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Gonadal Maturation and Spawning in *Penaeus semisulcatus* de Hann, 1844 by Hormone Injection

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Abstract: The effects of 3 hormones, HCG, LH-RH and serotonin (5-HT), on maturation and spawning of *Penaeus semisulcatus* de Hann, 1844 were investigated in 2 experiments in this study. The experiments were carried out in round (4 m diameter) fiberglass tanks for 50 days. In experiment 1, the effects of 3 dosages (1, 2 and 3 IU g^{-1} body weight (BW)) of HCG were compared against a sterile vehicle solution (0.85% NaCl) injected group (control group) and an eyestalk ablated group. In experiment 2, 3 dosages of both LH-RH (0.01, 0.1 and 0.2 mg g^{-1} BW) and serotonin (20, 50 and 100 µg g^{-1} BW) were tested against the control (0.85% NaCl) and eyestalk ablated groups.

In experiment 1, the molting interval did not vary significantly among the treatments ($P > 0.05$). The highest number of spawns was obtained from the ablated group and this was followed by the groups injected with 3 IU g^{-1} (2 spawns), 2 IU g^{-1} and 1 IU g^{-1} of HCG (1 spawn) respectively. The control group (injected with vehicle solution) did not spawn during the course of the experiment.

In experiment 2, no significant difference was established in the molting cycle among the groups ($P > 0.05$). A total of 28 spawns were obtained from the eyestalk ablated group during the experiment and repeated spawns occurred within the same molting cycle. Four females injected with 50-100 µg g^{-1} of serotonin matured and spawned. Again, 3 females from the group injected with 20 µg g^{-1} of serotonin matured yielding a total of 5 spawns. Three females spawned in the groups that received 0.01 mg g^{-1} and 0.1 mg g^{-1} of LH-RH. Only one female injected with 0.2 mg g^{-1} of LH-RH matured and spawned during the study. It appears that maturation and spawning of *P. semisulcatus* can be successfully induced by injection of these hormones in captivity, though eyestalk ablation remains the most reliable technique.

Key Words: *Penaeus semisulcatus*, maturation, spawning, hormone injection, eggs

Penaeus semisulcatus de Hann, 1844'ta Hormon Enjeksiyonu ile Gonad Olgunlaştırma ve Yumurtlama

Özet: Bu çalışmada üç farklı hormon, HCG, LH-RH ve Serotonin (5-HT)'in *Penaeus semisulcatus* de Hann, 1844'un gonad gelişimi ve yumurtlaması üzerine etkileri iki denemede araştırılmıştır. Denemeler yuvarlak (4 m çapında) fiberglas tanklarda 50 günlük sürede gerçekleştirilmiştir. Birinci denemede 3 farklı dozda HCG enjekte edilen [1, 2 ve 3 IU g^{-1} Vücut Ağırlığı (VA)] karideslerin üreme performansı serum fizyolojik solusyon (%0,85 NaCl) enjekte edilen grup ve göz sapı kesilen grup ile karşılaştırılmıştır. Ikinci denemede 3 farklı LH-RH (0,01, 0,1 ve 0,2 mg g^{-1} VA) ve Serotonin (20, 50 ve 100 µg g^{-1} VA) enjeksiyonu yapılan karideslerin üreme performansı serum fizyolojik solusyon enjekte edilen grup ve gözsapı kesimi yapılan grup ile karşılaştırılmıştır.

Birinci denemede kabuk değiştirme döngüsü muameleler arasında istatistiki olarak farklılık göstermemiştir ($P > 0,05$). En fazla yumurtlama gözsapı kesilen gruptan elde edilmiş ve bunu sırasıyla 3 IU g^{-1} (iki yumurtlama), 2 IU ve 1 IU g^{-1} HCG enjekte edilen grup takip etmiştir. Kontrol grubu karidesleri deneme süresince yumurtlamamışlardır.

Ikinci denemede, kabuk değiştirme döngüsü gruplar arasında farklılık göstermemiştir ($P > 0.05$). Deneme süresince gözsapı kesilen karideslerden 28 yumurtlama elde edilmiş ve yumurtlamalar aynı kabuk değiştirme döngüsü içerisinde tekerrür etmiştir. Yüksek dozda (50 ve 100 µg g^{-1}) serotonin enjekte edilen karideslerden dört dişi, düşük dozda (20 µg g^{-1}) üç dişi olgunlaşıp, toplam 5 kez yumurtlamıştır. Düşük dozda (0,01 ve 0,1 mg g^{-1}) LH-RH enjekte edilen karideslerden üç dişi, Yüksek dozda (0,2 mg g^{-1}) ise yalnızca bir dişi yumurtlamıştır. Bu hormonların kullanımıyla yetiştiricilik koşullarında *P. semisulcatus*'un olgunlaştırılıp yumurtlamaya teşvik edilebildiği görülmektedir. Bununla beraber, gözsapı kesimi halen bu türün olgunlaştırılması ve yumurtlatılması için en güvenilir tekniktir.

Anahtar Sözcükler: *Penaeus semisulcatus*, olgunlaşma, yumurtlama, hormon enjeksiyonu, yumurta

Introduction

The induction of ovarian maturation and spawning of female penaeid shrimps is mainly carried out using the unilateral eyestalk ablation technique (Primavera, 1985; Browdy, 1992; Ogle, 1992). Although this technique is used commonly worldwide in hatcheries, many problems, such as deteriorations in spawn, spawner and larval quality and quantity over time have been associated with it (Emmerson, 1980; Chamberlain, 1985; Primavera, 1985; Makinouchi and Primavera, 1987; Tsukimura and Kamemoto, 1991; Aktaş and Kumlu, 1999). Alternative techniques to the eyestalk ablation method, such as temperature and/or photoperiod manipulations, hormone injections and lobster ganglion implantations have been investigated in different shrimp species. Cripe (1994), with *Penaeus duorarum* (Burkenroad, 1939), and Aktaş et al. (2003), with *Penaeus semisulcatus* de Hann, 1844 studied the effects of temperature fluctuation on induced maturation and spawning with a high degree of success.

The effects of hormones on the maturation and spawning of penaeid shrimps have so far received little attention. The effect of progesterone injection was investigated on *Metapenaeus ensis* (de Hann, 1844) and the results showed that it is possible to induce maturation and spawning without statistical confirmation (Yano, 1985). Injections of 17 - alpha hydroxyprogesterone increased vitellogenin secretion in *Penaeus japonicus* Bate, 1888 (Yano et al., 1988), and also sperm quality in *Penaeus vannamei* Boone, 1931 (Alfaro, 1996). More recently, studies by Sarojoni et al. (1995) with *Procambarus clarkii* (Girard, 1852) and by Vaca and Alfaro (2000) with *P. vannamei* have shown that serotonin appears to induce maturation and spawning. HCG has been reported by Yano and Wyban (1993) to successfully induce maturation and spawning in shrimp and prawn. The effects of HCG on growth and body composition on *Penaeus indicus* H. Milne – Edwards, 1837 were tested by Jayaprakas and Sambhu (1998), and it has been established that HCG stimulates gonadal development. The studies with LH-RH (luteinizing hormone releasing hormone) on maturation and spawning have mainly focused on reproduction of teleosts. Gonadotropins such as FSH and LH have been investigated in sand shrimp, *Crangon crangon* (Linnaeus, 1758), and it was found that somatic cell numbers increased with both hormones (Bomirski and Klek-Kawinska, 1976; Zkowska, 1981). However, further studies are required to better understand the role of these hormones on the maturation of various shrimp species before any commercial application in shrimp farming sector is introduced.

The main objective of this study was to compare the effects of various hormones on gonadal maturation and spawning of *P. semisulcatus* by injection at various dosages.

Materials and Methods

Broodstock

Adult *P. semisulcatus* were collected from Yumurtalık Bight in the northeastern Mediterranean in May, 2001, by trawling and gill netting. Shrimps were transported in oxygenated seawater to the Yumurtalık Marine Research Station of the Faculty of Fisheries of Çukurova University and were selected for their length and healthy appearance. The animals were acclimated to the experimental conditions for 2 weeks prior to the experiments.

Experimental conditions

Experiments were conducted in round black fiberglass maturation tanks (4 m in diameter, 65 cm water depth, with a central outlet). All the tanks were covered with a green tent to reduce illumination and external disturbance. Water exchange rate was adjusted to 100% daily.

Shrimps were weighed to the nearest 0.01 g and measured for their total length (TL) from the tip of the rostrum to the end of telson, and was also measured carapace length from the post-orbital margin to the posterior end of the mid-dorsal line of the carapace using vernier callipers. Unilateral eyestalk ablation was done by first tying the eyestalk and then cutting it off with scissors. Injections were performed through the lateral second abdominal somite with a 1ml micro syringe. Each female was tagged individually by gluing different colored plastic labels onto the carapace and placing different colored plastic rings around the eyestalk to determine molting, maturation and spawning individually.

The females were checked externally by inspecting the size of the developing ovaries through the dorsal exoskeleton on a daily basis as described by King (1948). Females with stage III and IV ovaries were removed and placed individually into a 100 l spawning tank previously

filled with filtered (down to 1 µm) and UV irradiated seawater. Total numbers of eggs and fertility were estimated by counting 5 random samples in 100 ml beakers. Eggs were left in the spawning tank for 24 h to determine hatching rate. After hatching, the nauplii were concentrated in 10 l containers and 5 random samples of 2 ml were taken to determine the numbers of nauplii.

Shrimps were fed twice daily with squid (*Loligo vulgaris* Lamark, 1798), crabs (*Callinectes sapidus* Rathbun, 1896) and various fish species to excess in the morning and evening. Molts, dead shrimps and excess feed were removed every morning. Experiments were undertaken for 50 days.

Ranges of physicochemical parameters such as temperature, pH, salinity (YSI Model 30 salinometer, Yellow Springs Instruments USA) and dissolved oxygen (WTW Measurement Systems, Germany) were checked daily throughout the experiments. All experimental treatments and conditions are summarized in Table 1.

Experiment 1: HCG injection

Forty females, divided into 4 groups (HCG at 3 dosages, control and eyestalk ablated) and 20 males were stocked into one 10 m^2 round bottomed tank (Tank A). The female shrimps received 2 injections (days 1 and 21) of HCG (Pregnyl, 1500 and 5000 IU commercial packet) at 1, 2 and 3 IU g^{-1} BW or sterile vehicle solution (0.85% NaCl) (control group) (Table 1).

Experiment 2: Serotonin (5-HT) and LH-RH injection

Three serotonin (5-hydroxytryptamine, 5-HT, and creatinine sulfate complex, Sigma, St. Louis, MO, USA) doses (20, 50 and 100 µg g^{-1} BW) and 3 LH-RH (luteineizing hormone releasing hormone, des-Gly^{10} {D-Ala^6}) doses (0.01, 0.1 and 0.2 mg g^{-1} BW) were tested. Thirty serotonin injected females and 15 males were stocked into tank B, 30 LH-RH injected females and 15 males were stocked into tank C, and 10 ablated or 10 sterile vehicle solution (NaCl, 0.85%) injected females and 10 males were stocked into tank D (Table 1).

Table 1. Experimental conditions and various treatments applied to *P. semisulcatus*

Exp. No.	Tank	Treatment	No. of shrimps	No. of injections	Injection intervals (days)	Weight (g)	Total length (mm)
1	A	1 IU g^{-1} BW, HCG	8	2	10	45.69 ± 4.56	164.75 ± 6.71
		2 IU g^{-1} BW, HCG	8	2	10	42.49 ± 10.59	163.00 ± 13.14
		3 IU g^{-1} BW, HCG	8	2	10	47.71 ± 10.52	169.62 ± 11.92
		0.85% saline solution (control)	8	2	10	44.66 ± 8.07	164.50 ± 9.71
		Eyestalk ablation	8	-	-	45.64 ± 13.90	167.50 ± 14.78
		Males	20	-	-	22.50 ± 2.48	134.80 ± 7.80
2	B	20 µg g^{-1} BW, serotonin	10	3	10	38.76 ± 9.80	158.00 ± 11.58
		50 µg g^{-1} BW, serotonin	10	3	10	38.70 ± 7.86	158.20 ± 9.35
		100 µg g^{-1} BW, serotonin	10	3	10	38.93 ± 7.37	158.10 ± 11.76
		Males	15	-	-	25.33 ± 2.54	127.85 ± 5.67
2	C	0.01 mg g^{-1} BW, LH-RH	10	3	10	37.34 ± 4.98	160.90 ± 8.82
		0.1 mg g^{-1} BW, LH-RH	10	3	10	36.77 ± 8.53	155.40 ± 9.84
		0.2 mg g^{-1} BW, LH-RH	10	3	10	41.90 ± 7.64	163.70 ± 8.60
		Males	15	-	-	25.91 ± 2.75	128.60 ± 5.46
2	D	0.85% saline solution (control)	10	3	10	39.17 ± 5.03	160.20 ± 5.22
		Eyestalk ablation	10	-	-	36.20 ± 3.69	153.50 ± 7.18
		Males	10	-	-	38.60 ± 4.90	139.10 ± 4.53

Statistical analysis

Experimental data were analyzed using one-way ANOVA and any significant difference was determined at a 0.05 probability level using Scheffe's test after the normality and homogeneity (Bartlett's test) of the data were checked using Minitab statistical software. Statistical analyses were not performed for those data that could not be homogenized in any way.

Results

Throughout the experiments, salinity and temperature were recorded as 39-40 ppt and 28-29 °C, respectively. Dissolved oxygen varied between 5.0 and 5.5 mg l^{-1} and pH was 8.10-8.15 during the experiments.

The initial weights and total lengths of the females were not significantly different among the groups for each experiment (Table 1) ($P > 0.05$). A few females died after the first injections, and these were immediately replaced with females of a similar size.

Experiment 1: HCG injection

In this experiment, molting interval was 16.25 days for the eyestalk ablated, 18.42 days for the control, at 17.00, 19.00 and 16.00 days for the groups injected with 1 IU g^{-1}, 2 IU g^{-1} and 3 IU g^{-1} of HCG, respectively (Table 2). The molting interval did not vary significantly among the treatments ($P > 0.05$).

The highest number of spawnings was obtained from the ablated group and this was followed by the groups injected with 3 IU g^{-1} (2 spawning), 2 IU g^{-1} and 1 IU g^{-1} of HCG (1 spawning), in that order. The control group (injected with vehicle solution) did not spawn during the course of the experiment (Table 2). Statistical comparisons were not performed for the number of eggs, fertility rate, hatching rate or the number of nauplii produced due to the few spawns obtained from these groups. The percentage of fertilized eggs and hatching rate displayed the same trend among the groups (Table 2).

Experiment 2: Serotonin (5-HT) and LH-RH injection

The molting intervals were 20.79 days for the eyestalk ablated, 22.13 days for the control, and 20.00, 18.88, 19.20 days for the groups injected with 20 μg g^{-1}, 50 μg g^{-1} and 100 μg g^{-1} of serotonin, respectively (Table 3). Although the molting cycle decreased in the eyestalk ablated and serotonin injected groups compared with the control group, no significant difference was established among these groups ($P > 0.05$). The molting intervals for those groups injected with LH-RH were 22.33, 22.60 and 22.63 days from the lowest dose to the highest, and were the same among the groups ($P > 0.05$).

All the eyestalk ablated females matured and spawned at least once and at most 5 times during the course of the experiment. A total of 28 spawns obtained from 10 females during the experiment and repeated spawnings occurred within the same molting cycle (Figure 1). Four females injected with 50 μg g^{-1} of serotonin developed gonads and spawned. Again, 4 females from the group injected with 100 μg g^{-1} of serotonin matured yielding a total of 6 spawnings. Both the control group and those injected with 20 μg g^{-1} of serotonin produced 4 spawnings each. Three females spawned in the groups that received 0.01 mg g^{-1} and 0.1 mg g^{-1} of LH-RH. Only 1 female injected with 0.2 mg g^{-1} of LH-RH matured and spawned during the study (Figure). Some females from the hormone injected groups and eyestalk ablated group re-matured and spawned more than once within the duration of the experiment (Figure).

Table 2. Data regarding molting, spawning, fertilization, hatching rate and produced nauplii of *P. semisulcatus* in the HCG experiment.

Groups	Total length (mm)	Total weight (g)	Molting cycle (day)	No. of spawnings	No. of eggs	Fertility (%)	Hatching rate (%)	Nauplii produced
Control	164.50 ± 9.71	44.66 ± 8.07	18.42 ± 1.72	-	-	-	-	-
1 IU g^{-1} of HCG	164.75 ± 6.71	45.69 ± 4.56	17.00 ± 1.92	1	28,390	94.44	57.55	15,428
2 IU g^{-1} of HCG	163.00 ± 13.14	42.49 ± 10.59	19.00 ± 2.00	1	47,300	87.1	61.4	25,295
3 IU g^{-1} of HCG	169.62 ± 11.92	47.71 ± 10.52	16.00 ± 0.82	2	112,800	84.12 ± 8.65	55.99 ± 1.99	53,833
Eyestalk ablated	167.50 ± 14.78	45.64 ± 13.90	16.25 ± 1.39	5	197,498	73.65 ± 13.00	51.08 ± 23.83	70,685

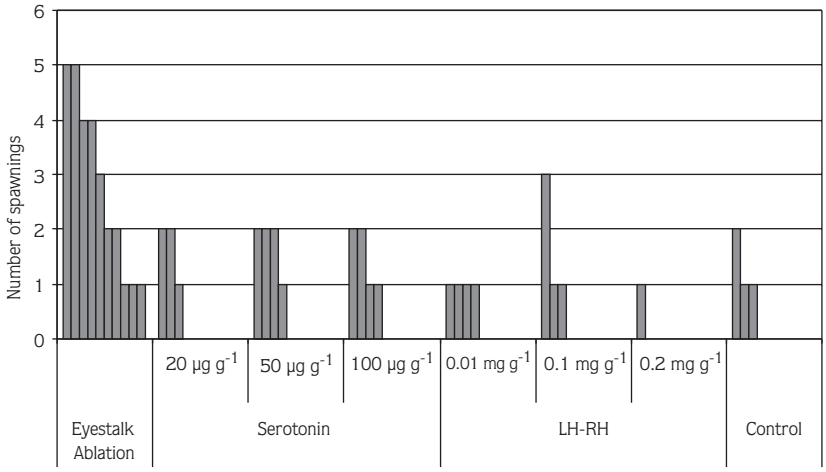

Figure. Number of spawnings of *P. semisulcatus* treated with different protocols.

The quality and quantity of spawnings were not compared statistically due to the low number of spawns obtained in some treatments. Eyestalk ablation was the most effective method in inducing spawning in *P. semisulcatus*. Nauplii production was 730,250 nauplii for the eyestalk ablated group, 68,475-336,000 nauplii for the serotonin injected groups, 64,500-310,950 nauplii for the LH-RH injected groups and 66,600 nauplii for the control group (Table 3).

Discussion

This study indicated that it is possible to induce maturation and spawning of *P. semisulcatus* by HCG injection (without statistical confirmation) in captivity. It was reported that HCG positively affected vitellogene synthesis in *Idotea balthica* (Pallas, 1772) and the sand shrimp, *C. crangon* (Laufer and Landau, 1991). Similarly, HCG was reported by Yano and Wyban (1993) to successfully induce maturation and spawning in shrimp and prawn. Zukowska (1981) found a stimulating effect of hypophysis gonadotropins (FSH and LH) on ovarian development in *C. crangon*. Jayaprakas and Sambhu (1998) showed similar patterns in ovarian development for *P. indicus* given food containing HCG. In addition, there are some unpublished data showing that *Penaeus chinensis* (Osbeck, 1765) can be maturated and spawned in captivity by HCG injection (Liao and Chien, 1990). The results of the above investigations and those in the current study reveal that the mammalian hormone (HCG) influences gonadal maturation and spawning in shrimps, including the penaeids. During our experiment, we did not obtain enough spawnings to clearly prove such effects, and further studies should be carried out with a different range of dosages of HCG on penaeid shrimps.

Table 3. Data about molting, spawning, fertilization, hatching rate and nauplii produced of *P. semisulcatus* in the serotonin and LH-RH experiment.

Groups	Total length (mm)	Total weight (g)	Molt cycle (day)	No. of spawnings	Number of eggs	Fertility (%)	Hatching rate (%)	Nauplii produced
Control	160.20 ± 5.22	39.17 ± 5.03	22.13 ± 2.23	4	252,150	73.39 ± 7.41	45.26 ± 9.20	66,600
20 µg g^{-1} of serotonin	158.00 ± 11.58	38.76 ± 9.80	20.00 ± 2.06	4	368,500	89.64 ± 10.21	56.95 ± 9.76	205,425
50 µg g^{-1} of serotonin	158.20 ± 9.35	38.70 ± 7.86	20.79 ± 2.59	7	550,049	85.77 ± 14.14	69.02 ± 6.07	336,000
100 µg g^{-1} of serotonin	158.10 ± 11.76	38.93 ± 7.86	19.20 ± 1.30	6	559,218	78.22 ± 22.47	58.10 ± 28.90	239,400
0.01 mg g^{-1} of LH-RH	160.90 ± 8.82	37.34 ± 4.98	22.33 ± 2.69	3	225,818	92.18 ± 8.63	79.71 ± 18.15	167,614
0.1 mg g^{-1} of LH-RH	155.40 ± 9.84	36.77 ± 8.53	22.60 ± 3.47	5	487,200	90.47 ± 6.77	73.68 ± 14.52	310,950
0.2 mg g^{-1} of LH-RH	163.70 ± 8.60	41.90 ± 7.64	22.63 ± 2.39	1	69,000	100.00 ± 0.00	93.47 ± 0.00	64,500
Eyestalk ablated	153.50 ± 7.18	36.21 ± 3.69	20.79 ± 3.20	28	1,733,829	78.18 ± 15.44	59.37 ± 22.71	730,250

The second experiment demonstrated that *P. semisulcatus* can mature and reproduce in captivity with serotonin injections. Similar findings have been reported for crayfish (*P. clarkii*) by Sarojoni et al. (1995), *Penaeus monodon* J. C. Fabricius, 1798 by Fingerman (1997) and *P. vannamei* by Vaca and Alfaro (2000). As a result, it appears that maturation and spawning of penaeid shrimps including *P. semisulcatus* can be successfully induced by serotonin injection in captivity. However, eyestalk ablation gives the highest and more predictable maturation and spawning in penaeid shrimps, as also demonstrated in the current study. The time between the commencement of the experiment and spawning was shortest for ablated females compared with those given hormone injection. This is assumed to be due to faster decreasing levels of GIH (gonad inhibiting hormone) in the hemolymph of the females following eyestalk ablation (Browdy and Samocha, 1985; Primavera, 1985; Dall et al., 1990). Serotonin injection resulted in the second best reproductive performance among the treatments in the present study. The quality and quantity of eggs for each spawning taken from ablated and serotonin injected females were fairly similar. Vaca and Alfaro (2000) who studied *P. vannamei*, reported similar results. The injection level of 50 µg g^{-1} BW gave the best results in the current study, confirming the results of Vaca and Alfaro (2000) for *P. vannamei*. It thus may be concluded that serotonin injection can be successfully applied to obtain offspring from *P. semisulcatus* broodstock. LH-RH did not have a clear influence on gonadal development and spawning in *P. semisulcatus*, at least not at the dose range tested in our study. However, fertility and hatching rates of the eggs taken from the females injected with LH-RH were observed to be better than those of the eyestalk ablated and control groups. Further research is needed to fully understand the effects of LH-RH on fertility and hatching rates of penaeid shrimps.

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