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Development Of Brood Stock From Farm Grown Shrimps

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Abstract

In recent years the wild catches of shrimp brooders have gradually found declined and quality wise were also found to be very poor, which ultimately affected the performance of shrimp hatcherie In Philippines, for the past several years, intensive research has been carried out towards the captive brood stock spawner development of penaeid shrimps. Several investigators have worked on the endocrine control of molting and reproduction in decapod crustaceans. As probiotics used in aquaculture contains many beneficial bacteria, it will maintain the water quality parameters thus enhancing growth rate in shrimps. In the present study the efficacy of probiotics in broodstock development was also tried.

Keywords: Penaeus monodon, broodstock, probiotics.

Introduction

Among the different species of penaeids Penaeus monodon and P. indicus reign supreme due to their export potential. Capture from wild not commensurating with demand, farming has picked up all along the coastline. To cater to the seed requirements of the farming sector, more number of hatcheries have been set up. Year round supply of quality and adequate quantity of brooders is highly essential for the successful operation of the hatcheries. In recent years the wild catches of shrimp brooders have gradually found declined and quality wise were also found to be very poor, which ultimately affected the performance of shrimp hatcheries. The prices of broodstocks have increased due to the demand and shortage of brooders. The cost of broodstock is about Rs.250 to 300 for 100 to 150 gm and Rs.500 to 700 for 150 to 200 gm sized females, while the cost of male is about Rs.100 to 150 for the size of 80 to 100 gm. To alleviate the constraints in the availability of brooders, more efforts have been focused on incaptive development of broodstock. For the success of the above, an understanding about the feeding, growth and reproduction is highly essential.

In Philippines, for the past several years, intensive research has been carried out towards the captive brood stock spawner development of penaeid shrimps. Several investigators have worked on the endocrine control of molting and reproduction in decapod crustaceans. Eyestalk ablation speeds up molting, maturation and spawning (Brown and Cunningham, 1939; Abramowitz an Abramowitz, 1940; Passano, 1960). Growth is a continuous process in shrimps

and interrupted only during molting period. After recovery from one molt, the organism proceeds to grow by metabolizing ingested food and accumulates the metabolic resources in preparation for the next molt. When the eyestalks are ablated, the Molt Inhibiting Hormone (MIH) is allowing the molting hormone to act upon shortening the inter molt period.

Under favorable environmental conditions, animals in general and crustaceans in particular grow and molt at a faster rate. The water quality parameters like salinity, dissolved oxygen, pH, temperature and ammonia at optimum levels, brood stock could be raised quickly.

Use of probiotics is helpful in maintaining the above parameters at the optimum levels. The "Probiotics" was originally used to describe substances produced by a protozoan, which stimulated another (Lilly and Stillwell, 1965). This was later tried as animal feed supplements on the host animals by increasing the gut flora which is considered to be beneficial in digestion. Generally in live stocks and poultry, a biological feed additive which has already been used successfully in animal husbandry is reported to hold immense potentialities for aquaculture. As probiotics used in aquaculture contains many beneficial bacteria, it will maintain the water quality parameters thus enhancing growth rate in shrimps. In the present study the efficacy of probiotics in broodstock development was also tried.

Material and methods

Experiment I

Four to five months pond reared P. monodon were collected from the culture ponds before harvesting using a cast net. The collected animals were sorted based on their sex and size ranging from 48 to 52 gm in the case of females and 38 to 41 gm in males (Plates 6a). The shrimps were packed in the oxygenated bags and transported to the laboratory (Plates 6b). On the arrival at the laboratory, the animals were gradually acclimatized to the laboratory conditions for one hour (Plate 6c, d). Then they were disinfected with 20 ppm formalin for 20 minutes and transferred to the circular pre cast cement tanks of one tonne capacity. They were then grown as brood stock by offering four different types of fresh feed combinations as squid and beef liver, squid and oyster, squid and crab and crab and beef liver at the rate of 15% of the body weight of the shrimps in the ratios of 1:1 (50% + 50%). 100% water exchange was carried out daily during the entire experimental period.

The water from Marakkanam estuary was pumped directly for exchange purposes and compressors were used for continuous aeration. Two water filters (10 microns) were placed in the inlet of hose for filtering the water. A generator was used as a standby. For the experiment eight tonne capacity cement tanks (1x1x1m), four 500 and ten 250 liter fiberglass tanks were used in the thatched shed (30' x 30'x 20' x 20').

Experiment II

The animals were collected from the culture ponds during sampling. The collected shrimps were treated with 50 ppm formalin for 20 minutes as means of disinfecting and slowly acclimatized to the laboratory conditions. The shrimps were sorted out based on their size and sex (Plate 7 a, b) and stocked in one tonne capacity cement tanks. Before stocking the initial weight of the individual shrimps was taken (females 48.3 to 51.3 gm and males 37 to 40 gm). Ten healthy

animals from each sex were selected and stocked. Animals were fed with different fresh feed combinations mentioned already daily at a rate of 15% of the body weight. Water exchange was done to a tune of 100% everyday during the experiment. Unilateral eyestalk ablation(Plate 7c) was performed in five shrimps from each sex by cutting the right eye stalk at its base with a fine sterilized scissors. The remaining non-ablated animals served as control.

Experiment III

Females of 40 to 45 gm weight and males of 30-35 gm weight were collected from the culture ponds. These shrimps were brought to the laboratory and acclimatized. They were separated based on their size and sex and stocked in circular fiber glass tanks of 250 l capacity. 4 sets of tanks were used. The first set serves as the control. Here the animals were not ablated and kept without the addition of probiotics. In the second set, shrimps were ablated and maintained in the probiotics medium. In the fourth set, the shrimps were ablated and maintained without probiotics. Feed combinations used were as in experiments I and II.

Activation of Probiotics

5 gm of commercially available probiotics (N.S super SPO) was dissolved in one liter of estuarine water in a two liter conical flask and activated for four hours (from 11 a.m. to 3 p.m.) with continuous aeration (Plate 7d). Foams were found continuously emerging during activation. The slurry was used at 4 p.m. The dosage of probiotics was gradually increased from 5 gm through 10 gm to 15 gm. To activate 10 and 15 gm of probiotics, two and three liters of estuarine water were used respectively.

The first experiment was carried out for 75 days. Second and third experiments were conducted for an intermolt duration. In all the three experiments, the water quality parameters were monitored. In the third experiment, the levels of nitrite, nitrate, total phosphate and reactive silicate were analyzed following Strickland and Parsons (1972). The level of total ammonia was determined using sea water method of Solorzano (1969) to test the effects of probiotics on water quality. Statistical analysis (ANOVA) was done to compare the growth rate of these shrimps.

RESULTS

Experiment I

Temperature ranged from 26 to 31°C and the salinity from 13 ppt to 28 ppt. The pH fluctuated between 7.5 and 8.1 and the dissolved oxygen was maintained above 4 ppm through continuous aeration. During the experimental period, highest mortality of 8% was observed among shrimps fed with crab and beef liver. The next highest mortality of 7% was recorded among the shrimps fed with squid and crab. The minimum mortality of 3% was observed in the case of shrimps fed with squid and beef liver and squid and oyster. In all the four feed combinations used, the average daily growth (ADG) rate was recorded on the 30^{th} day of culture (DOC). The maximum average daily growth was on the 30^{th} DOC and minimum on 75^{th} DOC. Among the different feed combinations squid and beef liver showed the maximum growth rate (0.49 gm) in female and

the minimum (0.26 fm) was observed in shrimps fed with squid and crab. The same trend was observed in male shrimps and the maximum growth rate was 0.28 gm and minimum 0.18 gm

The maximum weight gain was obtained in female and male shrimps fed with squid and beef and minimum in those fed with squid and crab. The two way analysis of variance showed that the growth rates among female and male shrimps fed with various feed combinations differed significantly ($P<0.05$)

Experiment II

The maximum average daily growth (0.62 gm) was observed in the ablated females fed with squid and beef liver and the minimum (0.47 gm) in non-ablated females. Among males, the maximum average daily growth (0.24 gm) was observed in ablated males fed with squid and crab.

The ablated females fed with squid and beef liver showed the maximum weight gain of 9.92 gm. The next highest weight gain of 8.1 gm was noticed in the shrimps fed with crab and beef liver. The minimum weight gain (7.2 gm) was observed in the shrimps fed with squid and crab. The same trend was observed in the case of non-ablated shrimps. The shrimps fed with squid and beef liver showed the maximum (7.8 gm) whereas the minimum (5.3 gm) was observed among shrimps fed with squid and crab.

The trend noticed among females was noticed in males also. The ablated males showed the maximum weight gain of 6.3 gm and the minimum of 4.1 gm. The non-ablated shrimps fed with squid and beef and squid and crab showed the maximum (3.8 gm) and the minimum (2.8 gm) weight gain respectively. The two way analysis of variance (ANOVA) showed that the growth rates in ablated and non-ablated females and males fed with various feed combinations differed significantly ($P<0.05$;).

Experiment III

The water quality parameters and growth rate of this set of experiment are given in figs. 120-125.The temperature ranged from 28 to 31ºC and there was no difference between control and experimental tanks. The same trend was observed with respect to salinity and dissolved oxygen which ranged from 24 to 27 ppt and between 5.3 and 5.6 ppm respectively.

The pH varied greatly between control and experimental tanks. It increased with the increase in probiotics concentration. The pH value increased from 7.4 to 7.7 in the third set, where shrimps were ablated, kept in probiotics medium and fed with squid and oyster. The maximum reduction of pH from 7.4 to 7.2 was noticed in the control.The total ammonia level in control tanks increases, but the level decreased in the tanks where probiotics was applied. The level of ammonia increased in tanks where the shrimps were fed with squid and crab (from 0.55 ppm to 0.95 ppm). However, in tanks where shrimps were ablated and kept in probiotics medium, the level declined from 0.71 to 0.25ppm. As in ammonia, range of nitrite level was recorded in all the sets. Built up of nitrite levels was observed in the tanks without probiotics and the reduction of the same was noticedin the tanks where probiotics was applied. The maximum reduction from 0.033 to 0.0022 was noted in the tanks where shrimps were fed with squid and beef liver and the maximum increase was observed in the shrimps fed with squid and crab (from 0.0029 to 0.0048 ppm in the control tanks).

The nitrate values increased in the case of probiotics applied tanks and decreased in the tanks without probiotics medium. The maximum increase from 0.0048 to 0.0087 ppm was recorded in the tanks of ablated animals kept in probiotics medium where the shrimps were fed with squid and crab. The maximum reduction from 0.0051 to 0.0021 ppm was noticed in the control sets where the animals were fed with squid and crab. The same trend was observed in the case of total phosphate. Tanks without probiotics medium showed maximum reduction and the increase were noticed in tanks with probiotics medium. The maximum reduction from 0.0012 to 0.00085 ppm was noticed in tanks where the shrimps were fed with squid and crab. The maximum increase in total phosphate from 0.0098 to 0.003 ppm was noticed in the probiotics medium. In tanks where ablated shrimps were maintained without probiotics medium and fed with squid and crab maximum reduction of reactive silicate from 0.0053 to 0.0024 ppm was noticed. The maximum increase was recorded in the tanks with ablated ones kept in probiotics medium and fed with squid and beef liver.

Among the four sets, ablated shrimps maintained with probiotics showed the maximum growth rate. The next highest growth rate was seen in ablated shrimps without probiotics. The minimum growth rate was noticed in shrimps in the control tanks.

The ablated females in probiotics medium fed with squid and beef liver showed the maximum ADG (0.56 gm) and ablated males in probiotics medium 0.34 gm. The next highest ADG (0.4 gm) in the set was recorded in the ablated females fed with crab and beef liver and the growth rate was observed in the ablated animals maintained without probiotics. The maximum ADG of 0.48 gm in females and (0.36 gm) in males was observed in shrimps fed with squid and beef liver. The minimum ADG of 0.26 gm in females and 0.18 gm in male were observed in the shrimps fed with squid and crab. The least growth rate was recorded in the control series, where the lowest maximum ADG of 0.23 gm was recorded in the shrimps fed with squid and beef liver and the minimum 0.16 gm in shrimps fed with squid and crab.

The ablated females in probiotics medium fed with squid and liver showed the maximum weight gain of 9 gm whereas it was 5.4 gm in males. The next highest weight gain of 6.4 gm was recorded in the females fed with crab and beef liver and the weight gain among males was 4.8 gm. Among these four sets the least weight gain was noticed among the control shrimps where fed with squid and beef liver showed the maximum weight gain was 3.6 gm and the minimum of 2.56 gm was observed among shrimps fed with squid and crab. The growth rate of the female and male shrimps between the treatments differed significantly at $p<0.005$ level (

6.4. Discussion

Feeding the organisms with nutritionally balanced feed is essential in the case of incaptive rearing. Very few works regarding brood stock development in India are available and no information on the growth rate of shrimps during brood stock development. This is mainly due to difficulties in holding the shrimps for duration of 3 to 4 months in the laboratory. Former also never attempts it after the culture period in the absence of information on assured returns. Keeping the above in mind, the present work was designed to find out the efficiency of different feed combinations in raising the brood stock. To reduce the holding period two techniques were tried (1) Eyestalk ablation and (2) Significance of the synergistic effect of eyestalk ablation and

probiotics. Maintaining of optimum environmental conditions and feeding with efficient feeds certainly lead to faster growth rate enabling rising of brood stock in the shortest possible period.

Experiment I

Pudadera et al., (1980b), Primavera (1985), Sang pradub et al., (1994) reported that the stocking density of P. monodon in tanks should be 2-7/m^2 depending upon the water quality and exchange rate and the biomass should not exceed 300-400 gm/ m^2. In the present study, the shrimps were stocked at a higher level of 9/m^2 in the female to male ratio of 2:1.

The safe levels of salinity, pH, DO and temperature for brood stock development are 15 to 25 ppt, 7 to 8.5, above 3ppm and 24 to 30ºC respectively. In the present study the water quality parameters such as salinity ranged from 10 to 25 ppt, pH was between 7.2 and 8.1., DO above 5 ppm and temperature in the range of 26 to 30ºC. Low level of salinity was observed in the source waterfor sometimes. However it improved to the desired range within a short period of time.

In the present study, shrimps were developed as broodstocks by feeding with suitable combinations of fresh feeds. Using the above mentioned fresh feed combinations females weighing 50 gm were grown to brood stock of 78.5 to 91.3 gm weight within 75 days and males from 38 gm to 56-61.7 gm. Among the feed combinations used, squid and beef liver yielded better as growth from it was 50 to 90 gm in 75 days showing the maximum average daily growth of 0.49 gm. The next highest average daily growth of 0.44 gm was observed in shrimps fed with squid and oyster. The minimum ADG and weight of the shrimps in different treatments and feed combination used could be the reason for differences in ADG and weight gain.

Experiment II

Several investigators dealt with the endocrine control of molting and growth in decapod crustaceans. The role of molting and growth is of great significance and regulated by two hormones like the molt inhibiting hormone and molting hormone. The molt inhibiting hormone is produced in the eyestalk and stored in the sinus gland and the molting hormone is produced by the Y organ (Passano, 1960 and Adiyodi and Adiyodi, 1970). When the eyestalk is removed, the molt inhibiting hormone is excluded thus allowing the molting hormone to act upon.

In the present study, it was found that an average daily growth of 0.62 gm was obtained in ablated females and ablated males (0.39 gm) fed with squid and beef liver. The non-ablated females showed an average daily growth of 0.49 gm whereas the males showed 0.28 gm with the same feed. It is in conformity with the previous investigators. Koshio (1985) reported that the higher growth rate is related to higher molt frequency and higher weight gain. Chakravarty (1992) suggested that eyestalk contains molt inhibiting hormone which delays molting and the removal of eyestalk shortens the intermolt period. According to Sindhukumari and Pandian (1987) eyestalk ablation can induce hyperghagic conditions to meet out the energy demand and thus helps in increasing the growth rate. Koshio et al., (1992) found out that ablated shrimps consume more food than non-ablated ones. Chakravarty (1992) reported that the ablated animals grew faster than non ablated animals and the intermolt period was shorter and growth rate more in bilateral eyestalk ablated prawns than unilaterally ablated ones. As per the present study, females weighing 50 gm could reach a size of 80 gm in 50 days and males weighing 35 gm could reach a size of 80 gm in 50 days and males weighing 35 gm a size of a 55 gm with unilateral

eyestalk ablation. The other eyestalk could be ablated to induce maturation and spawning at the latter stage of converting the brood stalk into spawner. The present study has proved that eyestalk ablation is helpful in reducing the duration of broodstock development.

Experiment III

Now a day, the main objective of the aquaculture industry is to grow the organisms to the desired size within the shortest possible period. By providing optimum ecological conditions and by increasing the feed acceptance by stimulating the bio-chemical process through eyestalk ablation the above objectives could be achieved. The above strategies could use for brood stock development. Therefore the present study was undertaken to ascertain the synergistic effects of probiotics and eyestalk ablation on the growth of the most important cultivable shrimp species (P. monodon) for an intermolt duration. The influence of probiotics over important water quality parameters, were clearly monitored.

According to Robertson et al., (1990) probiotics could reduce the percentage of mortality in salmon farming. Vanbella et al., (1989) stated that the probiotics inhibits the proliferation of pathogens (or) the production of toxins and also stimulates the immunological system of the host. Collington et al., (1990)reported that probiotics does not produce toxic residues in animal protests and improved the growth rate in turbot. However, it could not be decided whether its effect was due to a growth factor (or) to an antibiotic effect. The increase in the growth of organisms under probiotics medium is also attributed to the external nutrients supplied by probiotics strains besides maintenance of the water quality parameters (Deeth, 1984). The above results clearly indicate that probiotics is helpful in maintaining the water quality in the optimum levels and eyestalk ablation increased the growth rate of organisms. In the present study also the water quality parameters were in the optimal levels in experimental tanks and variations were observed in pH, ammonia, nitrite and nutrients between the control and experimental (probiotics applied) tanks.

The pH levels gradually increased in the probiotics applied tanks (fig.120) but never exceeded the optimum level. Thus addition of probiotics was found to be helpful in maintaining pH in the desired level.

Ammonia is the principal end product of protein catabolism of organisms and it is excreted through gill. Nitrite is the mid product in the nitrification process and both are toxic substances. Ammonia is more than nitrite. The safe level of ammonia is 2 ppm and nitrite is 0.1 ppm under farm conditions. During the course of this study, build up of ammonia and nitrite levels were observed in the experimental and control tanks. The reverse trend of reduction in the same was observed in tanks where the probiotics was added (figs. 121, 122). The results of the present study indicate the presence of nitrifying bacteria in the probiotics medium which initiate nitrification.

Nutrients levels in the experimental tanks where the probiotics was not used showed a decreasing trend but increasing trend was observed in the probiotics medium (figs 123-124). This is mainly due to the presence of beneficial microorganisms in the probiotics medium. Nitrate is the end product of nitrification process and phosphate is produced by phosphate producing bacteria and beneficial microbes which act on the decaying organic matter and are thus helpful in releasing the nutrients including silicate.

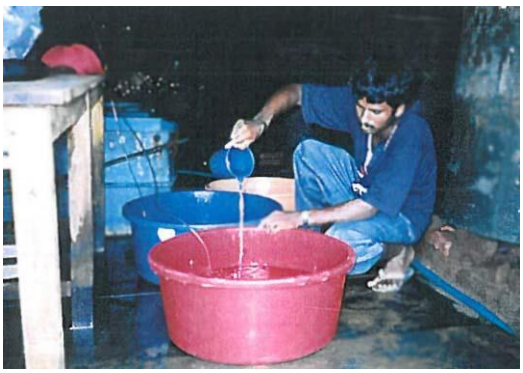
From the results of the present study it is evident that the probiotics enhanced both internal and external microbial environments (water quality parameters here) and the beneficial bacteria present in probiotics limit the establishment of pathogens in the culture environment, which is in agreement with the previous reports. Generally organisms grow and molt faster under favourable conditions. In live stock production, application of probiotics increases the growth rate of animals (Sisson, 1989). Dixon, (1993) and Fuller, (1977) stated that the addition of probiotics resulted in the changes of bacterial composition in the rearing medium and also in the gut flora of the organisms.

Growth in terms of weight is a continuous process in crustaceans and interrupted only by molting period. After the recovery from one molt, the organism proceeds to grow by consuming food and accumulates metabolic resources in the preparation for the next molt. In the present study the ablated animals maintained in the probiotics medium and fed with squid and beef liver showed maximum average daily growth and weight gain of 0.56 gm and 9 gm respectively. The next highest average daily growth rate of 0.42 gm was recorded in eyestalk ablated animals maintained without probiotics medium and fed with the same feed combination. The lowest growth was reported in the neither control tank where neither probiotics applied nor eyestalk ablation undertaken. The above trend was also observed in the case of males.

The synergistic effects of eyestalk ablation and probiotics are highly helpful in shortening the developmental period of broodstocks in captivity with suitable fresh feed combinations like squid and beef liver and squid and oyster. Therefore brood stock can be developed from farmed grown organisms as it has so many advantages compared to wild collection.

b) Broodstock collection

a) Transportation of broodstock



d) Acclimatization of broodstock

c) Broodstock acclimatized

a) Female and male

b) Weighing of broodstock

d) Eyestock ablation

c) Activation of probiotics