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A comparative study of quality of eggs produced from wild and captive spawners of *Penaeus indicus* and their bacterial populations

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ABSTRACT

Based on the morphology, the eggs of *Penaeus indicus* were classified into five different types : type A eggs with normal development; type B eggs with delayed or abnormal development; type C eggs with undifferentiated embryonic mass; type D eggs with unequal cells and type E unfertilised eggs with orange colour. The incidence of abnormal eggs was high in eyestalk ablated pond reared prawn. Statistical analysis revealed a significant difference in the percentage of abnormal eggs, hatching rate and bacterial numbers between eyestalk ablated pond reared and wild females of prawn. A significant correlation was found between the bacterial numbers and percentage of abnormal eggs, and bacterial numbers and hatching rate of eggs.

The seeds of *Penaeus indicus* were produced following the modified Galveston system at a hatchery located at Narakkal near Cochin, India from unilaterally eye stalk ablated wild females. Some adult prawns collected from the growout ponds attached to the hatchery were also used for breeding purpose. Poor quality eggs and abnormal nauplii were observed usually from the pond reared eye stalk ablated females during the period of investigation. The present study was carried out to describe different types of eggs based on morphology and to determine the bacterial load of eggs and its relationship with hatching rate.

The methods of breeding and larval rearing of *P. indicus* have been described in detail by Silas *et al.* (1985). For estimating the number of eggs produced, the eggs were counted in three random 1 litre samples after thoroughly mixing the water to achieve a uniform suspension of eggs. The total number of eggs was estimated by taking the average in the samples and multiplying by the total volume of water. The number of nauplii in the tank was estimated using the same procedure adopted for eggs and thus hatching rate was determined. The eggs were examined under a compound microscope for their development and viability. Eggs were classified based on morphological observations.

The samples (eggs and water samples) used for the estimation of aerobic

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heterotrophic bacterial population were collected from spawning tanks. The collected eggs were transferred to a sterile screw capped bottle containing sterile sea water. The bottles containing samples were transported to the laboratory in an ice box (4°C) within an hour of sampling.

The eggs were separated from the sterile seawater with a sartorius filter paper (0.25 µm pore size) and water adhering to filtered eggs was removed by means of sterile blotting paper. The eggs were weighed aseptically and ground in a sterile tissue homogeniser with 1 ml of sterile sea water. After homogenisation, the sample was serially diluted to 10^{-6}. Sterile aged seawater was used for serial dilution.

Zo Bell's 2116e agar and sea water nutrient agar were used for the estimation of bacterial flora. In the present study, the pour plate technique was followed for estimating the total number of aerobic heterotrophic bacteria. After incubation for 48-72 hrs at 29°C, plates with > 30 to 300 colonies were counted. Three replicates of each dilution was made.

The statistical significance of the differences between the two groups was calculated with the help of Student's t test. Correlation analysis was made between the percentage of abnormal eggs and bacterial numbers, and between the hatching rate and bacterial numbers. The Student's t test was used to test the significance of correlation.

The mean hatching rate of eggs produced by wild and pond reared ablated *P. indicus* were 62.86 and 23.38% respectively. The mean percentages of abnormal eggs encountered in the spawnings of wild and pond reared ablated *P. indicus* were 28.14 and 63.54 respectively. The incidence of abnormal eggs was high in the case of eyestalk ablated pond stock whereas it was low in ablated wild stock. Statistical analysis showed a significant difference in the means of percentage of abnormal eggs ($P<0.01$) and hatching rate between the two groups (Table 1). In the present study a highly significant correlation was observed between the abnormal eggs and hatching rate ($P<0.001$).

In the present investigation, several types of egg with normal and abnormal development were observed. On the basis of morphological features and pattern of development described by AQUACOP (1977) and Primavera and Posadas (1981), these eggs were grouped as follows.

Type A: Normal fertilised eggs, spherical with continuous external membrane were generally free of bacterial and other growth; dark-green colour;

TABLE 1. *Mean percentage of abnormal eggs, hatching rate and bacterial numbers of eggs of wild and pond reared eye ablated* Penaeus indicus

Particulars	Wild ablated female	Pond ablated female
No. of spawnings observed	7	13
Mean % of abnormal eggs[a]	28.14	63.54
Mean % hatching rate[a]	62.86	23.38
Mean bacterial numbers in eggs[b] (cfu/g)	1.07×10^6	2.40×10^9
Mean bacterial numbers in water[c] (cfu/ml)	2.30×10^4	1.23×10^6

Superscripts a and b denote statistical significance between wild and pond as $p < 0.01$ and $p < 0.05$ respectively. Superscript c denotes statistically not significant between the two groups.

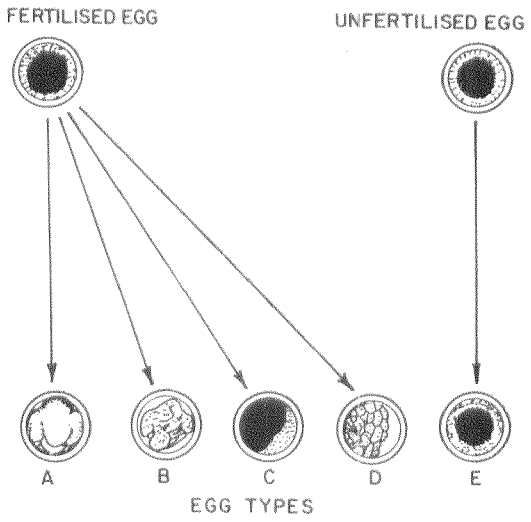

Fig. 1. Different egg types of *Penaeus indicus*.

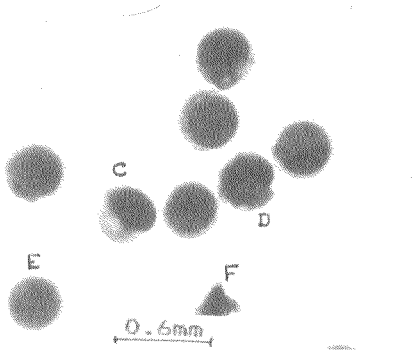

Fig. 2. Abnormal eggs of *Penaeus indicus*. Note fertilised egg with degenerating egg mass (C), fertilised egg with large and small irregular cell formations (D), unfertilised eggs with undifferentiated egg mass (E) and deformed nauplius (F).

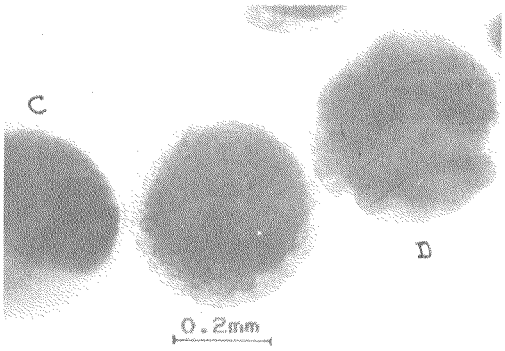

Fig. 3. Enlargement of the above Fig. showing C and D types of eggs.

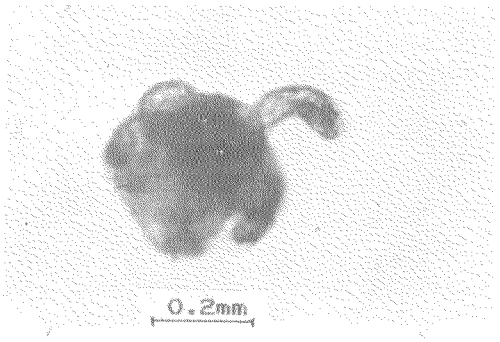

Fig. 4. Deformed nauplius of *P. indicus* with abnormal limbs.

distinct embryonic membrane and symmetrical naupliar structure seen clearly inside the egg. These eggs produced healthy nauplii.

Type B : Fertilised eggs, but showing delayed developments; external membrane continuous; asymmetrical naupliar structure seen inside the egg. These eggs produced nauplii with broken setae and unequal limbs.

Type C : Fertilised eggs with undifferentiated embryonic mass; the embryonic mass gradually degenerated in the course of time; road-shaped motile bacteria seen inside the egg.

Type D : Fertilised eggs but the cytoplasm divided into large and small irregular formations; bacteria seen inside the egg.

Type E : Unfertilised eggs, differentiated by orange colour; cell division was not observed and the embryonic membrane did not separate from the egg

membrane; rod-shaped motile bacteria seen inside the egg.

The mean bacterial numbers associated with eggs of wild and pond-reared eye stalk ablated shrimps were 1.07 x 10^6 and 2.4 x 10^9 cfu/g respectively. Statistical analysis showed significant differences in the number of bacteria associated with eggs of pond and wild ablated females ($P<0.05$) (Table 1). A highly significant correlation was found between the total number of bacteria and the percentage of abnormal eggs ($P<0.001$). At the same time a significant correlation was found between the number of bacteria and the hatching rate ($P<0.001$).

Poor quality of penaeid prawn eggs and the abnormal development in controlled spawning and rearing have been reported by several workers (AQUACOP, 1977; Primavera and Posadas, 1981; Tseng and Cheng, 1981; Primavera, 1985). Primavera and Posadas (1981) noted that eyestalk ablated pond reared females of *P. monodon* when used on spawner source, produced poor quality eggs, whereas eye stalk ablated wild females gave the highest proportion of viable eggs. AQUACOP (1977) also reported the incidence of unfertilised eggs and abnormal nauplii when the quality of brood stock was poor. In the present nauplii the quality of brood stock was poor. In the present investigation, the incidence of abnormal eggs and deformed nauplii were high when the eye stalk ablated pond reared spawners were used for breeding purpose.

Because of the presence of large number of rod-shaped motile bacteria inside the abnormal eggs, attempts were made to determine the bacterial load of egg. The bacterial flora estimated in the spawning tank water were relatively lower than that in the eggs. This might be due to the fact that the surface of egg provides a suitable microenvironment for bacterial growth (Stevenson, 1978). Further, interaction between egg surface and bacterial process of adhesion may also be responsible for association of greater numbers of bacteria with eggs.

In addition, dead eggs in tanks possibly due to poor egg quality, environmental conditions or husbandary practices may release nutrients into the tank water. These nutrients will enhance the bacteria that are already associated with eggs. Smith *et al.* (1986) demonstrated that shocked rainbow trout eggs released phosphate and amino acid, all of which enhanced bacterial growth and reproduction. Based upon the observation of higher bacterial number on dead eggs, the present study suggested the early removal of dead eggs and debris from the spawning tank to prevent bacterial proliferation and colonisation on larvae.

The eggs with high hatching rate contained less bacterial number whereas eggs with low hatching rate contained high bacterial numbers. Statistical analysis showed a significant correlation between the percentage of abnormal eggs and bacterial numbers as well as between bacterial numbers and hatching rate. Such a correlation was also found by Barker *et al.* (1989, 1991) and Sahul Hameed (1993) in the eggs of trouts and larvae of *P. indicus* respectively. The low hatching rate of eggs might be due to a variety of factors including the presence of large numbers of bacteria. However, further experimental studies on the effect of bacterial flora on the eggs of prawn are needed to confirm these observations.

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