CHAPTER - II

INFLUENCE OF ENRICHED *ARTEMIA* BIOMASS SUPPLEMENT ON MATURATION PERFORMANCE

The critical role played by nutritional factors in the reproductive process of penaeid species has been much emphasized (Harrison, 1990; Bray and Lawrence, 1992; Browdy, 1992; Primavera, 1985). Adequate nutrient and energy status is necessary for the onset of gonadal maturation. Under the induced maturation conditions (eye stalk ablation), the precocious gonadal development is induced even without the requisite accumulation of nutrients to support reproduction (Harrison, 1997). Thus, maternal nutrient intake during ovarian development, right up to spawning may be especially critical and influence the composition of ovaries and nutritional status of eggs (Goguenheim *et al.*, 1987).

The weight of the ovaries of maturing shrimp can increase four to nine fold in a week period (Jeckel *et al.*, 1989; Mourente and Rodriguez, 1991; Ravid *et al.*, 1999; Wouters *et al.*, 1999). Penaeid broodstock animals are typically fed a combination of natural feeds like oyster, mussel, squid, polychaete worms etc. and dry feeds or semi-moist pellets. The natural feeds with adequate nutritional profiles have an unpredictable supply with biochemical composition varying according to location, season of collection, method and duration of storage (Lytle *et al.*, 1990). The dry feeds and semi-moist pellets are not nutritionally complete (Harrison, 1997).

Lavens *et al.* (1986) first suggested the supplementation of shrimp broodstock diets with reproductively-active adult brine shrimp. The fresh or fresh-frozen *Artemia* biomass in addition to its fatty acid profile, the amino acid profile, high sterol content, carotenoids and possibly hormonally active substances assumed to ensure acceptable maturation and reproduction output. Bray *et al.* (1990a) reported good reproductive performance with *Litopenaeus stylirostris*, when fed with fresh-frozen squid, bloodworm, shrimp and whole adult *Artemia* in a ratio of 4:2:2:1. However, no specific *Artemia*-related effect was reported. Browdy *et al.* (1989) using frozen *Artemia* as a dietary supplement for *Penaeus semisulcatus*, reported an enhanced reproductive performance. The authors reported that results were not consistent and attributed it to the variable nutritional quality of the different batches of brine shrimp used.

Nevertheless, the variation between batches could be reduced as well as their nutritional quality can be enhanced by enriching live adult *Artemia* with nutritional supplement. Naessens *et al.* (1997) used frozen *Artemia* biomass for *L. vannamei* broodstock and concluded that the same may be useful as a supplement to or as a replacement for polycheates in maturation diets. Wouters *et al.* (1999) also successfully used enriched *Artemia* biomass for the maturation of *L. vannamei.*

The objective of the present study is to evaluate the supplementation of crude sardine oil-enriched, fresh frozen adult *Artemia* biomass in the maturation diet of both wild and pond-reared *Fenneropenaeus indicus.* The crude sardine oil was used to supply the n-3 and n-6 fatty acids for the enrichment of nutritionally-poor low-quality *Artemia.*

MATERIALS AND METHODS

Two experiments were carried out consecutively, the first with wild animals sourced from the sea just before the commencement of the experiment. The second experiment was with animals reared in captivity in the broodstock pond facility. Both experiment 1 and 2 were conducted with two replicates each for the control and experiment. The animals from both sources were acclimated to hatchery conditions for a week, before the start of the experiment. During the acclimation period, the animals were fed *ad libitum* on a diet consisting of frozen mussel, frozen oyster and fresh cuttlefish.

The average initial weight of the wild male shrimps was 36.55 ± 1.68 g (Mean ± S.D) and females 46.6 ± 2.10 g. In the experiments with pond-grown animals (experiment 2), the stocked male shrimps recorded an initial average weight of 31.2 ± 1.1 g and the female weight averaged 39.7 ± 1.82 g.

Maturation tank feeding

During the start of experiment, females of nearly similar weight were unilaterally ablated using red hot surgical scissors. The ablated females were randomly distributed in concrete circular tanks (3.5 m diameter with 80 cm water depth), each tank with 33 animals, with a sex ratio of 1:1.

In both the experiments, the control tanks were fed with mussel, oyster and cuttlefish at a feeding rate of 10%, 2.5% and 2.5% of the tank wet weight biomass per day. The experimental tanks were fed with control diet, 5% *Artemia* biomass per day. Feed quantity was adjusted during the course of experiment to maintain *ad libitum* levels as

well as to reduce excess feeding. From the day of eyestalk ablation, experiment 1 was run for 35 days and the second for 30 days. The water quality parameters measured were salinity (32-33 ppt), temperature (30 ± 1 °C) and pH (8.0-8.1). Light regime was maintained as 14:10 h light:dark using blue colored fluorescent tubes. The water exchange was 150% per day. The water exchange was usually stopped for 2 hours following each *Artemia* feeding to prevent the feed from being washed out. Every day the excess feed and faecal matter were siphoned out.

Artemia culture and enrichment

The low quality *Artemia* cysts of Great Salk Lake (GSL) origin were decapsulated (Sorgeloos *et al.*, 1977), incubated under optimal hatching conditions (Sorgeloos *et al.*, 1986) in 250 l cylindroconical tanks and after 24 hours of incubation, harvested at instar 1 stage. The nauplii were separated from the hatching debris and unhatched cysts and were rinsed with sterilized seawater thoroughly and transferred to indoor cement tanks with a stocking density of 1 *Artemia*/ml. The animals were reared till their reproductively-active stage with the diatom, *Chaetoceros muelleri* (strain CS-176, CSIRO algal culture collection, CSIRO Marine Laboratories, Tasmania, Australia) at a density 0.3 x 10^6 cells/ ml for the first day and the algal density raised gradually to 10 x 10^6 cells/ml at the end stage. The algae were supplied from the semi-continuous algae culture facility attached to the hatchery set up. The algae cultivated in 500 l photobioreactors (Solar Components Corp., NH, USA), illuminated by fluorescent tubes. The algae were grown using the f_2 medium (Guillard and Ryther, 1962). The cultures were maintained at 26 ± 1 °C temperature and 30 ppt salinity.

The *Artemia* were enriched from the day one using the local crude sardine oil, following the Daily Increasing Dosage (DID) method of Dhont *et al.* (1991) with slight modifications. The total quantity of oil was adjusted to give a final concentration of 0.85 ml/l (including emulsifier). The dosages were smaller during the initial period (0.012 ml/l) and increased gradually reaching bigger doses during the end (0.10ml/l). Each 5 ml sardine oil was emulsified with 1gram egg yolk and 94 ml seawater in a blender for 2 minutes and poured into the culture medium. The *Artemia* tank water was exchanged everyday (35% to 50%) to maintain the algal density (checked thrice a day) and water quality (checked twice a day). The reproductively-active *Artemia* (9 ± 0.5mm) were harvested and washed thoroughly with filtered freshwater.

Artemia Biomass

Artemia biomass was prepared in the following manner. Agar was dissolved in boiling water and cooked gently for 2 min. *Artemia* were subsequently poured into it (28 g agar for 1 kg *Artemia*), and mixed thoroughly for 5 min, stuffed in a plastic container, and again steamed for another 5 min. After cooling in open air, these were directly used or stored in refrigerator for future use.

Sampling and quality assessment

From the fourth day after ablation, the females in the tanks were checked for ovary development on alternate days and ready to spawn ones were transferred to individual 500 litre spawning tanks. After the examination of ovaries for completeness of spawning the animals were tagged and transferred to their respective tanks. The eggs were concentrated and rinsed thoroughly and after sampling (for egg quality and percentage fertilization determination) transferred to hatching tanks.

In the hatching tanks the nauplii were sampled during N_5 or N_6 stage and were then transferred to larval rearing tanks with algae where it was allowed to moult to zoea. The zoeae were sampled once all the nauplii had molted. Both the eggs and nauplii were sampled and examined under a stereoscope to determine the percent fertility and hatching.

For each of the experiment the reproductive performance was evaluated by studying the (1) spawn size (fecundity and nauplii and zoeae/spawn), (2) spawn quality (percentage fertility for the fertile spawns, egg hatching percentage and percentage metamorphosis to zoea) and (3) reproductive rate (average number of spawns/female/day, average number of eggs produced/female/day and average number of nauplii produced/female/day).

Student's t-test was used for comparing the parameters between the standard diet fed and *Artemia* fed treatments, and significance was considered at 5% level.

RESULTS

Experiment 1

The results from experiment 1, carried out with wild animals are summarized in table 7. The female survival rates at the end of the experiment was 91% in the control and 94% in the *Artemia* fed tanks. It is vivid from the data that improvement was achieved in all the criteria used for assessing the reproductive performance, when the diet regime included enriched *Artemia* biomass. The inclusion of *Artemia* induced more number of females to go for reproduction (52 against 43 from the controls) resulting in

Table 7. Reproductive performance of wild *F. indicus* broodstock fed standard broodstock diet or standard diet with enriched *Artemia* biomass supplement (Means ± S. E.).

Parameter	Standard diet	Standard diet and *Artemia*
Number of spawns	77	104
Spawns / spawner	1.8 ± 0.11^a	1.98 ± 0.1^a
Average number of spawns / day	0.034 ± 0.004^a	0.044 ± 0.005^b
Average number of eggs / day ($x10^3$)	3.81 ± 0.515^a	5.54 ± 0.55^b
Average number of nauplii / day ($x10^3$)	1.87 ± 0.21^a	3.31 ± 0.38^b
Average number of zoeae / day ($x10^3$)	1.39 ± 0.20^a	2.65 ± 0.31^b
Average number of eggs/spawn ($x10^3$)	114.28 ± 3.0^a	122.96 ± 2.0^b
Average number of nauplii/spawn ($x10^3$)	59.07 ± 3.3^a	74.94 ± 1.9^b
Average number of zoeae/spawn ($x10^3$)	47.12 ± 3.4^a	61.87 ± 2.2^b
Average fertility (%)	71.75 ± 2.2^a	79 ± 1.2^b
Average hatchability (%)	71.42 ± 2.8^a	78.38 ± 1.3^b
Average metamorphosis (%)	78.77 ± 3.2^a	83.06 ± 1.9^a

Mean values with a different superscript in the same row are significantly different ($P<0.05$).

more spawns/tank as well as more spawns/spawner ($P>0.05$). This was reflected in a increase in daily production of spawns ($P>0.05$), eggs ($P<0.05$), nauplii ($P<0.05$) and zoeae ($P<0.05$). Moreover, the inclusion of *Artemia* biomass in the maturation diet showed a trend towards increase in the number of eggs, nauplii and zoeae per spawn ($P<0.05$). Statistically significant ($P<0.05$) improvement was also noticed in spawn quality parameters, viz. percentage fertility and percentage hatchability with *Artemia*-fed tank. The increase in percentage metamorphosis was not significant ($P>0.05$).

Experiment 2

At the end of the experiment, the females' survival was 88% and 91% in the control and *Artemia* tanks respectively. Evaluation of reproductive parameters showed positive trend with addition of *Artemia* biomass as in the experiment 1 (Table 8). The inclusion of *Artemia* biomass in maturation diet regime improved all the parameters assessed. Number of spawners obtained from the control and *Artemia* tanks were 31 and 39 respectively. The inclusion of *Artemia* also resulted in significant increase ($P<0.05$) in spawns per female, reproductive rate (eggs, nauplii and zoeae per day) and spawn size (eggs, nauplii and zoeae per spawn). All the spawn quality parameters (percentage fertility, percentage hatchability and percentage metamorphosis) showed statistically significant increase ($P<0.05$).

Table 8. Reproductive performance of pond-reared *F. indicus* broodstock fed a standard broodstock diet or standard diet with enriched *Artemia* biomass supplement (Means ± S. E.).

Parameter	Standard diet	Standard diet and *Artemia*
Number of spawns	37	60
Spawns / spawner	1.2 ± 0.10^a	1.53 ± 0.14^b
Average number of spawns / day	0.018 ± 0.003^a	0.03 ± 0.011^b
Average number of eggs / day ($x10^3$)	1.37 ± 0.29^a	2.63 ± 0.55^b
Average number of nauplii / day ($x10^3$)	0.58 ± 0.13^a	1.48 ± 0.32^b
Average number of zoea / day ($x10^3$)	0.35 ± 0.09^a	1.05 ± 0.23^b
Average number of eggs/spawn ($x10^3$)	73.28 ± 3.20^a	88.89 ± 2.38^b
Average number of nauplii/spawn ($x10^3$)	32.68 ± 3.17^a	49.55 ± 2.60^b
Average number of zoea/spawn ($x10^3$)	23.37 ± 3.20^a	37.79 ± 2.44^b
Average fertility (%)	65.94 ± 3.50^a	74.71 ± 1.98^b
Average hatchability (%)	63.48 ± 4.00^a	74.63 ± 2.19^b
Average metamorphosis (%)	70.23 ± 3.49^a	76.00 ± 3.30^b

Mean values with a different superscript in the same row are significantly different ($P<0.05$).

DISCUSSION

Results from the two experiments indicated the presence of some nutritional source or sources resulting in the stimulation of reproductive performance. Published reports on the effect of *Artemia* biomass on the reproductive performance of shrimp are few in number. As with the present study, the increase in reproduction rate, spawn size and spawn quality has been reported with *P. semisulcatus* (Browdy *et al.*, 1989).

Naessens *et al.* (1997), with *L. vannamei,* reported significant increase in reproductive performance (mating success, percentage fertilization for the fertile spawns, fertile eggs per spawn, number of nauplii/female/day) when a squid diet was supplemented with enriched *Artemia* biomass. The *Artemia* biomass supplemented diet also outperformed the squid diet supplemented with polychaetes (*Glycera dibranchiata*), in terms of average number of nauplii produced per female per day ($P<0.05$). The authors also reported better maturation efficiency and mating success when a mixed diet was supplemented with enriched *Artemia* biomass.

Artemia biomass has been recommended to be included into artificial shrimp broodstock diets as a freeze-dried meal to increase diet ingestion and stimulate ovarian maturation (Wouters *et al.*, 2000). Addition of 17.5% of *Artemia* biomass in compounded diet for mud crab (*Scylla paramamosain*), resulted in increased fecundity, egg fertilization rate, hatching rate and zoeae per female (Djunaidah *et al.*, 2003).

Although *Artemia* is a source of various nutrients, various authors have stressed significance of n-3 highly unsaturated fatty acids (HUFA) like EPA and DHA. Numerous studies have been made on the nutritional requirements for shrimp maturation.

The lipid-specific requirement was shown by Middleditch *et al.* (1979, 1980) stressing the importance of dietary polyunsaturated fatty acids (PUFAs) for successful maturation. The dietary HUFA and phospholipids were also proved to affect the ovarian development of *Marsupenaeus japonicus* (Alava *et al.*, 1993a), egg composition of *L. vannaemei* (Cahu *et al.*, 1994). The dietary lipids also influenced the egg lipids in *F. indicus* (Galois, 1984; Cahu *et al.*, 1995), *L. vannamei* (Goguenheim *et al.*, 1987), *M. japonicus* (Teshima *et al.*, 1988) and in *F. chinensis* (Xu *et al.*, 1992, 1994a). Xu *et al.* (1994a) also examined the effect of the dietary lipid on egg hatchability. An improved reproductive performance was reported with *P. monodon,* when the broodstock diet was supplemented with cod liver oil as a source of n-3 HUFA (Millamena *et al.*, 1986).

The HUFA contribution of enriched *Artemia* has been confirmed by the study of Wouters *et al.* (1999). The authors reported improved maturation performance of *L. vannamei* fed with enriched *Artemia* biomass. However, after replacing the *Artemia* enrichment product by coconut oil free of HUFA and cholesterol, a decrease in egg fertilization, repeat performance and egg production/female were observed. Cholesterol has several important functions in crustacean endocrinology (Quackenbush, 1986) and in the biochemistry of crustacean reproduction. Shrimps cannot synthesize cholesterol *de novo* (Van den Oord, 1966; Zandee, 1967; Teshima and Kanazawa 1971b). Thus, cholesterol reserves from the body is insufficient to meet the reproduction requirements and the dietary source plays a vital role in the success of maturation.

Another reason for better results with *Artemia* supplementation could be the supply of carotenoids. The transfer of carotenoid from ovary to eggs has been documented in several crustaceans (Mantiri *et al.*, 1995). Vincent *et al.* (1988) have shown a nearly 12

fold increase in concentration and a greater than 100 fold increase in the total carotenoid levels in the ovaries by late vitellogenesis, reflecting its vital role in the gonadogenesis, embryogenesis and early larval development. The addition of carotenoids through the inclusion of paprika to maturation diet resulted in increasing nauplii quality, assessed by survival to zoea 2 (Wyban *et al.*, 1997). But, the effectiveness of different sources of dietary carotenoids on reproductive fitness needs to be determined.

Extra possible factor in *Artemia* adults that could have triggered reproductive performance may be of endocrine origin such as the hormonally-active substances suggested for *Artemia* biomass in reproductive stage (Naessens *et al.*, 1997) and bloodworm (Laufer *et al.*, 1998). The authors found that bloodworms contained methyl farnesoate, which is an ecdysome hormone that increased fecundity and hatch rate in cultured *L. vannamei* (Laufer *et al.*, 1997) and in *P. monodon* (Hall *et al.*, 1999). The hormone also enhanced ovarian development in other crustaceans (Laufer *et al.*, 1998).

A comparison of results between experiment 1 (wild) and 2 (pond-reared animals) clearly indicated that the percentage increase in reproductive performance (when compared to respective control) was better with the pond-reared animals. This may be attributed to the nutritional history of the animals in the broodstock pond. Farm-raised *P. monodon* has been found to have lower levels of phospholipids compared with wild adults (O'Leary and Matthews, 1990). It may be that the nutritionally- poor pond-reared animals fed *Artemia* biomass obtained much of the nutrients needed to go for reproduction, while the control tank did not. In the case of wild animals, even the control animal's body proper had most of the required nutrients and thus, there was only slight

difference in percentage increase of performance factors between control and experiment tanks.

To conclude, the improved performance after the inclusion of enriched adult *Artemia* could be due to the contribution of vital nutrients like HUFA, carotenoids, phospholipids and even maturation-stimulating neurosecretions The number of possible interpretations given here for the reproductive stimulation says the further need for fundamental research on prawn nutrition requirement and metabolism. In future experiments, the biochemical composition of *Artemia*, maturing females and their reproductive performance needs to be studied in detail and the active factors to be isolated. After which, controlled rearing of high quality *Artemia* as broodstock dietary supplement could be used to control reproduction of shrimp in captivity.