Indian J. Fish., 62(3) : 141-145, 2015

Note

Effect of eyestalk ablation on ovarian maturation and spawning in green tiger shrimp *Penaeus semisulcatus* (De Haan 1844)

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ABSTRACT

Effect of unilateral eyestalk ablation on ovarian maturation and spawning in *Penaeus semisulcatus* was studied in indoor cement tanks. Induction of maturation in ablated female shrimps was studied at two different light intensities with the experimental animals forming the following four groups: ablated shrimps maintained in tanks provided with shading (light intensity at 10 Lux) (T1), unablated females maintained in tanks with shading (T2), ablated shrimps in tanks with out shading (receiving light intensity at 400 Lux). (T3), and unablated females in tanks without shading (T4). Earliest spawning occurred on the 7, 14, 14, and 11th day in the treatment groups T1, T2, T3, and T4 respectively. Ovarian maturation in ablated shrimps maintained in tanks receiving dim light was significantly ($p<0.05$) faster than that in the other treatments, and females in T1 spawned repeatedly. The number of spawnings per female was also significantly affected by treatments ($p<0.05$), the average number of spawnings per female was 1.88±0.08 for ablated animals receiving dim light (T1), 0.08± 0.01 for non–ablated animals exposed to dim lighting (T2), 0.21±0.02 for ablated shrimps receiving bright light (T3), and 0.02±0.00 for non-ablated animals receiving bright light (T4). Eyestalk ablation was found to have no significant effect on egg quantity/spawning, egg hatching rate and nauplii quantity ($p>0.05$), while eyestalk ablated animals had significantly higher mortality rate ($p< 0.05$).

Keywords: Eyestalk ablation, Maturation, *Penaeus semisulcatus*, Spawning induction

Penaeus semisulcatus is widely distributed in coastal areas of South Africa, East Africa, Indonesia, Malaysia, Philippines, China and Japan (Liu and Zhong, 1986). It is one of the most promising species for aquaculture and commercially cultured around the world (Maheswarudu *et al*., 2013). The species has been reported to attain 7.7 g in 90 days of rearing, with a food conversion efficiency of 2.07 (Kumlu *et al*., 2000; Kumlu *et al*., 2003). In 145 days grow-out culture, survival and body weight of *P. semisulcatus* ranged from 46.4 to 98.5% and 10.2 to 18.78 g respectively, with FCR ranging from 1.9 to 2.6 (Maheswarudu *et al*., 2013).

Like most shrimp species, the wild caught gravid female *P. semisulcatus* is only seasonally available in the coastal areas of Hainan Province, P. R. China. In order to overcome the seasonal limitation and to meet the demands for post-larvae for commercial production, inducing ovarian development of *P. semisulcatus* in captivity is desired. Unilateral eyestalk ablation method has been proved to induce ovarian development, maturation and spawning under captive conditions in various species of shrimps/freshwater prawn *viz.*, *Penaeus orientalis* (Arnstein *et al*., 1975); *Penaeus monodon* (Santiago, 1977; Primavera, 1978; Emmerson, 1983; Shailender *et al*., 2013b); *Macrobrachium acanthurus* (Cristiane and Lidia, 2010) and *Macrobrachium rosenbergii* (Shailender *et al*., 2013b). This method has also been attempted in *P. semisulcatus* for small scale induced spawning experiments (Browdy *et al*., 1985; Aktas *et al*., 1999). However, no attempt has so far been made for large scale production of gravid females of *P. semisulcatus* in captivity.

This study was conducted to assess the effect of unilateral eyestalk ablation on ovarian maturation in *P. semisulcatus* for large scale production of gravid females under captivity aiming at off season larval production of the species.

The experiment was conducted at Anyou Research and Developemnt Centre, South China Sea Fisheries Research Institute, Sanya City, P. R. China. A total of 200 wild caught females and 100 males were collected locally. Only shrimps having intact appendages with no injuries, and strong leaping abilities were selected. The initial mean body weight was 86.6 for females and 41.5 g in case of males. Males and females were separately acclimated in two indoor cement tanks (4×3.5 m; length×width) for

5 days. Water temperature was maintained at 28°C, and the salinity and pH were 32‰ and 8.1 respectively. During acclimation, shrimps were fed with squid thrice a day and uneaten food and faecal matter were siphoned out daily. Continuous aeration was provided in the tanks from a blower, and 50% of water in the tanks was exchanged daily.

After acclimation, the experimental animals were divided into four groups in triplicates and stocked in indoor cement tanks (4×3.5 m, length×width), filled with filtered seawater at a depth of 50 cm. Six tanks were shaded with black cloth to maintain light intensity at 10 Lux, and another 6 tanks were unshaded, which received light intensity at 400 Lux. Pre-heated tweezers were used to perform eyestalk ablation through the middle of the eyestalk. A total of 90 females were unilaterally ablated and stocked in 6 maturation tanks (3 shaded and 3 unshaded) at a density of 15 females per tank. Fifteen unablated females each per tank were stocked in another set of 6 tanks (3 each under shaded and unshaded). Subsequently all the experimental tanks were stocked with 15 males each. The final stocking density was 3.2 shrimps m^{-2}. The treatment groups (each group having triplicate tanks) were designated as: T1 (ablated females in tanks under shade), T2 (non-ablated females in tanks under shade), T3 (ablated females in unshaded tanks) and T4 (non-ablated females in unshaded tanks). The experimental animals were fed *ad libitum* on live *Nereis succinea* three times per day at 8:00, 16:00 and 20:00 hrs and, uneaten food and faecal matter were siphoned out daily. Continuous aeration was provided in the experimental tanks and water exchange rate was maintained at 50% per day with pre-heated seawater. Experiment was conducted for a period of 30 days.

Indoor cement tanks (4×2.5 m) with cement bottom, filled with filtered seawater at a depth of 60 cm were used for spawning. The degree of ovarian development of females was assessed by macroscopic examination of the ovaries through the dorsal exoskeleton based on colour change and volume of ovaries (Browdy *et al*., 1985) at 19:00 hrs each day. Gravid female shrimps from the same group were moved to the same spawning tank, and were taken back to the maturation tank in the morning of the following day. After spawning, 10 samples each were taken from the spawning tanks randomly, in 100 ml glass beaker and the average number of eggs (N) per sample was determined. Total quantity of eggs was estimated as $6N\times10^4$ and the same method was followed for counting nauplii.

The number of spawned females, mortality rate (dead animals), and the total quantity of eggs and nauplii were estimated daily, from each group during the experimental period using the following equations:

Average quantity of egg (10^4) = 6N/no. of spawned shrimps

Total mortality rate (%) = Total no. of dead shrimps/45×100

Hatching rate (%) = Total no. of nauplii/ Total no. of eggs×100

The value of each variable was expressed as mean±SD and the difference between ablated and non-ablated groups was tested using one-way ANOVA. The effect of ablation, shading (light intensity) and ablation combined with shading on commencement of earliest spawning and spawnings per female were further analysed employing two-way ANOVA (PASW Statistics 18.0; Chicago, IL, USA).

Fast maturation in *P. semisulcatus* can be induced by unilateral eyestalk ablation. In this study, the earliest spawning occurred within 7, 14, 14, 11 days in the treatment groups T1, T2, T3 and T4 respectively (Table 1). The eyestalk ablated female shrimps also spawned repeatedly in the experimental tanks. During the 30 days experiment, a total of 71 spawnings were observed in group T1, which was higher than that in T2 (3 spawns), T3 (7 spawns) and T4 (1 spawn). A total 78 spawnings were observed in ablated females (T1+T3 combined) which were significantly higher than in non-ablated females (T2+T4). The total production of nauplii were $2151x10^4$, $92x10^4$, $170x10^4$ and $25x10^4$ in T1, T2, T3 and T4 respectively.

In this study, eyestalk ablation significantly affected mortality and number of spawnings per female shrimp ($p<0.05$). The total mortality of ablated female shrimps was about 2 times higher than that of non-ablated shrimps in shaded (T1 compared to T2) and non-shaded (T3 compared to T4) tanks. The average number of spawnings per female differed significantly ($p<0.05$) between treatments (1.88±0.08 in T1, 0.08±0.01 in T2, 0.21±0.02 in T3 and 0.02±0.00 in T4). Eyestalk ablation did not significantly affect the quantity of eggs per spawning and hatching rate of *P. semisulcatus* in the present experiment (Table 2). Further analysis showed that the earliest time taken to spawn and average number of spawnings per female were significantly affected by ablation; provision of shading for the experimental tanks and ablation combined with shading ($p< 0.05$).

This study demonstrated that *P. semisulcatus* can be induced to mature fast by unilateral eyestalk ablation in large indoor cement tanks. The total number of spawning females of *P. semisulcatus* in eyestalk ablated groups was 19 fold higher than that of non-ablated females. Average number of spawnings per female was 1.88±0.08 in group T1, 0.08±0.01 in T2, 0.21±0.02 in T3 and 0.02±0.00 in T4. There was significant difference between the treatments

Table 1. Number of spawned females, dead females, total number of eggs and nauplii of *P. semisulcatus* produced in the experimental groups

Days	Spawned females				Dead females				No. of eggs (x10^4)				No. of nauplii (x10^4)			
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
1					2		3									
7	3								140				128			
8	8				1				340				300			
9	11					1		1	490				415			
10	8				1		2		320				280			
11	6			1				1	240			30	200			25
12	3				2				95				75			
13	4								200				172			
14		1	2							32	52			28	45	
15	3	1	2						100	48	52		85	40	45	
16	3		1				2	1	96		29		85		20	
17	4								144				120			
18	2					1			48				32			
19	3								80				60			
20	2								56				40			
21			1								32				25	
22																
23						1										
24			1								40				35	
25	2						1		48				35			
26	1	1							28	28			20	24		
27	2				1			1	52				40			
28	2								60				44			
29	1								28				20			
30																
Total	71	3	7	1	7	3	8	4	2566	108	205	30	2151	92	170	25

Table 2. Details of maturation and spawning of *P. semisulcatus* in the different experimental groups

Parameter	Shaded tanks		Non-shaded tanks	
	Ablated (T1)	Non–ablated (T2)	Ablated (T3)	Non–ablated (T4)
Earliest spawning time (days)	7	14	14	11
Total spawning	71	3	7	1
Average number of spawning per female	1.88±0.08 [a]	0.08±0.01 [b]	0.21±0.02 [a]	0.02±0.00 [b]
Mortality rate (%)	15.5	6.6	17.7	8.8
Average number of eggs per spawning (x10^4)	35.62±11.20[a]	36.03±10.81[a]	29.26±10.22[a]	30.00±0.00[a]
Average hatching rate (%)	83.64±5.82[a]	85.16±2.60[a]	82.93±5.82[a]	83.34±0.00[a]
Average number of nauplii per spawning (x10^4)	29.86±6.23[a]	30.64±8.42[a]	24.28±4.22[b]	25.00±0.00[b]

Note: Values in same row with different superscript letters are significantly different between the ablated and non–ablated shrimps ($p < 0.05$).

($p<0.05$). Further analysis showed that spawning per female was significantly affected by ablation combined with shading ($F=5953.6 > F\text{-crit}$). Browdy and Samocha (1985) and Aktas and Kamlu (1999) reported that eyestalk ablated female *P. semisulcatus* spawned more number of times than non-ablated ones. In crustaceans, the sinus gland complex, also called X-organ, inside the eyestalks, secrete gonad inhibiting hormone (GIH), while brain and thoracic ganglion secrete gonad stimulating hormone (Otsu, 1963). After eyestalk ablation, the X-organ is destroyed partially, lowering the level of GIH in the hemolymph of the eyestalk ablated females leading to stimulation of ovarian maturation (Primavera, 1978).

In the present study, females in the shaded tanks (illumination intensity of 10 Lux) with unilateral eyestalk ablation were found to mature faster, with earliest spawning occurring within 7 days after ablation ($F=84.1 > F\text{-crit}$). This finding agrees with the results reported by Aktas and Kamlu (1999) in *P. semisulcatus*. Hoang *et al*. (2002a, b) found that bright light showed

an inhibitory effect, whereas dim light (2 Lux) favoured ovarian maturation and spawning in *Penaeus merguiensis*. However, such effects are more species dependent, for instance Hoang *et al*. (2002a, b) observed that light intensity does not significantly affect maturation and spawning performance in ablated female *P. merguiensis*. Results from the present study indicate that the process of inducing maturation of ablated female *P. semisulcatus* can be accelerated under dim light which is consistent with the finding reported by Radhakrishnan *et al*. (2000).

In this study, the mortality in eyestalk ablated shrimps was 20% which was significantly higher when compared to non-ablated animals ($p<0.05$), Similar result was also observed by Lumare (1979), who reported 18.2% mortality in ablated *Penaeus kerathurus* within 2 days after eyestalk ablation. Eyestalk ablation is a destructive operation which could cause the operated shrimp to lose equilibrium within a short period which is likely the cause of mortality. However, Aktas and Kamlu (1999) reported that unilateral eyestalk ablation in *P. semisulcatus* did not cause any mortality, which may be attributed to small number of animals (12 nos.) used in the study. Serotonin injection has been recommended for induction of ovarian development in shrimps without causing mortality (Vaca and Alfaro, 2000; Meeratana *et al*., 2006; Wongprasert *et al*., 2006). However, as the process is difficult to be applied in large scale production, it is rarely used for large scale ovarian maturation of shrimps despite low mortality rate.

The number of eggs per spawn corresponded to body weight and species of shrimp. For instance, *P. semisulcatus* with an average body weight of 46.2 g can produce 1.7×10^5 eggs per spawn (Aktas and Kamlu, 1999), *Litopenaeus vannamei* with an average body weight of 56 g produces approximately 2.1×10^5 eggs (Vaca and Alfaro, 2000), and *Penaeus monodon* having average weight of 80 g can produce 1.1×10^5 eggs (Wongprasert *et al*., 2006). In this experiment, shrimp with average body weight of 86.6 g produced about 3×10^5 eggs per spawn per female which suggests that female *P. semisulcatus* with larger size are suitable for induced spawning in captivity. Similar observation has also been made by Sampson Manickam *et al.* (1996).

The present study explored the inducing effects of unilateral eyestalk ablation on the ovarian development of *P. semisulcatus* for large scale production of larvae. The first spawning occurred within 7 days after eyestalk ablation in female shrimps maintained in shaded tanks. Ovarian development in ablated females kept in shaded tanks was quicker than in other treatments, and females in this treatment spawned repeatedly. The results indicate that maintenance of ablated females under dim light can lead to faster ovarian maturation and spawning in *P. semisulcatus.*

Acknowledgements

This project was funded by Sanya City Bureau of Industry, Information and Science Technology, China.

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Date of Receipt : 10.08.2014
Date of Acceptance : 20.01.2015