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Short Communication

Quality of eggs produced from wild and captive spawners of *Penaeus indicus* H. Milne Edwards and their bacterial load

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The seeds of *Penaeus indicus* H. Milne Edwards were produced following the modified Galveston system at a hatchery located at Narakkal near Cochin, India. Unilaterally eyestalk-ablated wild females were used for seed production. Some adult prawns collected from grow-out ponds attached to the hatchery were also used for breeding purposes. In all, 20 spawnings were observed in the present study. Poor-quality eggs and abnormal nauplii were observed, usually from the pond-reared eyestalk-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, Mohamed, Muthu, Pillai, Laxminarayana, Pandian, Thirunavukarasu & Ali (1985). To estimate the number of eggs produced, they were counted in three random 1-l 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 water volume. The number of nauplii in the tank was estimated using the same procedure adopted for eggs, and hatching rate was determined. The eggs were examined under a compound microscope for their development and viability. They were classified based on morphological observations.

Samples (eggs and water samples) used for the estimation of aerobic heterotrophic bacterial population were collected from spawning tanks. The collected eggs and water samples were transferred to a sterile, screw-capped bottle containing sterile sea water and to a 250-ml bottle respectively, and the bottles containing samples were transported to the laboratory in an ice box (4°C) within 1 h of sampling.

The eggs were separated from sterile sea water 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 homogenizer with 1 ml of sterile sea water. After homogenization, the sample was serially diluted to 10^{-6}. Sterile aged sea water was used for serial dilution.

ZoBell's 2116e agar and seawater 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 h at 29°C, plates with >30 to 300 colonies were counted. Three replicates of each dilution were made.

The statistical significance of the differences between the data of 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 the hatching rate and bacterial numbers. 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 63% (26–86%) and 23% (0–46%) respectively. The mean percentages of abnormal eggs encountered in the spawnings of wild and pond-reared ablated *P. indicus* were 28% (5–56%) and 64% (28–93%) respectively. The incidence of abnormal eggs was high in the case of eyestalk-ablated pond stock but low in the 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. A highly significant correlation was observed between the abnormal eggs and hatching rate ($P < 0.001$).

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 & Posadas (1981), these eggs were grouped as follows:

Type A: Normal fertilized eggs, which were spherical with continuous external membrane, generally free of bacterial and other growth; dark green; embryonic membrane distinct; symmetrical naupliar structure clearly seen inside the egg. These eggs produced healthy nauplii.

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

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

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

Type E: Unfertilized 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 eyestalk-ablated shrimps were 1.07×10^6 cfu g^{-1} (4.4×10^4 to 5.4×10^6) and 2.4×10^9 cfu g^{-1} (4.5×10^7 to 5.3×10^9) respectively. There was a significant difference in the numbers of bacteria associated with eggs of pond and wild ablated females ($P < 0.05$). A highly significant correlation was found between the total numbers of bacteria and the percentage of abnormal eggs ($P < 0.001$). At the same time, a significant positive correlation was found between the numbers of bacteria and the hatching rate ($P < 0.001$).

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

Because of the presence of a large number of rod-shaped motile bacteria inside the abnormal eggs, attempts were made to determine the bacterial load of the egg. The bacterial flora estimated in the spawning tank water was relatively lower than that in the eggs. This might be because the egg surface provides a suitable microenvironment for bacterial growth (Stevenson 1978). Further, interaction between the egg surface and the 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 husbandry practices, may release nutrients into the tank water, which would nourish the bacteria that are already associated with eggs. Smith, Armstrong, Springate & Barker (1986) demonstrated that shocked rainbow trout, *Oncorhynchus mykiss* (Walbaum), eggs release phosphate and amino acids, all of which enhance bacterial growth. The present study suggests the early removal of dead eggs and debris from the spawning tank to prevent bacterial proliferation and colonization of larvae.

Eggs with high hatching rate contained lower bacterial numbers whereas eggs with low hatching rate contained high bacterial numbers. Statistical analysis showed a significant positive 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, Smith & Bromage (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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