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Gonadal Maturation and Spawning of *Penaeus semisulcatus* (Penaeidae: Decapoda)

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Abstract: The effects of eyestalk ablation on maturation, moulting and spawning in *Penaeus semisulcatus* were investigated in this study. Twelve males and 12 eyestalk–ablated and 12 non–ablated, all individually marked females were stocked into a 12–ton maturation tank.

All the ablated females moulted in 9.08±1.68 days and first spawning occurred 16.75±3.41 days following eyestalk ablation. While all the ablated females spawned, producing an average of 177.000 ±133.340 eggs, only three non–ablated females spawned, producing average of 23.216±16.957 eggs during a period of 45 days ($P<0.05$). Throughout the experiment, the average number of spawnings per female was 3.17±1.40 for ablated and 1 for non–ablated ones. Multiple spawning (up to four times) occurred within the same moulting cycle in ablated females. Irrespective of the ablation treatment, the average fertility rate of the eggs was high, ranging between 84 and 87% ($P>0.05$). Average nauplii production per female was 81.574±55.126 for ablated and 13.397±11.535 for non–ablated ones ($P<0.05$).

This study demonstrates that eyestalk ablation is necessary to induce maturation and spawning of *P. semisulcatus* in captivity. By eyestalk ablation and management of environmental conditions, it should be possible to obtain seeds for shrimp farms to head–start on–growing in the early spring under the subtropical conditions of Turkiye.

Key Words: *Penaeus semisulcatus,* maturation, spawning, moulting, broodstock.

Penaus semisulcatus'ta (Penaeidae: Decapoda) Gonad Olgunlaştırma ve Yumurtlama

Özet: Bu çalışmada, gözsapı kesiminin *Penaeus semisulcatus*'un gonad olgunlaştırma, kabuk değiştirme ve yumurtlaması üzerine etkisi araştırılmıştır. Tümü bireysel olarak markalanan 12 adet gözsapı kesilen ve 12 adet gözsapı kesilmeyen dişi ile 12 adet erkek 12 tonluk olgunlaştırma tankına stoklanmıştır.

Gözsapı kesilen karideslerin hepsi 9.08±1.68 gün içinde kabuk değiştirmiş ve ilk yumurtlamalar gözsapı kesiminden 16.75±3.41 gün sonra gerçekleşmiştir. 45 gün içinde, gözsapı kesilen karideslerin hepsi yumurtlayarak dişi başına ortalama 177.000±133.340 yumurta üretmiş, ancak bu süre içinde gözsapı kesilmeyenlerin sadece üç tanesi yumurtlamış ve bunlar ortalama dişi başına 23.216±16.957 yumurta üretmişlerdir ($P<0.05$). Deneme boyunca, gözsapı kesilen dişiler ortalama 3.17±1.40 kez, kontrol grubu ise 1 kez yumurtlamıştır. Gözsapı kesilen dişiler, aynı kabuk değiştirme döngüsü içinde çok sayıda (4 defaya kadar) yumurtlama yapmışlardır. Hem gözsapı kesilenlerde ve hem de kontrol dişilerinde, yumurtalarda ortalama % 84–87 gibi yüksek bir döllülük oranı elde edilmiştir ($P>0.05$). Dişi başına ortalama naupli üretimi gözsapı kesilenlerde 81.574±55.126 iken, gözsapı kesilmeyenlerde 13.397±11.535 olarak gerçekleşmiştir ($P<0.05$).

Bu çalışma, tutsaklıkta *P. semisulcatus*'un gonadlarının olgunlaştırılması ve yumurtlatılması için gözsapı kesiminin gerekli olduğunu göstermiştir. Çevresel koşulların uygun tutulması ve gözsapı kesimiyle, Türkiye'deki yarı–tropik koşullar altında, ilkbahar başlarında karides çiftliklerindeki semirtme havuzlarına stoklanacak yavruların üretimi mümkün olabilecektir.

Anahtar Sözcükler: *Penaeus semisulcatus,* olgunlaştırma, yumurtlama, kabuk değiştirme, anaçlar.

Introduction

In tropical countries, a supply of mature broodstock may be possible throughout the year, but gravid females can only be obtained during certain seasons in subtropical Mediterranean countries. Hence, it is a prerequisite to induce the maturation and spawning of shrimps to supply enough seeds whenever needed for the shrimp industry.

The induction of maturation and spawning in female penaeids through unilateral eyestalk ablation of various species has been well documented (1, 2, 3, 4). Excellent reviews on the reproduction of penaeid shrimps in captivity have been published by Primavera (5), Bray and Lawrence (6) and Browdy (7). Considerable success has been achieved in the maturation and spawning of the

shrimp *Penaeus semisulcatus* in Israel (8, 9). This penaeid shrimp is one of the most promising species for aquaculture on the North–eastern coast of the Turkish Mediterranean. As the availability of gravid females in this area is seasonal (10), it is of importance to control their reproduction for a regular supply of seeds.

Hence, the present investigation was conducted to assess the effects of eyestalk ablation on the moulting, maturation and spawning of *P. semisulcatus* before large scale production can begin.

Materials and Methods

This study was conducted in a 12–ton round fibreglass maturation tank (4 m in diameter) at the Yumurtalık Marine Research Station at the Faculty of Fisheries, Adana, Turkiye. The shrimps were caught off the Yumurtalık Bight of the North–eastern Mediterranean by trawling. The animals were acclimated for two weeks in another 12–ton round fibreglass tank prior to starting the experiment.

All individuals were weighed to the nearest 0.01 g and vernier calipers were used to measure total length (TL) from the tip of the rostrum to the end of the telson, and carapace length (CL) from the post orbital margin to the posterior end of the mid–dorsal line of the carapace. Twelve male and 24 females were stocked into the maturation tank. The degree of sexual maturity of females was determined by inspection of the ovarium in live animals. None of the females stocked into the maturation tank had gonadal development at the beginning of the experiment. 50% of the females were unilaterally ablated by tying the eyestalk first and then cutting it off with scissors, while the rest comprised the control group (non–ablated).

The maturation tank was painted black and filled with 10 m^3 of water, attaining a depth of 70 cm. The tank was covered with a green tent to decrease illumination and external distrubance. The water exchange rate was 200% per day. Throughout the experiment, which lasted 45 days, salinity and pH were 38–39 ppt and 8.10–8.15, respectively. Dissolved oxygen ranged between 5.0 and 5.5 ppm, and the temperature was 28–29°C.

The animals were fed twice daily on small frozen fish of various species and squid in the morning, and frozen crabs *(Callinectes sapidus)* in the late afternoon. The moulting, maturation and spawning of each individual female were monitored daily. For this purpose, the females were marked by gluing different–coloured plastic labels onto the carapace and making coded cuts in one or more uropods (see Fig. 1). The degree of maturation was examined externally by inspecting the size of the developing ovaries through the dorsal exoskeleton on a daily basis. Each female with ripe ovaries was removed into a 100–L spawning tank previously filled with filtered (down to 1 µm) and UV–irradiated sea water. Upon spawning, the female was returned to the maturation tank and three 100–mL aliquot samples were taken for microscopic determination of the number of fertile and infertile eggs. Eggs were left in the spawning tank for 36 h to determine the hatching rate. Some of the nauplii were also allowed to develop to the protozoeal stage for a further 48 h.

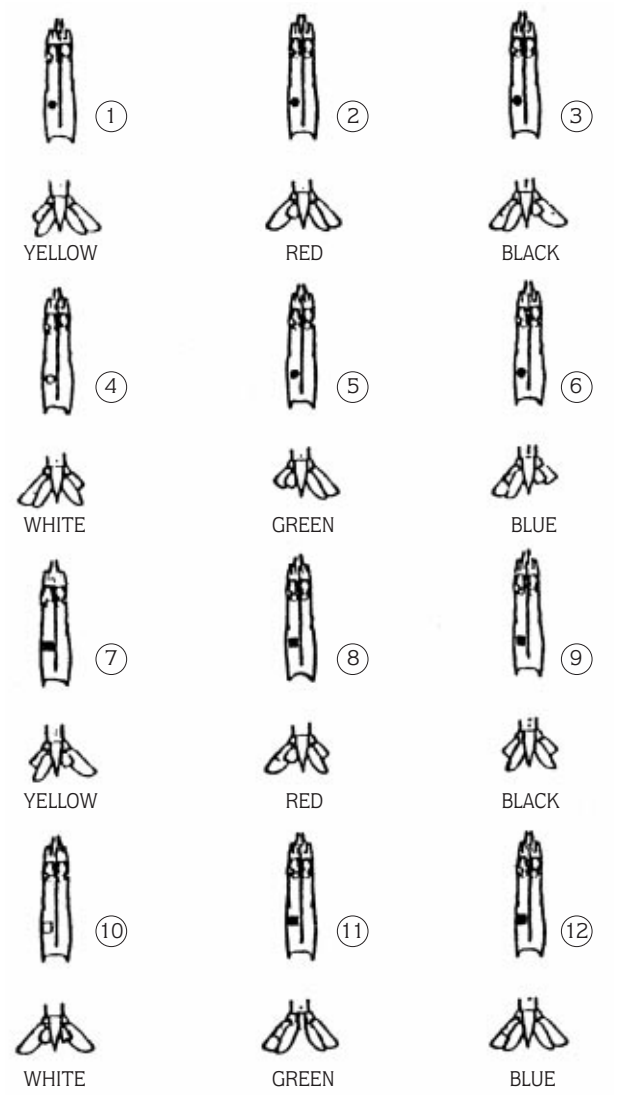

Figure 1. Marking of the carapace and coding of the uropods of *P. semisulcatus* females. Plastic labels for ablated and non–ablated females were glued on the left and right sides of the carapace, respectively.

Statistical analyses were carried out by one–way ANOVA in Minitab after the data were homogenised by logarithmic transformation.

Results

The initial weight, total length and carapace length of each individual female shrimp are given in Tables 1 and 2. Although the average weight of ablated females (46.25 g) was about 6 g higher than that (39.97 g) of non–ablated females, the difference was not significant (Table 3) (P>0.05). At the end of the experiment, the condition of ablated females was poorer than that of non–ablated females. A loss of equilibrium, black spots and missing appendages were observed in the ablated females.

From the onset of the experiment, irrespective of ablation treatment, all females moulted in 9.08±1.68 days when mating occurred. Following ablation, it was possible to achieve the first spawnings within 16.75±3.41 days. As shown in Tables 1 and 2, only three spawnings were detected in the non–ablated females in comparison to 38 spawnings for ablated females (Table 3) (P<0.05). Over the 45–day period, the ablated females spawned more than three times (average of 3.17±1.40), producing an average of 177.000±133.340 eggs per female, while only three non–ablated females spawned once each producing only an average of 23.216±16.957 eggs. The highest number of eggs (169.560 eggs) was produced by an ablated female (No: 7) (Table 2). Although the 2nd spawning date could not be determined during the experiment, many spawnings within one moulting period can still be deduced from Table 2. For example, the 1st shrimp in Table 2 appears to have spawned twice in the first moulting cycle and four times in the second moulting cycle. One mating was sufficient to fertilise the eggs up to four spawnings without significantly lowering the percentage of fertility (see Table 2).

The average percentage of egg fertility was high (83–87%) and did not differ significantly between ablated and non–ablated females (P>0.05). The average hatching percentages also did not differ significantly from each other (Table 3) (P>0.05). The average number of nauplii produced per ablated female (81.574±55.126) was significantly higher than that (13.397±11.535) of intact females. The moulting cycle of non–ablated females was 16.57±2.87 days (Table 3). 62 and 83% of the nauplii of the two ablated females (No 3 and 6) developed to protozoea 1 stage.

Discussion

This study demonstrates that *P. semisulcatus* mature and reproduce in captivity with or without unilateral eyestalk ablation. However, eyestalk ablation increases the number of spawnings and correspondingly the number of eggs and nauplii produced per female in comparison to non–ablated females. This is assumed to be due to lowering the level of GIH (gonad inhibiting hormone) and MIH (moult inhibiting hormone) in the hemolymph of the eyestalk ablated females (11).

Table 1. Moulting, spawning, number of eggs, hatching and fertility of non–ablated females of *P. semuslcatus* used in the experiment.

No	Weight (g)	Total length (mm)	Carapace length (mm)	Date of Ist moulting	Days between moulting and spawning	Date of spawning	Number of eggs	Fertility (%)	Hatching (%)	Date of 2nd moulting
1	30.90	135	34.00	22.07.97	10	1.08.97	3.648	87	64.57	–
2	34.90	147	34.65	22.07.97	–	–	–	–	–	–
3	36.30	154	36.00	23.07.97	11	18.08.97	33.600	81.81	37.10	7.08.97
4	36.93	158	35.75	23.07.97	10	17.08.97	32.400	90.79	78.3	7.08.97
5	38.19	154	35.75	17.07.97	–	–	–	–	–	–
6	39.70	155	36.60	23.07.97	–	–	–	–	–	7.08.97
7	41.36	154	36.35	17.07.97	–	–	–	–	–	8.08.97
8	42.05	160	38.20	18.07.97	–	–	–	–	–	–
9	42.33	163	40.00	23.07.97	–	–	–	–	–	7.08.97
10	43.19	158	37.45	23.07.97	–	–	–	–	–	–
11	43.97	162	38.80	24.07.97	–	–	–	–	–	8.08.97
12	49.68	166	40.75	20.07.97	–	–	–	–	–	3.08.97

Table 2. Moulting, spawning, number of eggs, hatching and fertility of ablated females of *P. semisulcatus* used in the experiment.

No	Weight (g)	Total length (mm)	Carapace length (mm)	Days between eyestalk ablation and moulting	Date of Ist moulting	Days between moulting and spawning	Date of spawning	Number of eggs	Fertility (%)	Hatching (%)	Protozoea (%)
1	36.44	149	33.0	8	21.07.97	5	26.07.97	38.640	–	8.33	
							30.07.97	–	–	–	
							11.07.97	3.950	98.70	70.00	
							14.08.97	42.396	93.75	58.11	
							17.08.97	16.400	87.80	46.87	
							21.08.97	12.900	79.47	36.20	
2	37.54	150	35.0	8	21.07.97	4	25.07.97	15.500	–	17.60	
							16.08.97	79.600	90.90	49.75	
3	40.05	152	38.0	9	22.07.97	8	30.07.97	128.400	95.00	5.00	
							1.08.97	30.396	80.65	64.24	61.90
							4.08.97	81.999	80.00	42.30	
4	41.09	148	37.0	12	25.07.97	8	30.07.97	–	–	–	
							7.08.97	13.988	100	87.00	
							21.08.97	55.560	79.47	36.20	
5	41.67	155	38.3	8	21.07.97	10	1.08.97	60.396	90.00	36.60	
							18.08.97	110.800	62.00	37.42	
							22.08.97	79.200	87.00	62.13	
6	43.68	158	37.0	7	20.07.97	10	30.07.97	134.400	90.22	47.01	82.80
							2.08.97	11.160	88.60	44.49	
							16.08.97	16.050	95.83	66.30	
							21.08.97	140.380	88.43	46.64	
7	43.96	159	38.0	10	23.07.97	6	30.07.97	169.560	90.41	44.03	
							3.08.97	5.100	56.00	39.39	
8	45.57	158	37.0	11	24.07.97	8	3.08.97	10.470	27.00	–	
9	48.48	160	38.8	8	21.07.97	15	7.08.97	12.798	90.00	83.34	
							11.08.97	8.100	90.74	85.71	
10	55.93	173	42.7	11	24.07.97	6	30.07.97	93.960	90.30	5.00	
							4.08.97	2.599	73.00	86.97	
							12.08.97	102.780	100.00	57.96	
							16.08.97	51.200	76.00	47.92	
							22.08.97	131.400	94.00	22.00	
11	60.19	175	43.55	10	23.07.97	8	31.07.97	13.500	80.00	12.50	
							17.08.97	32.400	93.80	76.48	
							21.08.97	36.660	87.80	68.97	
12	60.47	178	43.0	7	20.07.97	4	25.07.97	163.800	92.30	66.60	
							1.08.97	66.798	87.00	77.50	
							11.08.97	116.760	97.60	26.10	
							17.08.97	42.400	76.00	11.17	

The ablation method (cutting the eyestalk off after tying) used in the present study appears to be successful. Only one of the ablated females, which was replaced later on, died on the first day of the experiment, and this was due to handling stress rather than ablation. An electrocautery apparatus was also reported to be successful in ablation of *P. semisulcatus* (8). These methods prevent loss of hemolymph and lower the

	Ablated	Non–ablated
Initial weight (g)	46.25[a] ± 8.34	39.97[a] ± 4.91
Average number of eggs/female	177.700[a] ± 133.340	23.216[b] ± 16.957
Average number of spawns/female	3.17 ± 1.40	1
Average fertility (%)	83.81[a] ± 14.74	86.53[a] ± 4.51
Average hatching (%)	47.82[a] ± 24.43	59.99[a] ± 20.98
Average no of nauplii/female	81.574 ± 55.126	13.397 ± 11.535
Average days between ablation and moulting	9.08 ± 1.68	–
Average days between moulting and spawning	7.67[a] ± 3.08	10.33[a] ± 2.73
Moulting cycle (days)	–	16.57 ± 2.87

Table 3. Moulting, spawning and egg production of ablated and non–ablated *P. semisulcatus* broodstock. Means marked with different superscripts are significantly different (P<0.05).

chance of infection through the wounded area (4). Without eyestalk ablation, it does not seem to be possible to obtain a sufficient number of spawnings from *P. semisulcatus* in captivity. Only 25% of the intact females matured and spawned during the 45–day experimental period. In addition, the fecundity of these females was low compared to that of eyestalk–ablated females. Browdy and Samocha (8) also report a much lower number of spawnings in the control females than in eyestalk–ablated females.

Eyestalk ablation leads to predictable maturation and spawning in penaeids, but it is generally accepted that over–stimulation of reproduction in the broodstock ablated in captivity reduces the reproductive performance (low fecundity, low fertility and hatching rate) of the females of various penaeid species (2, 6). In spite of a few poor–quality spawnings, the present results do not show any decline in the reproductive performance in the broodstock of ablated *P. semisulcatus*, confirming the results of Browdy and Samocha (8) for the same species. The ablated *P. semisulcatus* had higher fecundity and equal fertility and hatching rates in comparison to non–ablated females in the present study. However, eyestalk ablation and coding the females by cutting one or more uropods lead to deterioration of quality (loss of balance, melanised regions, missing appendages). This may limit the use of broodstock, necessitating more frequent replacement. Hence, management of environmental factors (i.e. temperature, photoperiod and diet) may be used to stimulate maturation and spawning in *P. duorarum* (12) and *P. semisulcatus* broodstock without lowering the quality of the females (13).

The high fertility rate of eggs obtained in the current study indicates that the male–female male: ratio of 1:2 is adequate for *P. semisulcatus*. Alava and Primavera (14) report this as the best ratio for *P. monodon* with closed thelycum. Browdy and Samocha (8) obtained 86.7% spermatophore transfer in *P. semisulcatus* with a male: female ratio of 1:2.6. Hence, stocking a low number of males makes more efficient use of the maturation thanks. Repeated spawnings within the same moult cycle is quite common in *P. semisulcatus*. One mating is enough to fertilise the eggs up to 3–4 spawnings without considerably lowering the fertilisation.

This study demonstrates that *P. semisulcatus* females can readily mature and spawn in captivity with eyestalk ablation under the environmental conditions of the present study. Following ablation, it is possible to achieve the first spawnings within only 16 days. The ablation technique can be successfully used in order to supply the seeds enabling shrimp farms to get a head start on–growing in the early spring under the subtropical conditions of Turkiye.

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