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RESPONSES OF THE HARD CLAM *MERCENARIA MERCENARIA* (LINNÉ) TO INDUCTION OF SPAWNING BY SEROTONIN[1]

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ABSTRACT Clam size, sex of clam, concentration of serotonin, and site of administration of serotonin were found to influence the induction of spawning in the hard clam *Mercenaria mercenaria* (Linné). Overall, male clams greater than 36.4 mm thickness were more likely to spawn in response to serotonin injection at concentrations of 0.2 or 2.0-mM. Administration of serotonin by injection in the anterior adductor muscle resulted in significantly more spawnings than intragonadal injection or dispersal in water surrounding the incurrent siphon.

KEY WORDS: hard clam, *Mercenaria mercenaria*, spawning, serotonin

INTRODUCTION

The chemical serotonin or 5-hydroxytryptamine (5-HT) has been shown to be an effective inducer of spawning for many bivalves (Matsutani and Nomura 1982; Gibbons et al. 1983; Gibbons and Castagna 1984). Injection of serotonin into the gonad or adductor muscle of ripe bivalves will induce spawning without any additional stimuli; however, male bivalves spawn in greater numbers than females. This study examines the effectiveness of various concentrations of serotonin to induce spawning in male and female hard clams *Mercenaria mercenaria* (Linné) of three adult size classes. Different sites of administration of serotonin were also investigated.

MATERIALS AND METHODS

The method of individual spawning described by Castagna and Kraeuter (1981) was used to spawn all hard clams without any thermal shock or additional stimuli. Hard clams were spawned by placing individuals in glass dishes containing 1 *l* of 1-μm-filtered seawater (30 ppt) in water baths at 20°C. Crystalline serotonin (5-hydroxytryptamine, creatinine sulfate complex, Sigma Chemical Company, St. Louis, MO) was dissolved in 1-μm-filtered seawater to prepare the required concentrations of serotonin solutions.

A determination of ripeness and sex of hard clams was made by drilling a small hole through the shell and removing a biopsy of the gonad for microscopic examination (Castagna and Kraeuter 1981). Gravid hard clams were divided into six groups of 150 individuals by size and sex. Hard clams were classified by size as littlenecks (25.4-36.4 mm in thickness), cherrystones (> 36.4-41.2 mm), and chowders (> 41.2 mm). Small notches were filed into the valve margins of clams adjacent to the anterior adductor muscle. Hypodermic injections of 0.4 ml of 0 (control), 0.02, 0.2, 2.0, or 20.0-mM serotonin solutions were made into anterior adductor muscles. Each concentration of serotonin was given to 30 hard clams of each sex-size class. Observations were made of the time interval to spawning and the behavior of clams after injections. A three-way analysis of variance was used, after log (X+1) transformation of data, to test the effects of clam size, sex, and concentration of serotonin on numbers of hard clams induced to spawn (Sokal and Rohlf 1981).

The influence of administration site on the efficacy of serotonin to induce spawning was examined using 675 cherrystone clams. Three sites were tested using 225 clams each. The two groups received 0.4 ml of 2.0-mM serotonin solution by injection. One group of clams had notches filed into the valve margins adjacent to the anterior adductor muscle which allowed injection of serotonin into the muscle. The second group was injected intragonadally by inserting a needle between the valves immediately below the ligament on the posterior end of the clam. A new needle was used for each clam to prevent transference of gonadal products. The third group received 0.4 ml of 20.0-mM serotonin dispersed into the water entering the incurrent siphon. The efficacy of the various sites of serotonin administration upon induction of spawning was examined through the nonparametric Kruskal-Wallis test (Sokal and Rohlf 1981).

The possibility of using the foot as a site of serotonin injection was investigated using 380 male cherrystone and chowder clams. Small notches were filed into the lip of hard clam valves. Half of the clams were injected in the foot with 0.4 ml of 2.0-mM serotonin solution while the control group received 0.4 ml of 1-μm-filtered seawater. The G-test of independence and William's correction were used to test whether spawning was independent of serotonin injection into the foot (Sokal and Rohlf 1981).

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RESULTS

Hard clams reacted immediately to injection with serotonin into the anterior adductor muscle, gonad, and foot by increased siphoning, probing with an elongated foot, and adduction of the valves. Injection of serotonin in ripe hard clams induced spawning within 10-15 minutes and the majority spawned within 30-60 minutes. Control animals did not exhibit any of these behaviors and did not spawn. No mortality occurred in the 1,955 hard clams used in these experiments.

Sex of clams, size of clams, and concentration of serotonin were found to significantly influence ($P < 0.005$) the induction of spawning. Overall, male clams were seven times more likely to spawn than females upon injection with serotonin into the anterior adductor muscle (Table 1). For male hard clams, chowders and cherrystones were more likely to respond to serotonin injection by spawning than littlenecks. More specifically, male cherrystones (40.0%, 60.0%) and chowders (66.7%, 53.3%) spawned in significantly greater numbers ($P < 0.001$) at 0.2 and 2.0-mM serotonin solutions, respectively, than male littlenecks and female clams of all three size classes at any serotonin concentration. Of the female hard clams, chowders (23.3%) injected with 0.2-mM serotonin solution were the most likely to respond by spawning.

Occasionally both male and female hard clams would react to serotonin injections at concentrations of 0.2 to 20.0-mM by closing the valves on the end of the foot, often cutting the tissues (Table 2). This behavior is referred to as foot nipping. The effect of concentration was significant ($P < 0.001$), as greater numbers of clams displayed foot nipping at higher concentrations. Chowders of both sexes had greater tendencies ($P < 0.01$) to display this behavior than littlenecks or cherrystones. There was no significant statistical difference between the numbers of clams of either sex that showed foot nipping.

Injection of 2.0-mM serotonin solution into the anterior adductor muscle of the hard clam induced significantly more ($P < 0.001$) spawning than intragonadal injection or dispersal in water surrounding incurrent siphons (Table 3). Release of serotonin into seawater did not cause hard clams to display any behavioral responses to the serotonin or to spawn.

Injection of 2.0-mM serotonin into the foot of the hard clam induced significant numbers ($P < 0.001$) to spawn ($N = 86$). None of the control animals spawned. Hard clams reacted to insertion of hypodermic needles by withdrawing the foot.

TABLE 1.

Numbers of males and females of *Mercenaria mercenaria* of three size classes induced to spawn by injection of serotonin at five concentrations (N = 30 clams tested for each size class and concentration).

		Concentrations of Serotonin (mM)					
Sex	Size Class	0	0.02	0.2	2.0	20.0	Total
Male	Littleneck	0	3	1	2	0	6
	Cherrystone	0	6	12	18	12	48
	Chowder	0	4	20	16	9	49
Female	Littleneck	0	1	1	0	0	2
	Cherrystone	0	1	3	0	0	4
	Chowder	0	0	7	1	0	8
Total Number Males		0	13	33	36	21	103
Total Number Females		0	2	11	1	0	14
Percentage of Males		0	14.4	36.6	40.0	23.3	22.9
Percentage of Females		0	2.2	12.2	1.1	0	3.1

TABLE 2.

Numbers of males and females of *Mercenaria mercenaria* of three size classes responding to serotonin injection at five concentrations by nipping off the end of the foot (N = 30 clams tested for each size class and concentration).

		Concentrations of Serotonin (mM)					
Sex	Size Class	0	0.02	0.2	2.0	20.0	Total
Male	Littleneck	0	0	0	0	1	1
	Cherrystone	0	0	1	2	7	10
	Chowder	0	0	0	1	11	12
Female	Littleneck	0	0	1	0	3	4
	Cherrystone	0	0	0	1	6	7
	Chowder	0	0	1	4	20	25
Total Number Males		0	0	1	3	19	23
Total Number Females		0	0	2	5	29	36
Percentage of Males		0	0	2.2	3.3	21.1	5.1
Percentage of Females		0	0	1.1	5.6	32.2	8.0

TABLE 3.

Numbers of hard clams (*Mercenaria mercenaria*) induced to spawn by administration of serotonin by injection into the anterior adductor mucle, intragonadal injection, and dispersal in water surrounding the incurrent siphon (N = 25 clams tested for each site and replicate).

	Site of Serotonin Administration		
	Anterior Adductor Muscle	Gonad	Seawater
	6	5	0
	8	2	0
	9	6	0
	5	4	0
	7	1	0
	5	5	0
	5	4	0
	7	10	0
	9	4	0
Total number	61	41	0
Percentage	27.1	18.2	0

DISCUSSION

Serotonin, a molluscan neurotransmitter, occurs naturally in the visceral ganglia of *Mercenaria mercenaria* at mean concentrations of 40 $\mu g \cdot g^{-1}$ of fresh tissue (Welsh and Moorhead 1960). In the hard clam, laboratory studies have shown that serotonin increases the amplitude of the beating of isolated hearts, stimulates the beating of lateral cilia on the gill, and increases

the tone and induces rhythmic activity in the rectal muscle (Welsh 1957; Aiello 1970; Leake and Walker 1980). The role of serotonin in the spawning of bivalves is unknown.

Concentrations of 0.2 or 2.0-mM serotonin appeared to be most effective to induce ripe hard clams to spawn. Serotonin induced primarily male clams to spawn but similar numbers of males and females displayed foot nipping when exposed to high serotonin concentrations. Matsutani and Nomura (1982) found that intragonadal injection of ganglionic homogenates of the dioecious scallop *Patinopecten yessoensis* (Jay) induced spawning only in males. Injection of serotonin into the gonads of scallops induced spawning in both males and females, but males spawned more quickly and at lower serotonin concentrations.

Injection of serotonin solutions into the gonad or a blood sinus such as found in the anterior adductor muscle or foot induced spawning in ripe hard clams. The anterior adductor muscle proved to be a convenient and effective site for injection of serotonin. The shell margins adjacent to the muscle are easily notched with a file and the notch is repairable by the clam. A needle can be inserted directly into the anterior adductor muscle with certainty of destination as the resistance of the muscle is easily felt. Injection of serotonin solutions into the gonad or foot required a longer hypodermic needle. Hard clams reacted to insertion of the needle into the foot by withdrawing it. For intragonadal injection the needle must pass through overlying tissues when inserting it through a hole drilled in the shell over the gonad or between the valves on the posterior side of the ligament. Care must be taken to insert the needle into the gonad and not other surrounding organs.

Serotonin may be used to induce rapid and synchronous spawning of the hard clam. This technique may be easily applied to individual and mass spawning techniques. Since there is no need to heat seawater to thermally shock clams to spawn, bacterial growth in the culture of eggs may be reduced. Serotonin may also be used to induce spawning in other bivalves, such as the ocean quahog *Arctica islandica* (Linné), which are resistant to traditional spawning stimuli (Gibbons et al. 1983). This technique offers another way to selectively breed broodstocks for improvement or genetic research.

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