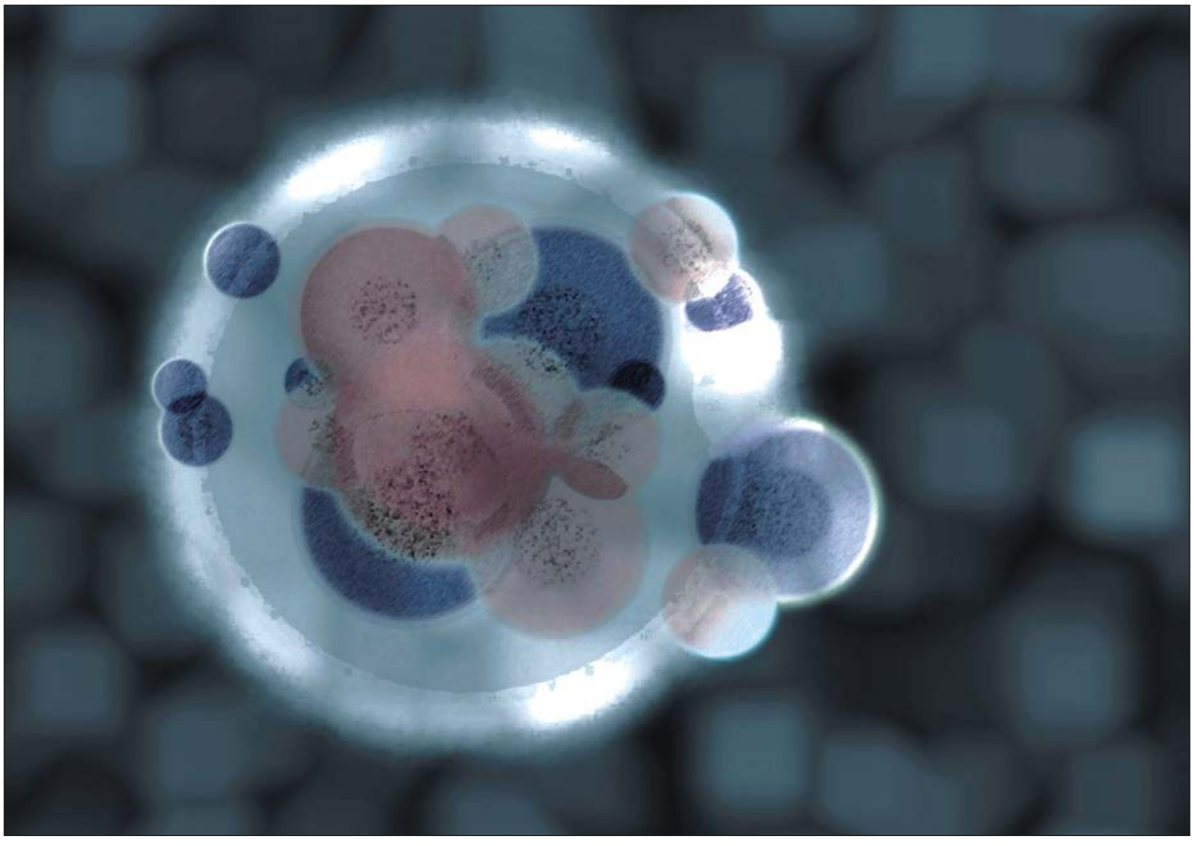

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Growth and Survival of Bilateral Eyestalk Ablated Small Male Freshwater Prawn *Macrobrachium rosenbergii* (DeMan)

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ABSTRACT

The freshwater prawn *Macrobrachium rosenbergii* exhibits three male morphotypes: Blue Claw (BC) male, Orange Claw (OC) male and Small Male (SM). Effects of bilateral eyestalk ablation on growth and survival of SM freshwater prawn were determined in monosex culture. The SM juveniles (9 g) were used in treatment bilateral eyestalk ablation and control (intact/unablated) at a hatchery. Ablated prawns were stocked at 5 prawn m^{-2} for 80 days. At the end of the experimental period, the average weight increased from 8.96±1.06 to 40.95±4.01 g, with bilaterally ablated prawns attaining the greatest growth. The final body weight of prawn at harvest was 40.95± 4.01 g in bilateral ablated SM and 22.69±2.41 g in unablated SM. The specific growth rate of bilaterally ablated prawns (1.90%) was significantly higher than unablated prawns (1.15%). The growth trend of SM prawn was increased significantly from the controls. The wet weight of ablated prawns doubled compared to the control but yield was not significantly different due to mortality. The survival of bilaterally ablated prawn was significantly low 50%. The potency of bilateral eyestalk ablation in enhancing growth rates may be limited due to high mortalities of ablated prawn.

Key words: Bilateral eyestalk ablation, freshwater prawn *Macrobrachium rosenbergii*, growth, survival

INTRODUCTION

Three distinctive adult male morphotypes coexist in a *Macrobrachium rosenbergii* population. Each morphotype represents a different reproductive strategy. Small Males (SM) while employ a sneak copulation strategy (Telecky, 1984), transform into Orange-Claw (OC) males. The OC males are characterized by rapid somatic growth and they do not exhibit courtship and mating behavior; the OC males transform into dominant Blue-Claw (BC) males while cease somatic growth and sequester, court; and mate with receptive females (Cohen *et al*., 1981; Ra'anan and Cohen, 1985). Transformation from SM to OC morphotype is gradual through intermediate forms resulting in the distinctively strong OC form while subsequently metamorphoses into BC form (Kuris *et al*., 1987). All males in the population are capable of transforming through all morphotype stages, exhibiting changes in claw shape and colouration (Kuris *et al*., 1987), growth rate and reproductive behavior (Ra'anan and Cohen, 1985), as well as in the anatomy and physiology of reproductive system and midgut glands (Sagi and Ra'anan, 1988; Sagi *et al*., 1988).

The eyestalk of the crustacean influenced to the endocrinological control of its growth and reproduction

(Kleinholz, 1976; Chandry and Kalwalkar, 1984). The eyestalk containing sinus gland complex (the neurosecretory cells, glial cells and axons of non secretory cells) while is a neurohemal organ (Skinner, 1985). Molt Inhibiting Hormone (MIH) is a neuropeptide while is produced from neurosecretory cells of eyestalk and suppressing the secretion of ecdysteroids by the molt gland. Many ecdysteroids, in several combinations, have been extracted from the mold organ (Lachaise *et al*., 1993). The result of the eyestalk ablation increased the level of ecdysteroids in the haemolymph and changed in crustacean metabolism and energy balance that related to its growth. The growth of crustacean may be increased by reducing of the cycle of molting, increasing size increments per molt (Mauviot and Castell, 1976; Ponnuchamy *et al*., 1981).

The regulatory endocrinological activity of eyestalk components in the freshwater prawn *M. rosenbergii* were investigated; on the histology of the eyestalk (Dietz, 1983), the influence of eyestalk ablation on growth (Chakravarty, 1992; Koshio *et al*., 1992; Karplus and Hulata, 1995), pigment metabolism (Maugle *et al*., 1980) and control of vitellogenin in haemolymph (Wilder *et al*., 1994). The enhancement of growth in unilaterally ablated over-unablated prawns and the shortening of the molt interval in ablated prawns were reported by Huang *et al*. (1981). Karplus and Hulata (1995) showed that the growth of male and female laggards was significantly increased by shortening molt interval and increased size increment per molt in unilaterally ablated prawns.

The Androgenic Gland (AG) secretes a hormone, Androgenic Gland Hormone (AGH) while is believed to act on the differentiation of primary, secondary and behavioral sex characteristics in most malacostracan crustaceans. AG function was first described by Charniaux-Cotton (1954) for the amphipod crustacean *Orchestia gummarella*. Hoffman (1968) found that eyestalk ablated prawns (*Pandalus platyceros*), exhibited hypertrophy of the AG and the prolongation of male phase. The removal of eyestalks from immature crabs, *Rhithropanopeus harriisii* and *Callinectes sapidus*, also induced hypertrophy of the AG with the involvement of hyperplasia (Payen *et al*., 1971). Androgenic gland factors control not only the differentiation of male secondary sexual characteristics but also morphotypic differentiation (Sagi *et al*., 1990; Okumura and Hara, 2004).

The growth enhancement of few lobsters by eyestalk ablation was demonstrated in *Panulirus argus* (Quackenbush and Herrnkind, 1981) and *P. homarus* (Vijayakumaran and Radhakrishan, 1984). The growth of spiny lobsters increased by bilateral eyestalk ablation but high mortalities in bilaterally ablated lobsters were reported by Juinio-Menez and Ruinata (1996).

The aim of the present study was to investigate the effects of bilateral eyestalk ablation on growth and survival of Small Male (SM) juvenile *M. rosenbergii* in monosex culture.

MATERIALS AND METHODS

Animals: The juveniles used in the present study were reared for 4 months. After sorting of BC and OC males, SMs were individually placed in fiberglass tank of 1 t containing 200 L of water under continuous aeration. Each tank was provided with a clay half-cylinder tube as shelter. Males were fed twice daily, morning and afternoon with commercial pellet (BLANCA, 7704, Star feedmills (M) SDN BHD).

Experimental design: The SM juveniles (9.0 g) were used in bilateral eyestalk ablation treatment and an unablated (control) at a hatchery. Eyestalk ablation was carried by cutting the base of eyestalk using a pair of sterilized scissors and forceps. By punching the base of eyestalk with forceps and cutting the eyestalk just above the position of the forceps with scissors was performed carefully. Each treatment had three replicates comprising 5 prawns each. The ablated prawns were transferred to three separate tanks of 1 t each. Left-over food was removed the next morning. The number of surviving prawns in each replicate was recorded daily. Individual weights were measured on monthly basis. The experimental period lasted 80 days, during which mortality was recorded once in 2 weeks.

Water samples were collected from the tanks every fortnight at 9 to 10 h to measure ammonia-nitrogen (mg L^{-1}), nitrate-nitrogen (mg L^{-1}), nitrite-nitrogen (mg L^{-1}) and phosphate-phosphorus (mg L^{-1}) using HACH Kit (DR/2010 model, HACH, Loveland, CO, USA, equipped with spectrophotometer). Water temperature and dissolved oxygen was recorded every morning at 8-9 h by dissolved oxygen (DO) meter (YSI MODEL 58) and pH using a pH meter (HACH). Samples were called to measure body weight and to readjust feeding rate once a month. Statistical analyses were carried with t-test.

RESULTS

Growth and survival: There were no significant differences ($p<0.05$) in the average initial individual weight of the animals among the three replicates in each treatment and between the two treatments at the start of the experiment. All of the prawns grew substantially during the experimental period. The mean final body weight of surviving individuals was significantly higher in bilaterally ablated prawns than with unablated prawns (Table 1). At the end of the experimental period, the average weight increased from 8.96±1.06 to 40.95±4.01 g, with bilaterally ablated prawns attaining the greatest growth. The growth increments of bilaterally ablated prawns were significantly higher than the control prawns. Although final individual weight of bilaterally ablated prawns was still higher, yield was not significantly different from either ablated or unablated prawns. However, survival of bilaterally ablated prawns (50%) was significantly lower than the control (100%) prawns. There were no signs of infection of the ablated eyestalks but death occurred while molting.

The specific growth rate of bilaterally ablated prawns (1.90%) was significantly higher than in unablated prawns (1.15%). The wet weight of ablated prawns doubled, compared to the control (Fig. 1). The growth trend of SM prawns increased significantly compared to the controls (Fig. 2). The effect of bilateral eyestalk ablation in increasing growth rates might be limited due to high mortalities of ablated prawns.

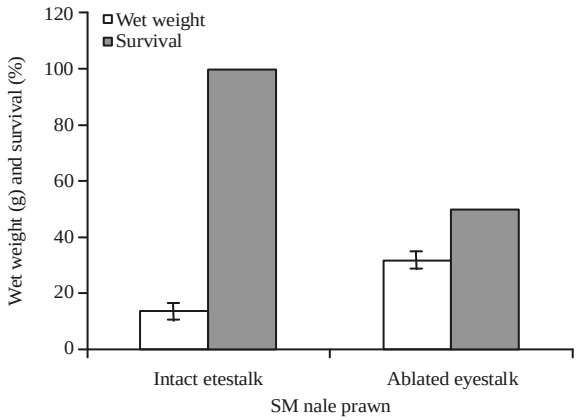

Fig. 1: Final wet weight and survival of SM prawns in treated and control tanks at the end of the experimental period

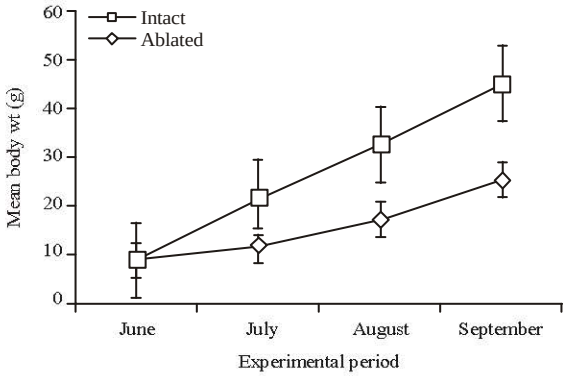

Fig. 2: Growth trends of SM prawn in treatments

Table 1: Growth parameters in eyestalk ablated and intact SM prawns

Parameters	Intact SM	Ablated SM	t-test
Initial individual weight (g)	9.06±1.21	8.96±1.06	NS
Final body weight (g)	22.69±2.41	40.95±4.01	S
SGR (% days^{-1})	1.15±0.16	1.90±0.18	S
Survival (%)	100	50	S
Yield (g m^{-2})	136.14±21	122.85±33	NS

NS: Not significant, S: Significant difference ($p<0.05$)

Table 2: Water quality parameters in treatment and control tanks

Parameters	Intact SM	Ablated SM
Temperature (°C)	26.84±0.45	26.86±0.44
pH	8.52±0.38	8.36±0.27
DO (mg L^{-1})	7.24±1.27	7.35±1.13
NH_3-H (mg L^{-1})	0.78±0.30	0.63±0.41
NO_2-N (mg L^{-1})	0.05±0.05	0.09±0.17
NO_3-N (mg L^{-1})	0.30±0.12	0.24±0.29
PO_3-P (mg L^{-1})	1.23±0.78	1.05±0.58

Water quality: Water quality of treatment and control tanks were within the optimum range. Dissolved oxygen levels were >4.0 mg L^{-1} and pH values were >7.00. Chemical parameters such as total ammonia, nitrate, nitrite and phosphorus concentrations were within optimum range. The details of water quality parameters are shown in Table 2.

DISCUSSION

The growth rate of crustaceans is controlled through molting with increment in size after each molt. The ecdysterone (which accelerates molting) and molt-inhibiting hormone (MIH which retards molting) influence molting. The latter, a peptide hormone (Freeman and Costlow, 1979) shown to be present in the sinus gland of crustaceans (Gersch *et al*., 1977; Bruce and Cheng, 1984). It is known to block the ecdysone-induced proecdysis and thereby delay the molt cycle (Quackenbush and Herrnkind, 1981, 1983). A vast majority of crustaceans particularly penaeid prawns are having sinus glands located in the eyestalks (Passano, 1960). Thus, result of eyestalk ablation can respond by increasing in growth rate due to molt frequency and greater increments per molt. The molting rate was high by increasing the level of ecdysteroids and decreasing the level of MIH secreted from the sinus glands in unilateral eyestalk ablation (Lachaise *et al*., 1993).

In the present study, individual growth of ablated prawns was higher than in unablated ones, similar to the finding of Karplus and Hulata (1995). Therefore, eyestalk ablation had significantly affected the growth of SM prawns. A significant growth enhancing effect of unilateral eyestalk ablation of *M. rosenbergii* juveniles was reported by Huang *et al.* (1981) and Koshio *et al.* (1992). However, despite the individual growth rates of bilateral ablated prawns being significantly higher compared to control, the total yield in biomass was significantly similar to the control due to low survival. Similar results were reported by Juinio-Menez and Ruinata (1996).

The survival of intact prawns was the highest, compared to ablated prawns during the study period. The reasons for the mortalities of ablated prawns could not be ascertained. There were few dead prawns in the experimental tanks due to cannibalism. In addition, some of mortalities of ablated prawns occurred just before or during molting. A 'molt death syndrome' was observed due to the lack of nutrition in juvenile *Homurus americanus* that was first described by Conklin *et al.* (1980). It was found in juveniles of *H. gammarus* (Ali and Wickins, 1994) and also in spiny lobsters (Booth and Kittaka, 1994). The high mortality during molting in juvenile *Panulius argus* suffered with diets consisted primarily of fish or fish meal which was reported by Lellis (1991). Since the natural diet of freshwater prawn contains mainly invertebrates and mussels in particular they have been used as food for cultured prawn, the nutritional requirement of the ablated prawns may not have been meet by commercial pellets used as food in the current study. Those factors may have caused the considerable mortalities. The feeding of nutritionally adequate natural diets (Trider *et al*., 1979), quality semipurified diets (Koshio *et al*., 1990) should decrease the high mortality of bilaterally ablated prawns. The highest survival of larger ablated lobsters was promoted by feeding of good diets (Castell *et al*., 1977). Thus, the low survival of bilaterally ablated prawns may have contributed with the nutritional deficiency of commercial feed in the present study.

In addition, the essential factor for high survival of ablated prawn is good quality water. Temperature and dissolved oxygen fluctuated widely in the ponds, compared to the more stable environmental conditions in tanks. The physiological and metabolic instability of prawn due to ablation might lead to high mortality under normally sub-lethal conditions (Waddy, 1988). A lethal low oxygen level for freshwater prawns is less than 4 mg L^{-1} (New, 2002) but it is still unknown for bilateral ablated prawns. A major cause of mortality in bilateral ablated *Panulius homarus* was the oxygen depletion, mainly during and just after molting (Vijayakumaran and Radhakrishan, 1984). The high oxygen requirement of bilateral ablated prawn might be due to their higher feeding and metabolic rates. The ablation caused fast growth of ablated lobsters might be attributed to more efficient energy utilization (Koshio *et al*., 1990).

CONCLUSION

In conclusion, eyestalk ablation has significant effect on growth rate through enhanced molting frequency. Ablation may have caused high mortalities in the present study. As the yield was not significantly different between treatments, the yield of ablated SMs may have twice compared to control by reducing the mortality. The mortality of prawns improved under taking some preventive measures for infectious diseases and high quality of nutritional diets. These findings will be used to design further investigation: Dietary high protein and calcium will mitigate stress due to eyestalk ablation.

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