tank.

During the control and test observation periods, the position and activity of the resident animal was recorded at one second intervals. The time that an individual spent out of its shelter, which was located in the center of the tank, was divided into four categories ---- 1. touching bottom in the nonstimulus half of the tank, 2. swimming (not touching bottom) in the nonstimulus half of the tank, 3. touching bottom in the stimulus half of the tank, 4. swimming in the stimulus half of the tank. The categories were weighted one through four respectively. A total score for each observation period consisted of the sum of the number of seconds of each activity multiplied by its weight. The scores from the "test observation period" were checked for significant difference from the "control observation" scores by the Wilcoxon matched-pairs signed-ranks test (Siegel 1956). In order to determine whether the test water was an attractant or repellent, the number of seconds of each activity during each "control observation period" was subtracted from that of each corresponding activity during the "test observation period". Values for the time spent on the bottom and for the time spent "swimming" were summed for the two halves of the tank for each test, and then compared by the Wilcoxon matched-pairs signed-ranks test.

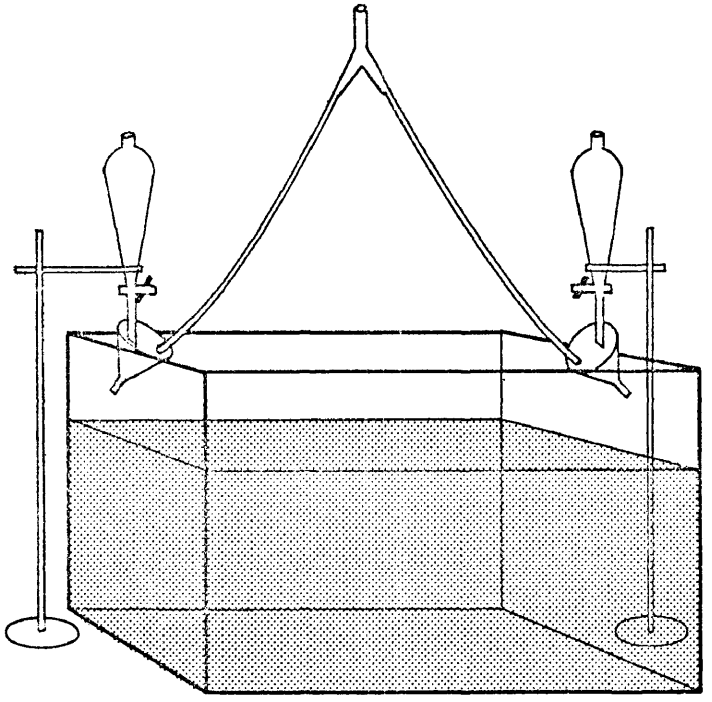

Figure 1. Shows the apparatus used for the introduction of test water and estuarine water.

A trial for an individual female was essentially the same as a trial for a male except that it was necessary to lengthen both the "control observation period" and the "test observation period" to 3.5 minutes each for the female trials, since the females were more reluctant to leave the shell.

The striped blennies were fed daily on small pieces of clams and oysters. The food was always placed in the center of the tank in order to avoid conditioning a preference for either end.

Experiment I: In order to determine the validity of the test conditions, clam juice was presented four times to each of the 11 isolated males. Clam juice was also presented four times to each of three randomly chosen females. The male and female controls were tested with estuarine water. The fish were not fed during the four days prior to testing with clam juice.

Both males and females significantly reacted and were significantly attracted to the clam juice (Tables 1-5). The controls as in all subsequent tests showed no significant reactions to the estuarine water.

Experiment II: The first test series consisted of presenting isolated ripe males with the following six water types: 1. male water --- water drawn from the tank of an isolated ripe male, 2. female water --- water drawn

from the tank of an isolated gravid female. The three following water types were obtained by introducing a gravid female into the tank of an isolated ripe male. Water was then drawn from the tank and characterized according to the behavior of the male at that time --- 3. no courtship water (male not courting), 4. courtship water (60 seconds after the male had initiated the head-shake display), 5. mating water (water drawn from a tank at least 50 minutes after oviposition had begun), 6. estuarine water. Water types were drawn from the constant-level siphon outlet to avoid disturbing the animal(s).

Both courtship water and mating water were presented seven times to each of the 11 isolated males. Each of the other water types was presented four times to every male. The control was always tested with estuarine water. In each test series, the individual tested, the order of presentation of the water types, and the end of the tank in which the test water was introduced, were chosen from a table of random numbers (Beyer 1966).

At least one hour elapsed between tests run on the same individual. This delay insured that the tank was completely flushed of the previous water type.

None of the 11 ripe males reacted significantly to any of the six water types (Table 5).

Experiment III: A series of tests was run to determine

if gravid females would react to any of the six water types. Eleven gravid females were isolated in separate tanks one week prior to testing. For each trial, the individual tested, the order of presentation of the water types, and the end of the tank in which the stimulus was to be introduced, were randomly chosen. Both courtship water and estuarine water were presented seven times to each of 10 gravid females and the four other water types were presented five times. The control was always tested with estuarine water.

The ten gravid females showed a significant reaction and attraction only to courtship water (Tables 5-7). Soon after the introduction of the courtship water, the female extended her head from the shell and faced the stimulus-end of the tank. She usually made several false starts out of the nest, i.e. she started to dart out of the shell, but then abruptly checked the movement and moved further back in the shell. Eventually she darted out of the shell, but quickly returned.

Experiment IV: A series of tests was run to determine if gravid females, which had been allowed to spawn, would still react to a water type which prior to spawning had evoked responses. Four females were randomly selected from the 10 gravid test females. Each of the four females and the control female was placed in a tank which contained an isolated ripe male. The animals were allowed to spawn. One hour after spawning had begun, the female was removed and returned to

her tank. The remainder of the test day was divided into four 90 minute intervals. During each interval each of the four females was presented with courtship water. The control female was tested with estuarine water. During each interval, the individual tested and the end of the tank in which the stimulus was introduced were randomly chosen. Part of the day following spawning was divided into seven consecutive 60 minute intervals. During each interval, each of the four females was tested with courtship water. The control female was tested with estuarine water.

Females tested on the day they spawned, showed no significant reaction to courtship water. Females which were presented with courtship water on the day following spawning showed a significant reaction and attraction to the stimulus water (Table 5).

Experiment V: A series of tests was conducted to determine if the active substance of a stimulative water type retained its effectiveness after having been stored for a period of 24 hours. Courtship water was stored in polypropylene bottles for 24 hours at the ambient water temperature (approximately 78 F).

Four females randomly chosen from the 10 gravid test females were tested with the stored water. The control female was tested with estuarine water that had been stored 24 hours in polypropylene bottles. The four test females

and the control female were tested during a period of one hour. Each of the test females was tested a total of four times over a period of four days with courtship water which had been stored 24 hours.

The test females showed no significant reaction to courtship water which had been stored 24 hours. Females tested with the same courtship water before it had been stored, showed significant reactions (Table 5).

Experiment VI: Tests were conducted to determine if olfaction was used in detecting the pheromone. Attempts were made to destroy the nasal epithelium of each of the 10 test females and the control by inserting a hot probe into the incurrent and excurrent nares of each animal. The chemical stimuli which previously had elicited reactions from uncauterized females, were then presented to the 10 anosmic females. The anosmic control was tested with estuarine water. Each cauterized female was exposed to the stimulus water type for four trials. The nasal epithelia of the 10 test animals and the control were examined for signs of regeneration.

None of the anosmic females showed a significant reaction to the courtship water. Examination of the olfactory chambers revealed that cauterization had produced scar tissue which had completely sealed both the incurrent and excurrent nares of four test females and the control. Pigment was present in only the innermost part of the nasal

chamber of these females. The incurrent nares of three females were plugged with scar tissue but the excurrent nares were not sealed. The incurrent and excurrent nares were plugged on only one side of the head of two females. None of the nares of one female were sealed.

Two males were cauterized. The following day a gravid female was presented to each of the anosmic males. The anosmic males still showed vigorous courtship.

DISCUSSION

At some time during the courtship display the male releases a pheromone which acts as an attractant to gravid females. The release of the pheromone is not necessarily coincident with the head-shake display and might be intermittent. In view of the existence of a heterosexual territorial system, a sexual pheromone which serves as an attractant could be of considerable value in bringing the two sexes together or in temporarily keeping them together.

Males of Chasmodes bosquianus, after having spawned, spend most of their time in the shell guarding their eggs. Several females may spawn their eggs in the nest of one male. Perhaps the pheromone attracts gravid females to the nest of an egg-guarding male as in the case of Blennius pavo (Kleerekoper 1969). The pheromone may increase the female's appetitive or searching behavior. The pheromone may exert stronger influence after the male and female have come in visual contact and may then reduce the female's tendency toward flight, thereby keeping her near the male until the appropriate releasing stimuli either further maintain the pair or initiate courtship behavior. The active substance of the pheromone is short-lived. A short-lived pheromone would have the advantage

of reducing the "chemical noise" in the environment.

Since females of C. bosquianus do not release all of their eggs at one spawning, it was not surprising to find that females were still attracted to the pheromone when tested the day after spawning. It is possible that the stress of being transferred from one tank to another on the day of spawning precluded response to the pheromone on that day. It is also possible that the internal conditions of the female are no longer "primed" just after spawning.

Cauterization of four females indicated that olfaction is used in detecting the pheromone. The stress of cauterization may have been responsible for the negative results, but this seems unlikely in view of the fact that the stress of cauterization in no way impared the courtship behavior of anosmic males.

TABLE 1

Values of male residents reactions to clam juice.

Replications		Male Residents										
		1	2	3	4	5	6	7	8	9	10	11
	control	279	207	346	150	0	252	228	200	17	111	273
1	test	411	330	359	410	150	312	440	244	39	416	380
	control	0	45	0	126	8	284	225	159	0	0	197
2	test	271	404	0	392	196	263	359	273	339	272	258
	control	2	345	246	390	251	283	201	220	0	202	111
3	test	298	257	162	359	119	257	323	469	25	461	210
	control	268	262	74	183	173	322	304	345	13	0	255
4	test	349	258	414	313	348	357	472	369	351	29	347

TABLE 2

Values of male residents attraction to clam juice.

Replications		Male Residents										
		1	2	3	4	5	6	7	8	9	10	11
1	*ns. half	- 24	- 30	- 27	-129	0	- 19	- 95	-109	22	- 23	- 39
	**s. half	41	35	27	129	50	21	95	47	0	109	39
2	ns. half	0	- 20	0	- 62	12	- 18	- 56	- 36	0	21	- 26
	s. half	87	125	0	108	57	12	63	43	104	77	30
3	ns. half	1	- 13	- 46	7	- 51	16	0	- 97	3	-124	- 8
	s. half	94	- 19	- 14	- 7	- 23	- 30	29	117	7	124	22
4	ns. half	- 38	- 40	- 63	- 56	- 32	- 21	- 72	- 58	42	0	- 39
	s. half	38	22	131	62	63	24	72	35	63	9	45

*non-stimulus half of tank
**stimulus half of tank

TABLE 3

Values of female residents reactions to clam juice.

Replications		Female Residents		
		4	6	7
	control	0	0	49
1	test	9	169	194
	control	0	0	0
2	test	0	63	102
	control	0	0	28
3	test	24	67	81
	control	0	0	0
4	test	32	13	43

TABLE 4

Values of female residents attraction to clam juice.

Replications		Female Residents		
		4	6	7
1	*ns. half	0	15	7
	**s. half	3	45	41
2	ns. half	0	0	22
	s. half	0	20	24
3	ns. half	1	0	- 2
	s. half	6	21	18
4	ns. half	0	2	0
	s. half	9	3	12

* non-stimulus half of tank

** stimulus half of tank

TABLE 5

Summary of probabilities (P) obtained by the Wilcoxon matched-pairs signed-ranks test for the six experiments.

Experiment		P	
		reaction	attraction
I	males	< 0.001	< 0.001
	females	0.002	0.002
II	male water	0.374	-----
	female water	0.194	-----
	no courtship water	0.289	-----
	courtship water	0.234	-----
	mating water	0.166	-----
	estuarine water	0.144	-----
III	male water	0.161	-----
	female water	0.392	-----
	no courtship water	0.479	-----
	courtship water	< 0.001	0.021
	mating water	0.490	-----
	estuarine water	0.201	-----
IV	day of spawning	+	-----
	day after spawning	< 0.001	0.006
V	fresh courtship water	0.009	0.046
	stored courtship water	0.368	-----
VI	anosmic females	0.288	-----

+ n too small for computation

TABLE 6

Values of female residents reactions to courtship water.

Replications		Female Residents									
		1	2	3	4	5	6	7	8	9	10
	control	0	285	210	22	88	0	9	0	210	210
1	test	0	342	230	37	347	117	37	73	554	376
	control	0	0	436	0	0	0	0	0	35	560
2	test	555	126	454	41	90	0	20	243	117	470
	control	0	0	0	0	0	0	0	0	0	630
3	test	96	291	6	0	0	47	3	29	0	630
	control	0	0	0	215	630	0	0	0	242	210
4	test	223	30	8	60	630	129	48	51	210	502
	control	0	210	210	0	0	330	0	164	0	352
5	test	2	210	230	539	0	310	67	274	11	573
	control	0	0	200	59	42	0	135	0	0	210
6	test	311	103	342	467	136	183	267	0	106	374
	control	0	0	0	0	210	39	0	0	33	244
7	test	0	0	57	59	210	72	37	34	203	633

TABLE 7

Values of female resident's attraction to courtship water.

Replications		Female Residents									
		1	2	3	4	5	6	7	8	9	10
1	*ns. half	0	0	- 10	- 15	- 19	0	22	73	-172	- 83
	**s. half	0	19	10	10	92	39	2	0	172	83
2	ns. half	0	0	43	23	0	0	20	0	- 35	45
	s. half	185	42	- 9	6	30	0	0	81	39	- 45
3	ns. half	96	0	0	0	0	2	0	29	0	0
	s. half	0	97	2	0	0	15	1	0	0	0
4	ns. half	1	0	5	- 75	0	0	48	0	16	-146
	s. half	74	10	1	- 24	0	43	0	17	- 16	146
5	ns. half	2	0	- 9	2	0	10	4	137	2	-106
	s. half	0	0	9	179	0	- 10	21	- 9	3	106
6	ns. half	0	73	-200	- 24	93	0	- 33	0	72	- 79
	s. half	102	10	114	144	0	61	55	0	11	79
7	ns. half	0	0	0	26	0	33	34	25	194	-193
	s. half	0	0	19	11	0	0	1	3	- 8	193

* non-stimulus half of tank
** stimulus half of tank

TABLE 8

Values of female residents reactions to courtship water one day after spawning.

Replications		Female Residents			
		1	3	5	8
	control	0	0	49	165
1	test	49	130	58	210
	control	0	0	0	0
2	test	19	27	35	0
	control	210	0	0	0
3	test	0	11	43	291
	control	0	0	0	0
4	test	49	150	0	138
	control	35	0	0	0
5	test	123	0	137	0
	control	0	210	0	210
6	test	0	255	216	452
	control	0	0	0	210
7	test	113	22	47	498

TABLE 9

Values of female residents attraction to courtship water one day after spawning.

Replications		Female Residents			
		1	3	5	8
1	*ns. half	16	28	9	35
	**s. half	11	34	0	0
2	ns. half	1	0	17	0
	s. half	6	9	6	0
3	ns. half	0	2	0	0
	s. half	- 70	3	12	97
4	ns. half	0	9	0	0
	s. half	15	47	0	46
5	ns. half	- 18	0	17	0
	s. half	35	0	40	0
6	ns. half	0	- 69	51	-121
	s. half	0	38	55	121
7	ns. half	7	4	2	-144
	s. half	32	6	15	144

* non-stimulus half of tank
** stimulus half of tank

SUMMARY

1. Ripe males of Chasmodes bosquianus release a sexual pheromone during courtship. The pheromone acts as an attractant to gravid females.

2. Females tested 24 hours after spawning still significantly respond to the pheromone.

3. Females detect the pheromone by olfaction.

4. The "active substance" of the pheromone appears to be short-lived. Females showed no significant response to courtship water which had been stored in polypropylene bottles for 24 hours at the ambient water temperature.

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