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Ovarian maturation and spawning in the tiger shrimp, *Penaeus monodon* by serotonin and dopamine injection

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Abstract

The effect of serotonin (5-hydroxytryptamine, 5-HT) injection on the ovarian maturation and spawning of wild *Penaeus monodon* was investigated. The neurotransmitter was evaluated at 20 µg g-1 body weight (b.w) and 50 µg g-1 (b.w) applied at day 2, 12 and 22. The effect was compared against a control group, which received the injection of the sterile vehicle solution (NaCl 0.85%), and an unilaterally eyestalk ablated group. 5-HT induced ovarian maturation and spawning at both doses tested, generating more spawnings at 50 µg g-1 (b.w). However, unilateral eyestalk ablation induced a sooner and a higher rate of maturation and spawning. Dopamine reduce ovarian maturation and spawning in *P.monodon* compared to 5-HT.Our findings may be the result of 5-HT interaction with the release of different neurohormones and inhibition of methyl farnesoate synthesis. Additionally, gonad inhibiting hormone may have an intense control over ovaries and hepatopancreas. Spawning obtained by serotonin treatment showed excellent quality, and it was not statistically different (P>0.05) from unilaterally eyestalk ablated females. Further research is necessary in order to increase the spawning activity and define the duration of the reproductive performance of serotonin and dopamine treated females.

Key-Words: *Penaeus monodon*, Reproduction, Serotonin, Dopamine, Spawning

Introduction

Many studies have reported the opposing effects that 5-HT and DA have on crustacean reproduction. For example, the administration of 5-HT into male fresh water crayfish, *Procambarus clarkii*, induces testicular maturation and development of androgenic glands (1), while in females it stimulates ovarian maturation and ovulation (2). In contrast, DA can inhibit testicular maturation in the fiddler crab, *Uca pugilator* (3), and the crayfish, *P.clarkii* (4). In the freshwater prawn, *Macrobrachium rosenbergii*, treatment with 5-HT significantly increases the vitellogenin level in the hemolymph at ovarian stage IV. Whereas DA has an opposite effect (5). In addition, females injected with 5-HTexhibit significantly shorter periods of ovarian maturation, embryonic period and decrease of oocyte diameter (6). Worldwide commercial maturation of female penaeids relies almost exclusively on the technique of unilateral eyestalk ablation (7); the technique gives predictable peaks of maturation and spawning, but many associated problems have been reported, like deterioration in spawn quality and quantity over time (8, 9, 10), and conflicting results on spawn size, hatch success and other variables (7).

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Eyestalks are the endocrine center for regulating many physiological mechanisms, such as molting, metabolism, sugar balance, heart rate, pigments, and gonad maturation. DA is widely distributed in the crustacean nervous system and has a diverse array of physiological effects as reviewed by (11). DA has been reported to stimulate the release of both the pigment concentrating hormone (2, 12) and the distal retinal pigment dark-adapting hormone (13) in the fiddler crab, *Uca pugilator*; to effect the release of crustacean hyperglycemic hormone from the x-organ- sinus gland complex of *Orconectes linosus* and ovarian maturation in *U.pugilator* (14). Therefore, unilateral eyestalk ablation affects all aspects of shrimp physiology (15). Predictable induced reproduction in captive penaeids without the use of eyestalk ablation was considered a long term goal for shrimp mariculture (16). Various alternatives to ablation have been evaluated, based on accumulated knowledge about environmental control and crustacean endocrinology. Photoperiod and temperature manipulations, based on seasonal natural variations of these parameters, have been successful in controlling maturation of unablated *P. japonicus* (17), *P. stylirostris* (18), and *P. setiferus* (19); however, photoperiod control seems to be more important for subtropical species (for review: 2). Implantation of

thoracic ganglia from a mature female into an immature female stimulates vitellogenesis(20). This principle was tested on *P.monodon* by implanting thoracic ganglia from maturing *Homarus americanus* (21); the report indicates that the implantation technique generates maturation, but the experiment is based on a low number of replicates, without statistical analysis. Moreover, the current knowledge on tissue recognition between different crustacean species is limited, so there is no scientific evidence to support the assumption that tissue from a lobster would be recognized as self by a shrimp. Steroids are biologically active in crustaceans and they have been found in ovarian tissue of *P. monodon* at different stages of vitellogenesis (22). The authors proposed that ovarian development and oocyte maturation in crustaceans may be regulated by steroid hormones similar to teleost fish and amphibia. Complementary, it has been established that the mandibular organ of crustaceans synthesizes and secretes steroids and terpenoids, which may play a role in ovarian stimulation (13). This study found that 17 alpha-hydroxyprogesterone, methyl farnesoate and juvenile hormone III significantly increased *P. monodon* oocyte diameter, in vitro. Injection of progesterone was tested in *Metapenaeus ensis* (23); the study shows a possible induction of maturation without statistical confirmation, and quality of spawnings could not be evaluated. Complementary, 17 alpha-hydroxyprogesterone induced vitellogenin secretion in *P. japonicus* (24). Other roles played by 5-HT are migration of the proximal retinal pigment, pericardial organ neurohormone, stomatogastric ganglion neuromodulator or neurohormone, behavioral responses, osmoregulation, and mechanoreception. As an effort to develop alternatives for the commercial reproduction of penaeid shrimp, this study was conducted to evaluate the effect of two doses of 5-HT on *P. monodon* maturation and spawning quality, compared to unilateral eyestalk ablation. Recently, it has been reported that GnRH plays an important role in the development of ovarium in *P. monodon* (25), and serotonin induces ovarian maturation by increasing vitellogenin accumulation in *Fenneropenaeus indicus* (26).

Material and Methods[9-11]

Broodstock

Adult *P. monodon* with a mean weight of 56 g for females and 40 g for males, were obtained by Arambakam near Chennai, India. Selection was done based on size, stage of ovarian maturation, and healthy appearance.

Experimental conditions

The animals were kept in ten plastic tubes containing water at depth 10 lit, with adequate aeration, and 20% of water being changed daily. Commercial shrimp feed was provided at 3% body weight daily. They were acclimatized under the natural light-dark cycle for 2 weeks before the experiments. Molting and reproduction are major metabolic processes in decapod Crustacea (27); therefore, in penaeid shrimp ovarian maturation and spawning occur during the intermolt (7). The molt cycle duration of adult *Penaeus* is 6–8 days (28); at postmolt females are weak and at premolt maturation is delayed. During the adaptation period (2 weeks) to the experimental environment, molting stages of females were determined by uropod analysis, based on the technique described by (28). Intermolt females (2–4 days) Were randomly distributed in six plastic tubes with six treatments each and 6 females per treatment-tube, for a total number of 36 females per treatment. Each female was individually eyestalk tagged; tube density was adjusted to 6 animals/m^2 using non-treated animals, with 1:1 sex ratio. Treatment A consisted of unilateral eyestalk ablation by cutting the right eyestalk with red-heated scissors; animals in treatment B received three injections days (2, 12, and 20) of 5-HT (Sigma, St. Louis, MO, USA) at 20 µg g^{-1} body weight (b.w., 3). Treatment C received three injections (days 2, 12 and 20) of 5-HT at 50 µg g^{-1} b.w. Animals in treatment D and E received three injections days (2, 12, and 20) of DA (Sigma, St. Louis, MO, USA) at 20 µg g^{-1} and 50 µg g^{-1} body weight. Treatment F served as control receiving three injections of sterile vehicle solution (NaCl 0.85%), at the same experimental days. Volume of injection was 0.07 ml per 56 g; the study was undertaken for 46 days. Ovarian maturation was evaluated by external observation of ovarian size and color as described by (29, 22) with slight modifications:

Stage I. The ovary is transparent with no distinguishable outline.

Stage II. The ovary is visible as a thin opaque line along the dorsal central axis.

Stage III. The ovary is visible as a thick and yellow band.

Stage IV. The ovary is turgid, broad and dark orange. Mating and spawning are imminent.

Quality of spawns was evaluated in terms of number of eggs per spawn, percentage of fertilized eggs, hatching rate, and number of nauplii per spawn. Mature and mated females were selected and placed in individual spawning tubes; 10 h after spawning, the eggs were washed and concentrated in a 10-l container, three 1-ml samples were taken to asses total number of eggs,

additional samples were taken for a microscopic investigation of the proportion of normally embryonal eggs vs. non-em- bryonal eggs (30). Eggs were treated with formalin (37%) and iodine (1%) at 0.26 ml 1^{-1} and 0.20 ml 1^{-1}, respectively; then moved to hatching containers (60 l) at salinity of 30 ppt. Thirty-four hours after spawning, nauplii were collected and concentrated in 15 l containers; three samples of 1 ml were randomly taken to count number of nauplii. Hatching rate was estimated based on number of eggs and nauplii produced per spawn.

Statistical analysis: A multiple comparisons (Duncan's) test was conducted to compare significant differences among treatments using the SPSS software and differences were considered significant when $P<0.001$.

Results and Discussion

Mortality during experimental period was similar between treatments, no statistical differences were measured ($P>0.05$); however, eyestalk ablation gave the highest variance between tanks (Table 1). *P. monodon* was induced to mature by eyestack ablation as well as by 5-HT. Table 1 shows the rate at which females were induced to mature and spawn successfully under the protocols tested. All the eyestalk ablated females reached the spawning condition, whereas the injection of 20 µg g^{-1} and 50 µg g^{-1} b.w. of 5-HT induced spawning of 20% and 35% of the females, respectively. As it can be seen in Table 1, ablation allowed a great proportion of females to re-mature and spawn many times, and 5-HT only induced re-maturation and spawning in few animals (6%–7%) during the 46 days of experimentation. Administration of DA did not result in any significant changes in the ovarian maturation in intact prawn when compared with respective control prawn during the experimental duration. Table 2 presents a statistical comparison in spawning activity for weekly intervals; the analysis indicates that the spawning activity for ablated females increased to a maximum performance of 95% of females at week 4. The spawning activity for serotonin treated females was maximum at week 4 and 6 for both dosis. Complementary, in the control group only two spawns occurred, representing 6.7% of the group. Quality of spawnings is presented in Table 3; the variation of the number of eggs obtained were not statistically different between treatments for first spawnings nor between first, second, third, fourth and fifth spawnings for the ablated animals ($P>0.05$). Second spawnings for the other treatments were not statistically analyzed because of the low number of replicates. A similar trend was observed for the percentage of fertilized eggs, percentage of hatch and number of nauplii per spawn.

To our knowledge this is the first report that demonstrates maturation and spawning by 5-HT injection in a penaeid shrimp. Based on the findings by (2), 5-HT activated the release of GSH, producing gonad maturation and spawning in *P.monodon*. Ablated females started to mature 3 days after ablation and they kept high rates of maturation until day 41 (week 6); during week 7 no maturation was observed in ablated females. Similar patterns have been previously reported, for example, Chamberlain and obtained spawning activity during 45 days for ablated *P. stylirostris*, previous studies in decapods crustaceans, including *P. clarkii, U. pugilator, litopenaeus stylirostris, L. vannamei and P. monodon*, have shown that 5-HT is stimulatory while DA is inhibitory to gonadal development in both males and females (22, 31, 32, 33, 3, 4, 34,35). As well, in female *M. rosanbergii*, administration of 5-HT resulted in shortening the period of the ovarian development, as well as increased gonado- somatic index and oocyte diameters (6, 36). 5-HT also significantly increased hemolymph vitellogenin (vg) level, wheras This seems to indicate that the intense spawning activity induced by ablation generates a physiological unbalance, possibly involving nutrient depletion from the hepatopancreas (37). This disorder deactivates vitellogenesis, stopping the ovarian maturation process until the female re-establish an acceptable physiological balance. On the other hand, serotonin treatment gave spawning activity at week 7, which could indicate that more spawnings would be expected in the following weeks; however, research has to be implemented to define if serotonin can generate a longer reproductive performance than eyestalk ablation. Removal of one eyestalk induced a sooner and higher rate of maturation and spawning compared to 5-HT treatment. This observation may indicate that GIH control over ovaries and hepatopancreas (19) is very intense; however, other factors should be considered. Serotonin induces hyperglycemia in decapods by releasing the crustacean hyperglycemic hormone (CHH; 38); on the other hand, eyestalk ablation induces hypoglycemia (39). Since it has not been established any direct effect of CHH on decapod maturation (40, 2), the elevated glucose level measured in the hemolymph of *P.monodoni* after 5-HT injection may have a negative effect on maturation (41) since 5-HT could also play a role as a stress modulator (42). Additionally, the inhibitory effect of 5-HT on MF synthesis may have contributed to the lower rate of maturation and spawning in the 5-HT treatment. 5-HT

stimulation on the release of MIH (43) may have a positive effect on maturation because MIH inhibits the secretion of ecdysone by the Y-organ, delaying the molting event (44). However, ecdysteroids seem to play a role in ovulation and embryonic development in Crustacea (45), so during ovarian maturation the molting hormone (20-hydroxyecdysone) secretion is inhibited, but other ecdysteroids are being accumulated in developing oocytes (45). The data on spawning quality presented in this report, demonstrate that once the gonad maturation mechanism is activated by (46) 5-HT, the animal will generate eggs and nauplii at commercial numbers, similar to previous reports for eyestalk ablated *P. monodon*. Regardless of treatment and re-maturation, 56 g females generated an average of 200,000 eggs per spawn, with a 50% hatching rate; these values agree with the commercial production of 55,000 to 150,000 nauplii per spawn for ablated *P.monodon* the number of eggs per spawn reported by (47, 48) of 119,000 and 152,432, respectively. Based on our experimental data, a serotonin injection program seems to be a practical alternative to eyestalk ablation; however, further research is required to increase the spawning activity and evaluate the duration of the reproductive performance for serotonin treated females. The release of different neurohormones GSH, MIH, CHH, RPDH, and NHH. may be controlled by a difference in the threshold of 5-HT; therefore, it would be interested to screen the effect of different 5-HT concentrations on shrimp maturation. The doses used in this study seem to be pharmacological; 15 μg g^{-1}b.w. was based on (3) for *Pr. clarkii*. To our knowledge the physiological level of 5-HT in *P. monodon* hemolymph has not been reported; in the crab, *Libinia emarginata*, 10^{-8} M 5-HT inhibited MF synthesis by 20%–35%, 10^{-6} M 5-HT enhanced the contraction of foregut neuromuscular preparations of *Panulirus interruptus* and *Cancer magister*, a high dose of 5-HT (10^{-4} M) caused extreme leg flexion in *Carcinus maenas* (for review: 15).

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Table 1 Rate of females reaching spawning during the experimental period for *P. monodon* treated with different protocols b.w.: body weight

Variable	Treatment group					
		Serotonin		Dopamine		
	Ablation (mean ± s.e)	50 µg/g b.w. (mean ± s.e)	20 µg/g b.w. (mean ± s.e)	50µg/g b.w (mean±s.e)	20 µg/g b.w (mean±s.e)	Saline solution (NaCl 0.85%) (mean±s.e)
Females in first spawning (%)[a]	100a	38.6±2.8b	23.5±21.6b	7.2±41.2b	3.3±8.3a	6.1±10.5c
Females in second spawning (%)	80.3±4.8	9.4±7.3	10.5±4.3	0	0	0
Females in third spawning (%)	42.6±59.7	0	0	0	0	0
Females in fourth spawning (%)	25.8±22.8	0	0	0	0	0
Females in fifth spawning (%)	10.5±6.9	0	0	0	0	0
Mortality (%)[a]	46.4±31.5a	38.1±19.2a	35.3±8.3a	34. 7±8.3a	32.3±8.3a	33

[a]Means with different letters indicate statistically significant differences ($P<0.05$).

Table 2: Weekly spawning rate of *P.monodon* treated by different protocols Means with different letters within row indicate statistically significant different protocols ($P<0.05$) .b.w body weight

Treatment group	Females (%).			
	Week 2[a] (mean ± s.e).	Week 4[a] (mean ± s.e).	Week 6 (mean ± s.e).	Week 8[a] (mean ± s.e)
Ablation	65.7±6.4 a	98.3±22.8b	73.8±2.2 c	0 a
n	31	28	22	20
5-HT 50 µg/g b.w.	0a	16.8±5.0 bc	25.6±5.0d	0a
n	30	27	24	2
5-HT 20 µg/g b.w.	0 a	14.5±2.2c	22.6±16.7bc	5.7±6.4ab
n	25	25	24	24
DA 50 µg/g b.w.	0 a	10.3±3.5a	0a	0a
n	30	27	24	22
DA20 µg/g b.w.	0	9.4±5.7a	0 a	0a
n	30	27	24	22
Saline solution (NaCl 0.85%).	0 a	12.6.7±11.5a	0a	0a
n	29	26	24	24

[a]Weeks when injections were applied

Table 3: Reproductive performance variables of *P.monodon* females treated with different protocols Means for each variable where not statistically different (*P*>0.05). between treatments for first spawning, and between spawnings for ablated females. b.w.s body weight

Variable	Treatment group									
	Ablation		5-HT 50 µg/g b.w.		5-HT 20 µ g/g b.w.		DA 50 µg/g b.w		DA 50 µg/g b.w		Saline (NaCl 0.85%.)	
	n	Mean± s.e.	*n*	Mean± s.e.	*n*	Mean± s.e.	*n*	Mean± s.e.	*n*	Mean± s.e.	*n*	Mean± s.e.
Eggs/spawn												
First spawning	34	224652 ±46428	21	230103±55597	9	239037±77079	5	241346±120208	5	243643±582597	5	248464±35294
Second spawning	27	237031±21476	5	183451±4315	5	198639±23570	2	–		–		–
Third spawning	16	193026±81340		–		–		–		–		–
Fourth spawning	11	239063±21533		–		–		–		–		–
Fifth spawning	5	172664±7042		–		–		–		–		–
Fertilized eggs (%)												
First spawning		76.5±74.1		73.8±57.73		79.6±82.6		52.9±17.3		54.5±49.4		56.4±17.3
Second spawning		63.9±76.1		89.1±24.6		74.6±38.1		–		–		–
Third spawning		72.3±60.4		–		–		–		–		–
Fourth spawning		55.7±24.9		–		–		–		–		–
Fifth spawning		54.3± 63.4		–		–		–		–		–
Hatching rate (%)												
First spawning		64.1± 32.7		73.2± 98.2		72.1± 3.7		63.7± 9.4		71.4± 9.4		74.3± 9.6
Second spawning		56.4± 35.6		73.8± 39.2		–		–		–		–
Third spawning		69.5± 42.6		–		–		–		–		–
Fourth spawning		48.3± 43.8		–		–		–		–		–
Fifth spawning		68.9± 52.6		–		–		–		–		–
Naupliirspawn												
First spawning		120479± 63018		129345± 34695		130612± 48552		143631± 45329		145633± 56349		156313± 35629
Second spawning		120632± 32529		119365± 25762		60301± 34272		–		–		–
Third spawning		139393± 25491		–		–		–		–		–
Fourth spawning		10351± 28242		–		–		–		–		–
Fifth spawning		113035± 26365		–		–		–		–		


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