<u>Experimental studies on acclimatization of marine shrimps, *Penaeus monodon* and *Metapenaeus monoceros* to freshwater</u>

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<u>Abstract</u>

The adult shrimps, *Penaeus monodon* and *Metapenaeus monoceros* were induced to mature and spawn by eyestalk ablation. The larvae of both the species of shrimps were reared up to post larval stage on a diet of the phytoplankton, *Chaetoceros calcitrans*. The post larvae of *M. monoceros* were fed with artificial feeds only, whereas the post larvae of *P. monodon* were fed on small quantities of Artemia sp. in addition to artificial feed. After post larval stage, PL-20, the post-larvae were acclimatized to freshwater. Acclimatization was tried for different periods ranging between 2 and 20 days. The best survival rate was obtained for an acclimatization period of 5 days. Results obtained so far showed that the growth of *P. monodon* was significantly higher in freshwater. The methodologies of larval rearing, acclimatization and the results obtained on the growth of *P. monodon* in freshwater and seawater are described.

Keywords: Marine shrimp, eyestalk ablation, post larvae, phytoplankton, acclimatization, freshwater, seawater.

1. Introduction

In recent years, tremendous developments have been made in farming of shrimps all over the world. The giant tiger shrimp, *P. monodon,* is the major species cultured and accounts for 58% of total shrimp production from farms worldwide (Rosenberry, 1996). It has the fastest growth rate among a number of penaeid species reared in captivity (Forster and Beard, 1974) and is the largest species of shrimp in the world. *P. monodon* is markedly euryhaline and tolerates wide variations in salinity. *M. monoceros* is also highly tolerant to salinity variations and is a candidate species for culture in low saline areas preferably under extensive systems.

Different ranges of salinity have been reported to support the survival and growth of *P. monodon* (Chanratchakool *et al.*, 1994; Karthikeyan, 1994). A high rate of survival and good growth of *P. monodon* in a freshwater lake has been reported from the Philippines (Pantastico, 1979). Monoculture of *P. monodon* in rivers, irrigation channels and groundwater has been successfully accomplished in Thailand (Raghunath *et al.*, 1999). Since culture of *P. monodon* in extremely high salinities of over 30 ppt may cause disease problems, particularly white spot or yellow head virus or luminescent bacteria, more shrimp farmers keep moving towards brackish water or freshwater areas (Chanratchakool, 2003; Laxminarayana, 2001). The culture of *P. monodon* in freshwater, a distant dream of scientists and farmers, has become a reality in Andhra Pradesh and in some places in Kerala (Laxminarayana, 2001), Saha *et al.* (1999) observed faster growth and survival of *P. monodon* in low salinity and freshwater. Monoculture of *P. monodon* has been successfully carried out in a hardwater pond in India (Athithan *et al.*, 2001). The farming of marine shrimp, *Litopenaeus vannamei*, in recirculating freshwater systems has been successfully achieved in Florida (Scarpa, 1998). Studies have shown that *L. vannamei* can be successfully farmed in freshwater raceways and ponds (Scarpa and Vaughan, 1998; Scarpa *et al.*, 1999).

The present work was undertaken on the acclimatization of marine shrimps, *P. monodon* and *M. monoceros* to freshwater. A comparative study on the growth of the marine shrimp *P. monodon*, in freshwater and seawater was undertaken.

2. Materials and methods

2.1 Induced breeding

The adult shrimps, *P. monodon*, weighing 90g and above, were collected by seining operations, at night, at Bambous Virieux in the southeast region of Mauritius during November, 2004. The adult *M. monoceros* weighing 14g and above were also collected from the same region by seining operations. The shrimps were maintained in 2-tonne tanks filled with filtered seawater and provided with aeration for a period of two days.

Unilateral eyestalk ablation of impregnated females of *P. monodon* and *M. monoceros* was carried out using an electric cautery. The eyestalks of males were not ablated. After ablation, the females were introduced into a 2-tonne tank filled with filtered seawater and provided with continuous aeration.

In one 2-tonne-tank, two ablated *P. monodon* females and two males were kept. In the case of *M. monoceros*, ten ablated females and five males were kept in a 2-tonne tank. The maturation tanks were kept in the dark in the maturation house. 70% of the seawater in the tanks was changed daily. The shrimps were fed on clam, mussels, squid, beef liver and polychaete worms at 12% of the body weight, twice daily.

The stages of ovarian maturity were monitored daily after 3 days of ablation by using an underwater flashlight. The flashlight was tied to a pole held close to each female so that the light was perpendicularly to the upper body portion where the ovaries are located. The fully mature female shrimps were kept for spawning in cylindroconical tanks filled with 200 litres of filtered seawater. The mature shrimps normally spawned either on the same day or on the next day. After spawning, the females were removed and the eggs and the nauplii were counted by volumetric method.

The larval rearing was carried out in one 2-tonne concrete and one 2.7-tonne FRP rectangular tanks. Healthy nauplii of the shrimps were selected by using positive phototactic response of the larvae. The nauplii were stocked in one tonne of filtered seawater on the second day of hatching. The culture of *Chaetoceros calcitrans* was added to the larval rearing tanks to maintain the concentration of the microalgae in the tanks at 20,000 – 25,000 cells/ml. This concentration of *C. calcitrans* was maintained in the larval rearing tank up to the post larval stage. From the post larval stages onwards, *M. monoceros* were fed with artificial feeds only whereas the post larvae of *P. monodon* were fed on small quantities of freshly hatched out artemia nauplii at the rate of one individual per 4ml, in addition to the artificial feed. The daily water exchange was in the range of 50% to 70%. Antibiotics were not used throughout the period of larval/post larval rearing but the fungicide, treflan was used at a dosage of 0.05 ppm, whenever needed, up to the post larval stage.

2.2 Acclimatization

The postlarva-20 was acclimatized to freshwater by gradual reduction of the salinity. Acclimatization was tried for different periods ranging between 2 and 20 days. During the period of acclimatization, the post larvae were fed on artificial feed. 70% of the water in the tanks was changed daily. Antibiotics and fungicides were not used during the period of acclimatization.

The juveniles of the shrimp, *P. monodon* acclimatized to freshwater were stocked in three concrete tanks of $500m^2$. Borehole water was used in the three freshwater culture tanks. A $500m^2$ concrete tank filled with seawater was stocked with *P. monodon* juveniles and reared as the control. The stocking density in each tank was 7 individuals per metre square. The parameters monitored were the nitrate and phosphate levels, pH values and the temperature. The wet weight of the shrimps was regularly recorded by random sampling at predetermined time intervals.

The growth parameters of the shrimps in both freshwater and seawater ponds were analysed using the software SPSS 10.0. One-way ANOVA, the Dunett T3 test, was used to compare the weight gain of shrimps in freshwater and seawater at different time intervals. A regression analysis was also performed to determine the regression equation and growth of shrimps in freshwater and seawater.

3. Results

3.1 Induced breeding

All the ablated *P. monodon* matured and spawned within 10 to 27 days after eyestalk ablation. One female spawned three times, two spawned twice and one spawned only once after ablation. The eggs were fertilized and healthy nauplii were obtained. One female of *M. monoceros* matured and spawned 8 days after ablation producing healthy nauplii.

Eight larval rearing trials were conducted for *P. monodon* and five for *M. monoceros*. All the larval rearing trials resulted in the production of healthy post larvae. The survival rate from nauplii to PL 20 varied from 50% to 75% in the case of *P. monodon*. A survival rate of 55% to 61% was obtained from nauplii to PL 5 in the case of *M. monoceros*.

3.2 Acclimatization

The PL 20 of *P. monodon* was subjected to acclimatization to freshwater for different periods ranging from 2 to 20 days. The best survival rate of 95% was obtained when the acclimatization period was 5 days and the lowest survival of 75% was obtained when the period of acclimatization was only 2 days. When the acclimatization period was 20 days, the survival was 80%.

In the case of *M. monoceros*, the best survival of 93% was obtained when the period of acclimatization was 5 days and the survival was 80% when the period of acclimatization was only 2 days.

3.3 Comparative study of the growth of *P. monodon* grown in freshwater and seawater

The average weight gain for a culture period of 80 days was 3g for shrimps cultured in seawater. In the freshwater ponds the shrimps attained an average weight of 5.3g, i.e. an average weight gain of 5g as presented in figure 1 and table 1.

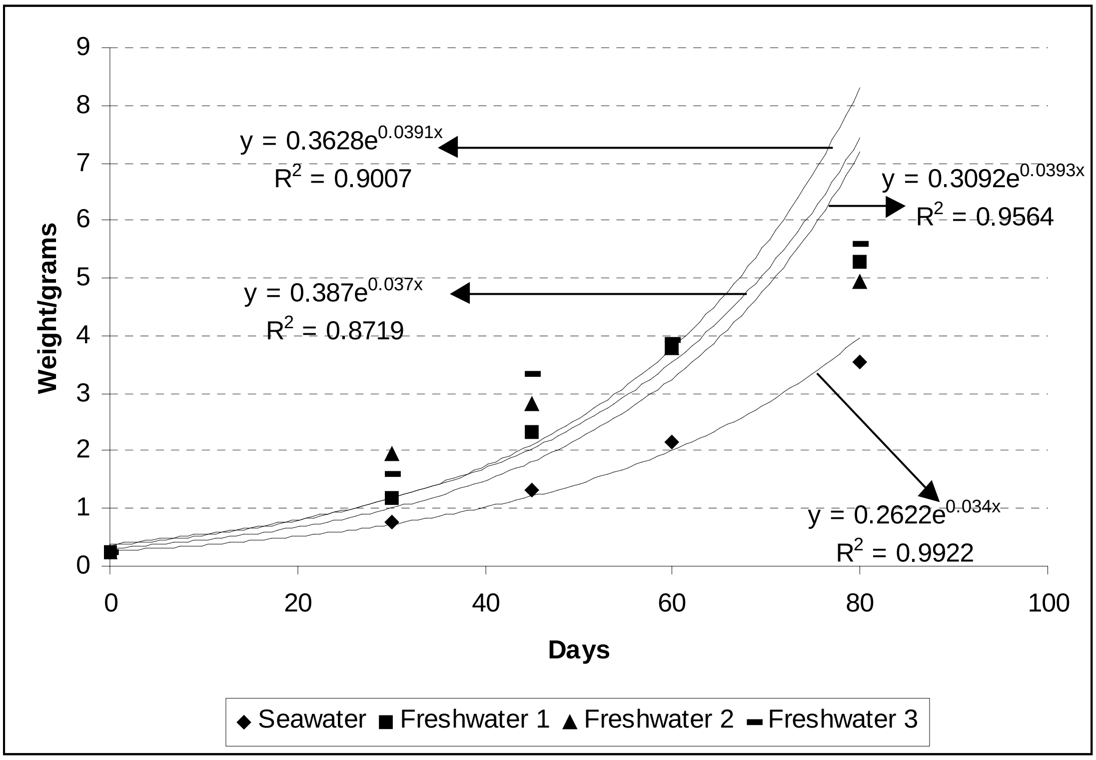


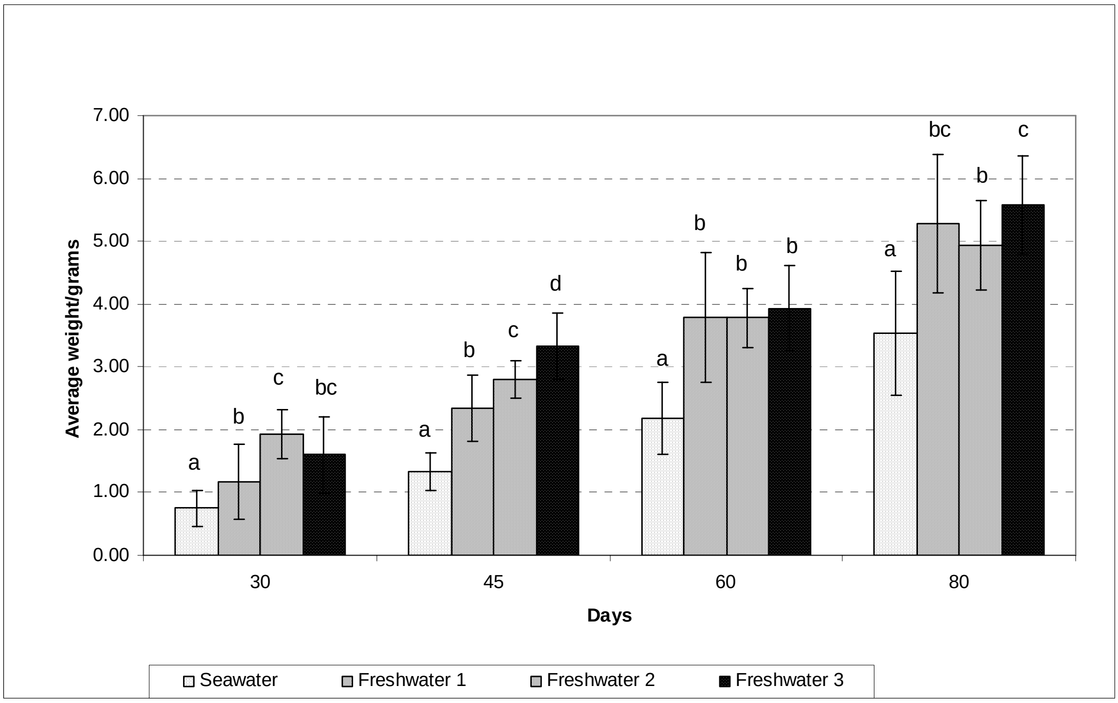
Figure 1: Growth rate of *P. monodon* cultured in freshwater and seawater

Regression equations after natural logarithm transformation of data gave three linear lines of good fit with the following R^2 values 0.95, 0.87 and 0.9 for the three freshwater ponds 1, 2 and 3 respectively. Linear regression for the seawater pond gave an R^2 value of 0.99. The regression equation gave a growth of 0.04 g/day for shrimps cultured in freshwater and 0.03g/day for shrimps grown in seawater according to the slope of the linear regression equation.

Table 1: Average weight of *P. monodon* cultured in freshwater and seawater

	Seawater pond		Freshwater pond 1		Freshwater pond 2		Freshwater pond 3	
Days	Mean weight	St. dev	Mean weight	St. dev	Mean weight	St. dev	Mean weight	St. dev
0	0.24[a]	± 0.04	0.24[a]	± 0.04	0.24[a]	± 0.04	0.24[a]	± 0.04
30	0.75[a]	± 0.28	1.17[b]	± 0.60	1.93[c]	± 0.40	1.6[bc]	± 0.61
45	1.32[a]	± 0.30	2.33[b]	± 0.53	2.80[c]	± 0.29	3.32[d]	± 0.52
60	2.17[a]	± 0.57	3.78[b]	± 1.03	3.78[b]	± 0.47	3.93[b]	± 0.68
80	3.54[a]	± 0.99	5.28[bc]	± 1.11	4.93[b]	± 0.71	5.58[c]	± 0.79

Values with the same letter as superscript are not significantly different (p>0.05)



Values with the same letter for each time intervals are not significantly different (p>0.05)

Figure 2: **Average weight of *P. monodon* cultured in freshwater and seawater**

The analysis of data on the average weight gain of shrimps clearly indicated that the mean weight of shrimps grown in freshwater was significantly higher as presented in figure 2 and table 1. However, within the freshwater ponds, there was significant difference in mean weight except in the case of 60 days of culture as shown in figure 2 and table 1.

During the period of culture, the temperature in the freshwater ponds ranged from 20^0C to 27^0C, the pH values were between 8.6 and 9.3, nitrate between 0.2 and 1.7 mg/l and phosphate between 0.1 and 1.15 mg/l. The temperature in the seawater pond varied from 20.5^0C to 28^0C, pH values were between 8.3 and 8.4, the nitrate level was between 0.1 and 0.5 mg/l and the phosphate level was between 0.03 and 0.17 mg/l.

5. Discussion

Acclimatization of marine shrimps, *P. monodon* and *L. vannamei* to freshwater was undertaken in several countries. Athithan *et al* (2001) acclimatized the Pl-

35 of *P. monodon* to freshwater by gradually reducing the salinity of the seawater at the rate of 5 ppt/week. Wyk and Davis (1999) recommended a duration of 48 hours to acclimatize Pl-13 of *L. vannamei* from 32 ppt to freshwater. Main *et al* (1999) suggested once the post larvae reach Pl-12 to Pl-14 stage, they can be acclimatized to freshwater. At this stage, the gills are developing and they can withstand the osmotic stress (Scarpa, 1998). Main *et al* (1999) mentioned that the gill development should be checked prior to acclimatizing post larvae to salinities less than 15 ppt. The larvae should have branched gill filaments before any attempt is made to acclimatize them to low salinities. The osmoregulatory capability of post larval shrimp is related to the amount of gill surface available for osmoregulation. Prior to Pl-10, the gills have very little branching and shrimp have limited tolerance to low salinities. Branching is usually quite evident by Pl-10. At Pl-12, the shrimp usually exhibit extensive branching of the gill filaments and can easily be acclimatized to freshwater.

Different farms have different protocols for acclimatization of shrimps but the overall result is positive with a shrimp survival exceeding 90% (Jory, 2004). The results obtained in this study are in agreement with this observation. In the present study on *P. monodon* and *M. monoceros*, the acclimatization was tried for different periods ranging from 2 to 20 days. Results of the study as revealed by the survival, indicated that the best acclimatization period was 5 days.

The results of the study on the culture of marine shrimp *P. monodon* in freshwater are in agreement with the work done on freshwater culture of *P. monodon* and *L. vannamei* in different countries (Pantastico, 1979; Raghunath *et al.*, 1999; Laxminarayana, 2001; Athithan *et al*; 2001; Shivappa and Hamprey, 1999; Saha *et al.*, 1999; Scarpa, 1998; Scarpa and Vaughan, 1998; Scarpa *et al.*, 1999). The mean weight of *P. monodon* cultured in the 3 freshwater ponds was significantly better than those cultured in seawater. The difference in the growth of the shrimps in the 3 freshwater ponds is related to the differences in water quality parameters such as nitrate and phosphate levels and pH values. Since the water temperature was low during the culture period, the weight gain was slightly lower in all the culture ponds. Henning and Andreatta (1998) observed that the low water temperature resulted in slow growth and low survival in the shrimp, *P. paulensis*. The shrimps reared at 18^0C were lethargic and mortality cases occurred during the entire experimental period. Increased fouling of carapace due to decrease in moulting frequency and lack of feeding contribute to mortality (Henning and Andreatta, 1998).

Pantastico (1979) reported a high rate of survival and growth of *P. monodon* in a freshwater in the Philippines. Raghunath *et al.* (1977) reported the successful culture of *P. monodon* in rivers, irrigation channels and ground water in the inland area of Thailand. Although farmers in the inland areas of Thailand occasionally achieve yields of 6-8 t/ha/crop under ideal conditions, yields of 3-4 t/ha/crop are common at the intensive stocking densities of 30-40 juveniles/m^2. Saha *et al* (1999) reported a growth ranging from 28.43g to 25.64g in 35 days. Athithan *et al* (2002) reported a production of 208.8 kg/ha/100 days in a freshwater pond with a stocking density of 1.5 individuals/m^2. The successful culture of *P. monodon* in freshwater ponds of Andhra Pradesh and Kerala, where the production ranged between 1500 and 2000/ha/crop, was reported by Laxminarayana (2001).

From the results obtained, it was evident that an acclimation period of 5 days is the most suitable one for both *P. monodon* and *M. monoceros*. The results of this preliminary study also clearly indicated that the growth of *P. monodon* is better in freshwater than in seawater. The growth rates obtained in both freshwater and seawater, in the present study, is generally lower compared to the results obtained in other countries. This is due to low water temperature prevailing during May to July. Therefore, the shrimp culture in Mauritius needs to be carried out during January to April, when the water temperature will be 28 to 29°C. This will lead to better growth, survival and higher production of shrimps.

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