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Off-season Maturation and Spawning of the Pacific White Shrimp *Litopenaeus vannamei* in Sub-tropical Conditions

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Abstract

This study deals with investigations on how to control off-season maturation and spawning of the Pacific white shrimp *Litopenaeus vannamei* by using various maturation techniques. For the experiment, the broodstock were separated into five groups (Group 1: Control, Group 2: Serotonin-injected, Group 3: Ablated, Group 4: Temperature-fluctuated, and Group 5: Another ablated groups). Each of the first four groups were stocked into a 2-m diameter round tank at density of 9.44 shrimps per m^2 (2:1, female/male), while Group 5 were stocked into a 3-m diameter tank at density of 5.67 shrimps per m^2 (1:1, female/male). The experiment continued for 2 months until maturation in a recirculation system. Each female was tagged and any ripe female carrying a spermatophore was removed to spawn individually in a spawning tank. The first spawnings occurred on 25-28th days of the experiment in all the groups. The highest female spawning rate (55-90%) and fecundity (79,778-125,015 eggs) were obtained in the eyestalk-ablated groups ($P<0.05$). Serotonin (Group 2) induced ovarium development in 35% of the females, generating 60,277 eggs per female. Cyclic temperature fluctuation (Group 4) stimulated ovarium maturation in 39% of the females with a mean fecundity of 28,500 eggs per female ($P<0.05$). Mean egg fertility rates ranged from 63.08% to 96%, and hatching rates from 8.53% to 31%. Spawning, fecundity and hatching rates were found to be different between the two eyestalk-ablated groups (Group 3 and 5), and the reasons were thought to be due to tank size and/or shrimp stocking density. Our broodstock displayed poor reproductive performance with abnormal egg morphology and low egg hatching rates. The stress caused by off-season reproduction and low genetic variation due to past selective breeding programs might have seriously hampered the reproductive performance of our broodstock. The results of this study has demonstrated that, under Mediterranean climatic conditions, the broodstock of this non-indigenous shrimp species can be readily matured and spawned out of season in recirculating systems.

Keywords: Shrimp, *Litopenaeus vannamei*, reproduction, maturation, spawning, egg.

Pasifik Beyaz Karidesi (*Litopenaeus vannamei*)'nin Yarı-tropik İklim Koşullarında Mevsim-dışı Olgunlaştırılıp Yumurtlatılması

Özet

Bu çalışma, Pasifik beyaz karidesi *Litopenaeus vannamei*'nin farklı teknikler kullanılarak mevsim-dışı olgunlaştırılması ve yumurtlatılması ile ilgili araştırmaları kapsamaktadır. Deneme için anaçlar, 5 ayrı gruba (1. Grup: Kontrol, 2. Grup: Serotonin enjekte edilenler, 3. Grup: Gözsapı kesilenler, 4. Grup: Sıcak/soğuk dalgalanması uygulananlar ve 5. Grup: Diğer gözsapı kesilenler) ayrılmıştır. İlk dört grubun her biri 2-m çapında birer adet yuvarlak tanka m^2'ye 9,44 karides (2:1, dişi/erkek oranı), 5. Grup ise 3-m çapında bir tanka m^2'ye 5,67 karides (1:1, dişi/erkek oranı) olacak şekilde stoklanmışlardır. Anaçlar; kuluçkahanede kurulan bir resirküle sistemde 2 ay süren deneme boyunca olgunlaştırılmışlardır. Her dişi karides markalanmış ve olgun-spermatofor taşıyan dişiler anaç tanklarından yumurtlama tanklarına alınarak bireysel olarak yumurtlatılmışlardır. Tüm gruplarda da ilk yumurtlamalar denemenin 25-28. günlerinde elde edilmiştir. En yüksek dişi yumurtlama oranı (%55-90) ve yumurta verimliliği (79.778-125.015 adet) gözsapı kesilen gruplarda kaydedilmiştir ($P<0,05$). Serotonin uygulaması (Grup 2) dişilerin %35'inde ovaryum gelişimini uyarmış ve bu grupta her dişi ortalama 60.277 yumurta üretmiştir. Deneme boyunca Sıcaklık dalgalanması (Grup 4) dişilerin %39'unda ovaryum gelişimini uyarmış ve bu grupta ortalama dişi başına 28.500 adet yumurta elde edilmiştir ($P<0,05$). Tüm gruplarda yumurta döllülük oranı %63,08 ile %96, açılma oranı ise %8,53 ile %31 arasında çıkmıştır. Gözsapı kesimi yapılan gruplarda (Grup 3 ve 5) yumurtlama oranı, yumurta verimliliği ve açılma oranı açısından önemli farklılıklar belirlenmiş ve bunun nedenlerinin tank büyüklüğü, anaç stok oranı ve cinsiyet oranından kaynaklandığı yargısına varılmıştır. Anaçlarımız, anormal yumurta morfolojisi ve düşük açılma oranları şeklinde kendini gösteren zayıf bir üreme performansı sergilemişlerdir. Anaçların mevsim-dışı üremeye zorlanmaları neticesinde ortaya çıkan stresin ve ayrıca anaçlardaki düşük genetik varyasyonun yumurta kalitesini düşürerek yumurta açılma oranını olumsuz etkilediği sonucuna varılmıştır. Bu çalışma *L. vannamei*'nin Akdeniz iklim koşullarında resirküle sistemler kullanılarak özellikle gözsapı kesim tekniğiyle mevsim dışında rahatlıkla olgunlaştırılıp yumurtlatılabileceğini göstermiştir.

Anahtar kelimeler: Karides, *Litopenaeus vannamei*, üreme, olgunlaştırma, yumurtlatma, yumurta.

Introduction

Availability of gravid broodstock in the wild is possible throughout the year in tropical countries, but mature females can only be obtained during certain seasons in the sub-tropical Mediterranean countries (Aktaş and Kumlu, 2003). In such countries, the broodstock have to be matured and spawned out of season in temperature-controlled recirculation systems in captivity (Kumlu *et al*., 2003). Off-season maturation and spawning, that would allow larval and nursery culture of shrimps to be carried out in greenhouses in winter months, can provide opportunity to extend production season of shrimp farming to a few months in temperate regions.

In the last decade, farming of the Pacific white shrimp *Litopenaeus vannamei*, of which fast growing and disease resistant strains have been developed by selective breeding programs, has been expanding throughout the world, especially in the far-eastern countries such as Thailand, Vietnam, Indonesia, China and India. This species can be readily reproduced in captivity, has wide tolerance to environmental parameters, better utilizes low-protein containing diets, and grows fast compared to other penaeid shrimp species (Wyban, 2007). It is well known that shrimp growth is fast during the warm season (25-34°C) between May and October (5-6 months) in the Mediterranean region (Kumlu *et al*., 2003; Kumlu and Kır, 2005). If this species is to be cultured sucessfuly in the subtropical regions of Turkey, its reproduction has to be fully controlled and its seed production must be shifted towards winter or early-spring in the year. We carried out several investigations on off-season maturation and spawning (Aktaş and Kumlu, 1999; Aktaş *et al*., 2003), and over-wintering possibilities of the green tiger shrimp (*Penaeus semisulcatus*), a native species to North-eastern Mediterranean (Kır and Kumlu, 2006; 2008a,b). However, no research has been yet conducted in detail to obtain knowledge on reproduction performance of off-season maturation and spawning of *L. vannamei* in sub-tropical conditions.

Induction of maturation through eyestalk ablation technique is extensivelly used by almost all commercial hatcheries and research facilities worldwide (Caillouet, 1973; Muthu and Laximinarayana, 1977; Lumare, 1979; Hillier, 1984; Browdy and Samocha, 1985; Browdy, 1992; Aktaş and Kumlu, 1999). Despite its several advantages, this technique produce poorer quality larvae after successive spawnings and the spawners have to be discarded due to rapid loss of condition (Emmerson, 1980; Chamberlain, 1985; Primavera, 1985; Makinouchi ve Primavera, 1987; Aktaş and Kumlu, 1999). Alternative techniques to control shrimp reproduction have received little attention, and such studies have mainly concentrated on the injection of various hormones or manipulations of temperature/photoperiod regimes.

Serotonin (5-hydroxytryptamine; 5-HT), a neurotransmitter, has shown to induce reproduction in the crayfish *Procambarus clarkii* and the lobster *Homarus americanus* (Kulkarni *et al*., 1992; Fingerman, 1997), the giant freshwater shrimp *Macrobrachium rosenbergii* (Meeratana *et al*., 2006), and some penaeid shrimps such as *P. monodon* (Wongprasert *et al*., 2006), *L. vannamei*, L. *stylirostris* (Vaca and Alfaro, 2000; Alfaro *et al*., 2004) as well as *P. semisulcatus* (Aktaş and Kumlu, 2005). Serotonin is reported to inhibit GIH (gonad inhibiting hormone), secreted from the X-organ/sinus complex, or stimulate GSH (gonad stimulating hormone) in decapod crustaceans (Sarojini *et al*., 1995; Fingerman, 1997; Tinikul *et al*., 2008). Recently, it has been reported that GnRH plays an important role in the development of ovarium in *P. monodon* Ngernsoungnern *et al*., 2008), and serotonin induces ovarian maturation by increasing vitellogenin accumulation in *Fenneropenaeus indicus* (Santoshi *et al*., 2009). Similarly, progesteron was also found to influence ovarian development and spawning in *Metapenaeus ensis* (Yano, 1985), and improve sperm quality in *L. vannamei* (Alfaro, 1996). Yano and Wyban (1993) stated that HCG stimulates reproduction in shrimps, while Aktaş and Kumlu (2005) found unclear effects of HCG and LHRH-a on the reproduction of *P. semisulcatus*. Up to now, no study has shown the effects of such hormones on off-season maturation and spawning in *L. vannamei*.

It is well known that some environmental factors have effects on reproductive performance of penaeid shrimps in hatcheries. In general, long photoperiods and high temperatures were reported to be required for reproduction in *P. japonicus* (Laubier-Bonichon, 1978), *P. setiferus* (Brown *et al*., 1979), *P. esculentus* (Crocos and Kerr, 1986) and *P. duorarum* (Cripe, 1994). Low temperatures (<25°C) are known to discourage mating, gonad development and spawning in *P. stylirostris* (Robertson *et al*., 1991), *P. esculentus* (Crocos and Kerr, 1986), and *P. semisulcatus* (Aktaş *et al*., 2003). Cycling temperature fluctuations between 20 and 28°C induce maturation and spawning in *P. duorarum* (Cripe, 1994) and *P. semisulcatus* (Aktaş *et al*., 2003). The cycling temperature fluctuation has been suggested to be an effective technique in obtaining off-season reproduction in the green tiger shrimp *P. semisulcatus* by the latter authors. As a result, this study aimed also at investigating if this technique would also be effective in inducing off-season maturation and spawning in the pacific white shrimp *L. vannamei* before this species can be suggested for farming in sub-tropical regions in Turkey.

Therefore, this study was conducted to assess the effects of three different induction methods (eyestalk ablation, serotonin injection, and cycling temperature fluctuation) in an attempt to control off-season reproduction in *L. vannamei* broodstock. The main purpose was to shift maturation and spawning towards winter or early spring in order to enable hatcheries to complete the whole hatchery cycle (maturation, spawning, hatching, larval and nursery culture) before new on-growing period begins in May, when the temperature is warm enough for fast growth in ponds.

Materials and Methods

Experimental Conditions

The experiment was conducted at Yumurtalık Marine Research Station of the Faculty of Fisheries, Adana, Turkey, for 60 days between 15 April and 15 June, 2009. The pacific white shrimp (*Litopenaeus vannemei*) post-larvae (PLs) at PL8 were imported from Thailand in the first week of July 2008 and reared in earthen ponds until the end of October, 2008 (4 months), during which the animals reached above 20 g. They were then over-wintered until April, 2009 in two 3-m diameter tanks in a green house in recirculation system before the initiation of the experiment.

Prior to start of the experiment, all individuals were weight to the nearest 0.01 g and shrimps (mean weight 33.5-34.5 g) were randomly allocated to each maturation tank in five groups as described in Table 1. None of the females stocked into the maturation tanks had gonodal development at the beginning of the experiment. Small maturation tanks (2 m diameter black fibreglass tanks) connecting to a central bio-filter were preferred to facilitate easier temperature and broodstock control for the Groups 1-4, while a bigger tank (3 m diameter) detached to the recirculation system was allocated to Group 5 to see the effects of tank size and lower shrimp stocking densities on the reproduction of the shrimps. Broodstock stocking density was maintained as 9.44 shrimps per m^2 in Groups 1-4 and 5.67 per m^2 in Group 5. A sex ratio of 1:2 (male/female) in Groups 1-4 and 1:1 in Group 5 was used (Table 1).

The shrimps were acclimated to final experimental conditions for a period of 7 days, water temperature in tanks were maintained at 28°C and shrimps were fed with fresh feeds (squid, crab and mussel) and occasionally frozen feeds prior the start of the experiment.

After the acclimation period, the animals were allocated to groups as follow;

Group 1 (Control): Half of female broodstock (10 shrimps) were injected with normal saline solution (8.5‰ NaCl) and the rest were remained without any treatment.

Group 2 (Hormone injected): Serotonin (5-hydroxytryptamine, 5-HT, and creatinine sulfate complex, Sigma, St. Louis, MO, USA) hormone injection was made with 1 ml micro-injector at a dose of 50 µg g^{-1} to each female individual from the sternite of the second abdominal segment as in Aktaş and Kumlu (2005).

Group 3 (Eyestalk ablation): Females in this group were unilaterally ablated by tying the eyestalk first and then cutting it off with scissors.

Group 4 (Temperature fluctuation): Water temperature (28°C) was lowered to 20°C at a rate of 2°C day^{-1} and then kept for 2 days prior to increasing the temperature to 28°C at the same rate. The animals were exposed to 28°C for 2 days before the temperature was lowered to 20°C again. This procedure was repeated in ten days intervals, until the first ovarian development and spawning occurred (Cripe, 1994; Aktaş *et al*., 2003).

Group 5 (Eyestalk ablation): The same procedure was applied as explained before for the Group 2, except that the stocking density and sex ratio in this group were 5.67 shrimps per m^2, and 1:1 (male/female), respectively.

Each maturation tank was painted in black and had a central outlet. In Goups 1-4, drained seawater was recirculated through coarse filters, a submerged bio-filter and a water heating system prior to entering into each tank. All tanks were connected to a cooling-heating system in order to control water temperature independently in each tank when needed. Each tank was fitted with 300–600 W aquarium heaters as well. Recirculation rate was adjusted to 1000% of each tank volume per day. In addition, 5-10% fresh sea water was supplied to recirculation system to avoid high nitrate concentrations. Fluorescent bulbs (80 W) were hung 0.5 m above each tank to obtain the desired photoperiod (16 h light: 8 h dark) and light intensity. Each maturation tank was covered with a thick black polyethylene to avoid excessive light. Light intensity on water surface was measured with a light-meter (Model Li-250, USA) and adjusted to <5 µE m^{-2} s^{-1} as referred by Vaca and Alfaro (2000).

Moulting, maturation and spawning of each individual female were monitored and recorded daily. For this propose, females were marked by placing plastic rings of various colours around the eyestalk. Shrimps were fed four times a day (at $09:^{00}$, $12:^{00}$, $19:^{00}$ and $24:^{00}$) until satiation with fresh squid, mussels, and crab (*Callinectes sapidus*), which were caught daily by gillnetting during the experimental period. All the remaining wastes were removed by siphoning the tanks before the morning feeding.

The degree of maturation was examined externally by inspecting the size of the developing ovaries through

Table 1. Experimental design used in the current study (Group 1: Control*, Group 2: Serotonin injected, Group 3: Eyestalk ablated, Group 4: Cycling temperature fluctuation, Group 5**: Eyestalk-ablated)

Groups	Stocking density (shrimp per m^2)	Mean weight of females (g)	Sex ratio (Male/Female)	Temperature (°C)
Group 1*	9.55	34.30±3.99	1:2	28
Group 2	9.55	33.49±3.61	1:2	28
Group 3	9.55	35.25±3.40	1:2	28
Group 4	9.55	35.08±3.01	1:2	20-28 (cycles in 10 days)
Group 5**	5.67	34.60±3.09	1:1	28°

* Half of the shrimps were injected with a serum solution while the other half are not treated in any way.

** This group of shrimps held in 3 m^2 maturation tank, while the rest of the groups were matured in 2 m^2 tanks.

the dorsal exoskeleton on a daily basis in the evenings (between 19.^{00} and 21.^{00} h). Each female with ripe ovaries and carrying spermotophore was removed into a 200 L spawning tank previously filled with filtered (down to 1 µm) and UV-irradiated seawater. EDTA (20 ppm) was added into the spawning tanks to bind possible heavy metals. Upon spawning, the female was returned to the maturation tank and five 100 ml aliquot samples were taken to determine fecundity, and the number of fertile and infertile eggs under a microscope. The eggs were siphoned onto a 100 µm plankton mesh and then treated with 100 ppm formalin for 30 seconds and 50 ppm iodine for 60 seconds before being placed into a hatching tank (500 L) for further 36 h to determine hatching rate. An antibiotic (furazolidone) was used in prophylactic dose (0.5 ppm) in the hatching tanks (Anonymous, 2005; 2007). After the hatching, five 100 ml aliquot samples were taken to count the nauplii under a microscope.

Salinity, temperature, dissolved oxygen level of each tank were monitored daily and pH, total ammonia, nitrate, nitrite concentrations were monitored weekly. Salinity was measured with a refractometer, O_2 and temperature with an O_2 meter (OxyGuard, Denmark) and pH with (Thermo Orion Star 3, USA). Total ammonia, nitrate and nitrite levels were measured with a photometer (Nova 60, Merck).

Statistical Calculations

Data were analyzed using one-way ANOVA and any significant difference was determined at 0.05 probability level by Scheffe`s test after normality and homogeneity (Duncan's test) of the data were checked in SPSS version 17 statistical software.

Results

The initial weights for female shrimps were 34.53 ± 0.81 g while this value was 32.15± 0.75 g for males (Table 1) (P>0.05).

Throughout the experiment, water temperatures remained between 27.5-28.5°C and 26.5-28.5°C for Groups 1-4 and Group 5, respectively. During the experimental period, (15 April and 15 June 2009) ambient water temperature ranged between 18 and 22ºC. All maturation tanks were aerated with oxygen by supplying air continuously through air-stones from an air-blower, thus dissolved oxygen (DO) levels remained >5 mg L^{-1}. pH and salinity levels of the water were measured as 8.0-8.2 and 38-39.4‰, respectively. Ammonia (NH_3-N), nitrite (NO_2–N) and nitrate (NO_3-N) were 0.04-0.09, 0.05-0.17 and 0.5-0.6 mg L^{-1}, respectively, throughout the study.

First ovarian maturation was observed in the eyestalk-ablated groups (Group 3 and 5), but still the first successful spawning occurred between the 25th and 28th days of the experiment, in all the groups. It was observed that females at final maturation stage mated around 19.^{00}–21.^{00} h and received spermatophores. When placed individually into spawning tanks, these females spawned about midnight.

Throughout the experiment, a total of 9 spawning were recorded in the control group (Group 1), 4 of which were spawned by the females injected with saline solution (8.5‰ NaCl) and the rest were produced by those not treated in any way (Figure 1). 40-50% of the females spawned during the experimental period in this group. Mean fecundity was calculated as 48,483 for non-treated and 52,000 eggs for saline-injected females. Mean fertilization rate was 86-96%. Hatching rate ranged between 16 and 31% (Table 2).

In the hormone-injected group (Group 2), a total of 8 spawning occurred and the mean fecundity was calculated as 60,278 eggs per female. The first spawning was recorded on the 28th day, (after the third hormone injection) in this group (Figure 1). Fertilization and hatching ratios were 63.08% and 18.5%, respectively (Table 2).

In the eyestalk-ablated shrimps (Group 3), a total of 9 females spawned and mean fecundity was 79,778 eggs per female. Eyestalk ablation stimulated ovarian development from the first week of the experiment; nevertheless, first successful spawning occurred on the 25th day (Figure 1). The highest fecundity per spawning was recorded as 121,100 eggs in this group. Mean fecundity ratio was above 88%, however, hatching rate remained at just 8.53% (Table 2). In this group, 55% of the females spawned during the experimental period.

In Group 4 (temperature fluctuation), 7 females spawned producing an average of 28.500 eggs per female. Ovarian development was stimulated after the third temperature fluctuation and the first spawning occurred on the 25th day (Figure 1). The highest number of fecundity per female was recorded as 62,000 eggs. Fertilization and

Table 2. Number of spawns, spawning rate, fecundity, fertilization rate, hatching rate of the females during the experiment

Groups	Number of spawns	Spawning rate (% female)	Mean fecundity (per female/spawn)	Mean fertilization rate (%)	Mean hatching rate (%)
Group 1	5	50.00	48,483±12,980^{bc}	86.25±11.25^{a}	31.00±7.12^{a}
	4*	40.00	52,000±16,513^{bc}	95.75±5.06^{a}	16.22±6.73^{b}
Group 2	6	35.29	60,278±17,745^{bc}	63.08± 13.23^{b}	18.50±3.81^{b}
Group 3	11	55.00	79,778±19,933^{b}	88.37±8.02^{a}	8.53±2.15^{c}
Group 4	7	38.89	28,500±18,624^{d}	86.54±12.57^{a}	10.13±1.83^{c}
Group 5	18	90.00	125,015±29,276^{a}	79.06±13.21^{ab}	28.55±7.06^{a}

In Group 1, * indicates the number of saline solution-injected females, while the rest are non-treated females. Each value for fecundity, fertilization and spawning rate is a mean ± standart deviation. Means marked with different letters at the same column are significantly different from each other (P<0.05).

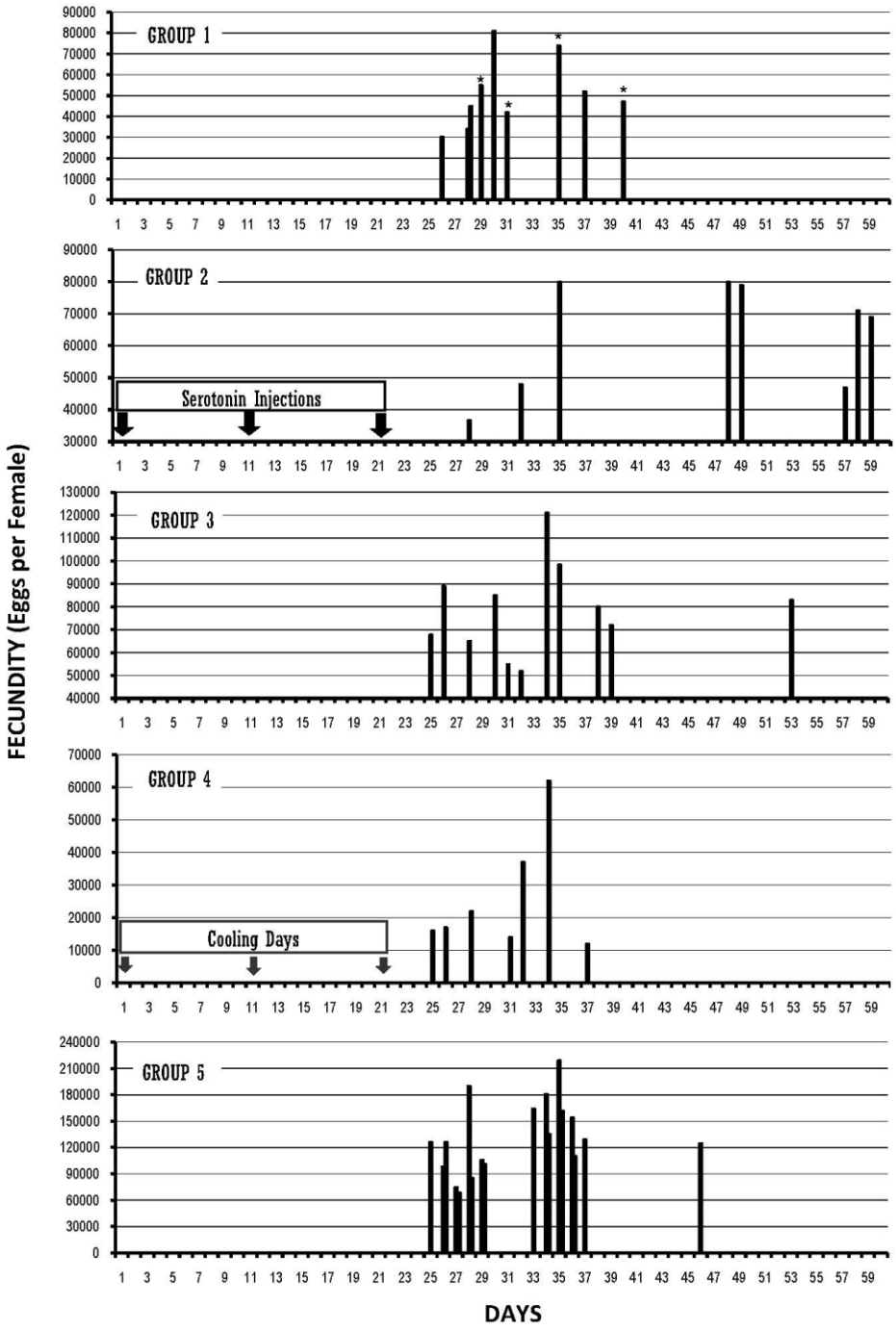

Figure 1. Fecundity (eggs per female) and spawning time of the individual shrimps over the entire experimental periods.
Group 1: Control, Group 2: Serotonin injected, Group 3: Eyestalk-ablated, Group 4: Cycling temperature fluctuation, Group 5: Eyestalk-ablated
In Group 1, * indicate the fecundity values of saline solution-injected individuals, while the rest are non-treated females.

hatching rates were 86% and 10%, respectively (Table 2). %39 of females in this group spawned during the experimental period.

In the second eyestalk-ablated group (Group 5), a total of 18 females (%90 female spawning rate) spawned generating the highest mean fecundity of 125,015 eggs per female ($P<0.05$). Eyestalk ablation stimulated ovarian development from the first week of the experiment; but the first successful spawning occurred on the 25th day (Figure 1). The highest fecundity (219,000 eggs per female) was recorded in this group ($P<0.05$). Fertilization and hatching rates were 79% and 28.55%, respectively (Table 2). The highest hatching rate was determined as 32.24%.

At overall, the highest (125,015 eggs) and the lowest (28,500 eggs) average fecundity were observed in Group 5 and Group 4 ($P<0.05$), respectively, during the experimental period (Table 2). More females spawned and had better overall reproductive performance in Group 5 compared to other treatments. Fertilization rate (63.08%)

was found to be significantly lower in the serotonin-injected group (Group 2) among all the treatments ($P<0.05$). In general, hatching rates ranged between 9% and 30%, and were lower than expected. The highest hatching rates were observed in the control (Group 1; in non-treated shrimps) and Group 5 (29-31%) and the lowest were found in Group 3 and Group 4 ($P<0.05$). Throughout the experimental period, broodstock survival rate remained above 90%.

In the microscopic examinations, even in the best spawnings, morphological apperances of some of the eggs were observed to be abnormal (ellipsoid shapes, extremely large or small in size, punctured membranes etc.). These abnormalities continued during the course of experiment. A part of eggs, showing normal embryonic development, hatched out successfully and the larvae could be cultured up to post-larvae.

Discussion

Eyestalk ablation is still the most effective and common method used for the induction of ovarian maturation in penaeid shrimps. As with other species (Browdy and Samocha, 1985; Bray and Lawrence, 1992; Browdy, 1992; Aktaş *et al*., 2003), the eyestalk ablation was found to be the best technique in the off-season maturation and spawning of the Pacific white shrimp *L. vannamei* in our study. Despite being in off reproduction season, 55-90% of the eyestalk- ablated females were able to successfully develop their ovarium and spawn within the 2-months experimental period. The shrimps in Group 3 (eyestalk-ablated) displayed the best performance in terms of the rate of spawned females and fecundity compared to the other groups which matured at the same tank size and stocking density (Groups 1, 2, and 3). Throughout the experiment, 90% of the females in Group 5 spawned and produced an average of 125,000 eggs per female with 79% fertilization rate. In agreement with Bray and Lawrence (1992), Browdy (1992), and Aktaş and Kumlu (1999), the eyestalk-ablation generated more spawnings and egg-production, but not higher fertilization or hatching rates in our study.

Size of maturation tanks and broodstock stocking density are known to influence matings and ovarian development in shrimps (Primavera, 1979; Crocos and Kerr, 1986). In our study, a significant difference in the reproductive performance found between the two eyestalk-ablated groups (Group 3 and 5) might be due to tank size and/or stocking density. In fact, these factors are known to have different effects on reproductive performance of shrimps. For example, in a study we carried out with the green tiger shrimp (*P. semisulcatus*), we had good results in 1.2 m diameter tanks at 1:2 male/female ratio and 10 shrimps per m^2 (Aktaş *et al*., 2003). In this study, we found similar reproductive performances (spawning rate, fecundity, fertilization and hatching rates) in this species in either small (1.2-m) or large (4-m) broodstock tanks. In the current study with *L. vannamei*, however, the females in 3-m tank at 5.67 shrimps per m^2 (Group 5) produced significantly (>36-60%) more eggs per female and spawning rate (90%) compared to those held in 2-m tanks at 9.55 shrimps per m^2 in the Group 1 (control) or Group 3 (eyestalk-ablation). Yet, tank size, sex ratio, and/or stocking density did not have any positive effects on either fertilization or hatching rate in the present study. Based on our results and those at the literature (Browdy and Samocha, 1985; Crocos ve Kerr, 1986), it can be concluded that broodstock tanks of not smaller than 3 m in diameter have to be preferred for the successful reproductive performance of *L. vannamei*. Chen *et al*. (1991) suggested the use of at least 6 m^2 of tank bottom for *L. vannamei* broodstock.

Our broodstock size (30-35 g) is similar to what Yano (1993) suggested to be optimum (25-35 g) for *L. vannamei*. A sex ratio of 1:1 (male/female) is very common, but some hatcheries prefer 1:1.5-2.5 (male/female) ratio for this species. Stocking density can be between 5 and 10 animals per m^2 (Chamberlain and Lawrence, 1985; Treece, 1999). In a recirculation system, Chen *et al*. (1991) obtained good results with 10 shrimps per m^2 stocking density, which we also used in Group 5 in our study.

In general, long photoperiods and temperatures above 25°C are known to be suitable for maturation of many shrimp species such as *Penaeus japonicus* (Laubier-Bonichon, 1978), *P. setiferus* (Brown *et al*., 1979), *P. esculentus* (Crocos and Kerr, 1986), *P. duorarum* (Cripe, 1994) and *P. semisulcatus* (Aktaş *et al*., 2003). In sub-tropical regions, temperatures <25°C, encountered during late-autumn, winter or early-spring, depress gonad development and spawning in shrimps (even in eyestalk-ablated females) (Crocos and Kerr, 1986; Robertson *et al*., 1991; Aktaş *et al*., 2003). Despite providing optimal conditions, shrimp broodstock may not readily develop ovaries and spawn in off-reproductive season in captivity, but applying cyclic temperature fluctuations between optimal and sub-optimal levels (20 and 28°C) have proven to induce maturation and successful spawnings in *P. duorarum* by Cripe (1994), and in *P. semisulcatus* by Aktaş *et al*. (2003). The latter researchers obtained similar results with this technique as eyestalk-ablation even in winter months (off-season) in *P. semisulcatus*. However, although cyclic temperature fluctuations appeared to induce maturation and spawning after the third cycle in *L. vannamei*, the effect on reproductive performance was very poor ($P<0.05$). Therefore, *L. vannamei* seems to be responding differently to this treatment than the other shrimp species, and that the cycling temperature fluctuation technique, as we used in this study, is not recommended for off-season maturation and spawning of this shrimp species.

Several studies have shown some positive effects of hormone injections on reproduction of decapod crustaceans (Vaca and Alfaro, 2000; Alfaro *et al*., 2004; Aktaş and Kumlu, 2005; Ngernsoungnern *et al*., 2008; Santoshi *et al*., 2009), but there is no study dealing with the influence of hormones on off-season maturation in penaeid shrimps. Although serotonin was found to induce maturation and spawning in *L. vannamei* (Vaca and Alfaro, 2000) and *P. semisulcatus* (Aktaş and Kumlu,

2005), this hormone poorly induced maturation and spawning in *L. vannamei* in the current study. The reason for this might be due to some unknown seasonal response (winter conditions) of this shrimp species to hormone.

Time between induction and first spawnings in off-season studies depends on the treatments and may change from one shrimp species to another. Aktaş *et al*. (2003) obtained the first spawnings on the 13th and 33rd days in eyestalk-ablated and temperature-fluctuated groups, respectively, in *P. semisulcatus*. Crocos and Kerr, (1986) also reported similar response for *P. esculentus*. In the cyclic temperature fluctuated group, Cripe (1994) obtained the first spawnings on the 20-25th days of the experiment in *P. duorarum*. In our study with *L. vannamei*, despite much earlier ovarian development (first week) we observed in the eyestalk-ablated groups, the first spawnings occurred between the 25th and 28th days of the experiment, irrespective of the maturation treatments.

Throughout the experiment, some of the spawnings resulted in poor egg quality regardless of the experimental groups. Even in the best spawnings, morphological apperances of some of the eggs were observed to be abnormal (ellipsoid shapes, large or small sizes, punctured membranes etc.). In general, fertility rates were high but hatching rates were unexpectedly low. Many factors such as low water quality, inappropriate photoperiod, insufficient quantity or quality of the feeds or even genotype of the broodstock migh account for low hatching rates (Menasveta *et al*., 1994). It is well-known that nutrition is one of the main factors influencing gonad development in shrimps. In commercial hatcheries, broodstock are generally fed on fresh seafoods (mussel, oyster, squid, crab or sea worms) and sometimes artificial feeds until satiation for successful maturation and spawnings (Primavera, 1978; Chamberlain, 1985; Makinouchi and Primavera, 1987; Palacios and Racotta, 2003). Similarly, in our study, we also fed the broodstock *ad libitum* on fresh and occasionally on frozen squid, crab and mussel to provide nutritionally balanced diet. In our earlier studies, with similar feeding regimes we obtained good reproductive performances in *P. semisulcatus*, even in off-season periods (Aktaş and Kumlu, 1999; Aktaş *et al*., 2003).

In addition to water temperature, photoperiod, light intensity and quality, many other water quality parameters (i.e. salinity, pH, dissolved oxygen, nitrogenous wastes, heavy metals etc.) might also influence spawning success in penaeid shrimps (Primavera, 1985; Harrison, 1990). In order to ensure best water quality, we recirculated the seawater through sand filters, 1 µm cartridge filters, and a UV system at least 5-6 times prior to using in the spawning and hatching tanks. EDTA, an antibiotic (furazolidone) and povidone iodine were also used to chelate heavy metals and control infections (Anonymous, 2005; 2007). As a result, we never observed any kind of disease throughout the experiment. The photoperiod, temperature, salinity, pH and all other environmental parameters were adequate for good hatchery practices.

The stress encountered in captivity is know to exert negative impacts on reproductive performance and gamet quality in both males and females of shrimps (Chen *et al*., 1991; McVey, 1983). In addition to the stress that the broostock are facing in captivity, forcing them to reproduce out of the season might have also exacerbated the problem. In a study we carried out with *P. semisulcatus* in winter months, despite maintaining adequate maturation and spawning conditions, we also faced with problems of low hatching rates, which we were unable to rectify during the entire study (Aktaş *et al*., 2003).

In general, the use of pond-reared broodstock in hatcheries results in lower reproductive performance compared to wild counterparts. Benzie (1997) reported serious larval quality problems from the domesticated broodstock of *P. japonicus* or *L. vannamei*, both of which are considered to be relatively easy shrimp species to reproduce in captivity. It is also know that inbreeding depressions might often be seen when pond-reared animals are used as broodstock for several generations (Sbordoni *et al*., 1986). When a new species is imported into a country and its life-cycle is closed, the reproductive performance might seriously be hampered due to low genetic variation (Goyard *et al*., 2003). In fact, in the breeding programs with low genetic variation, inbreeding depressions migh occur even after 1 to 2 generations. Moss *et al*. (2008) found 33.1-47.1% lower hatching rates in *L. vannamei* due to inbreeding depressions even after two generations. These researchers reported that the stress exerted by environmental parameters worsen the situation further. As a result, the low hatching rates we obtained in the current study migh have been influenced by the stress due to captive conditions, season, as well as genotype of the broodstock of *L. vannamei*.

Recently, faster growing and disease-resistant strains (SPR: Specific Pathogen Resistant of SPF: Specific Pathogen Free) through breeding programs have been developed in many countries (Cuzon *et al*., 2004). Some commercial companies are known to produce and sell off their post-larvae from genetically modified broodstock, and when the purchased seeds are grown in ponds and re-used again as broodstock by the farmers, immediate inbreeding deformations occur. Therefore, if *L. vannamei* is to be produced in the sub-tropical regions in Turkey, we have to either bring the fast growing SPF or SPR post-larvae for only on-growing purposes from abroad, or establish our own selective breeding program for this shrimp species.

The results of this study has demonstrated that, under Mediterranean climatic conditions, the broodstock of this non-indigenous shrimp species can be readily matured and spawned out of season in recirculating systems, but further research has to be carried out to improve hatching rate.

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