SUMMARY

This study was carried out in a commercial shrimp hatchery located in the Arabian sea port city of Mukalla, in the Republic of Yemen (15° 38N, 44° 21E).

In this investigation, a few of the distinguishable critical points in the commercial hatchery production process of postlarvae of the Indian white shrimp *Fenneropenaeus indicus* were selected. These selected critical points either directly or indirectly affect the overall performance of the hatchery and health of the larvae produced. Regulation of each critical control point was attempted, applying innovative biotools. The results from each of the experiments were analysed for their merits and drawbacks. The positive results obtained could be introduced into the prevailing larviculture practices to improve the hatchery performance.

Usage of pond-reared broodstock with known health history is well recommended to avoid entry of pathogens into the hatchery. However, generally the performance of pond-reared broodstock is reported to be poorer than the wild. An effort was made to compare the reproductive performance, egg and larval quality of both the wild and the pond-reared broodstock. Biochemical analysis confirmed lesser amounts of vital highly unsaturated fatty acids (EPA, DHA and ARA), glucose, triglycerides and total carotenoids in eggs from pond-reared spawners, resulting in their poor performance and of lecithotrophic nauplii. The results in turn suggested inadequate nutritional supply to the pond-reared broodstock.

especially during maturation, resulting in poor spawn quality. The results also emphasize the need to supply a balanced diet to pond broodstock even at the time of rearing as some of the nutrients rich in the wild environment are not available in the pond.

As nutritional quality of the maturation diets directly influence the reproductive performance of broodstock, achieving better results was targeted by supplementing sardine-oil enriched *Artemia* biomass to regular maturation diet. The supplementation of biomass prepared using reproductively-active stage *Artemia* at a level of 5% body weight/day, resulted in the improved reproductive performance and egg as well as larval quality of wild and pond-reared broodstock. A comparison of results clearly indicated higher percentage increase in performance of the pond-reared animals than the wild. It could be suggested that the nutritionally poor pond-reared animals obtained much of the needed nutrients from *Artemia* biomass for reproduction.

An endeavour was made to cure the 'pigment deficiency syndrome' of pond-reared broodstock and resultant poor egg quality and larval survival in hatchery. Biochemical studies confirmed low levels of total carotenoids in the eggs from affected females. The spray-dried blue green algae *Spirulina* was tried as carotenoid source. *Spirulina* inclusion in the diet of affected females indicated reversal of pigment deficiency symptoms after four weeks resulting in improved ovary colour, spawn quality, egg colour and egg and nauplii quality. With normal wild females also, *Spirulina* inclusion from the beginning of the experiment resulted in improved maturation performance, egg and nauplii quality. This study confirms the suitability of *Spirulina* as a carotenoid source for the shrimp broodstock.

and recommends the regular usage of the same in diet to preclude the appearance of 'pigment deficiency syndrome" and other pigment deficiency related problems.

The ablation of eyestalk to induce maturation is a traumatic method leading to numerous physiological anomalies. As an alternative, injecting prostaglandins and steroids were tried out. Prostaglandin (PGE_2) and steroids (17 α-hydroxy progesterone and human chorionic gonadotrophin) were injected seperately or in combination into wild females. Six week long experiment confirmed that the injections did not evoke any response from the animal, casting doubt over the use of these substances as maturation inducing hormones.

The green flagellate *Tetraselmis suecica* known for its antibacterial properties was assessed for controlling the *Vibrio* spp. population in maturation and larval rearing tanks. When algae was fed into maturation tanks, it showed a reduction in *Vibrio* count in the maturation and spawning tank water, gut content and egg and nauplii samples. Egg hatching and broodstock survival also improved significantly. In larval experiments, the addition of *Tetraselmis suecica* (either xenic or axenic) was found to reduce the *Vibrio* count in the rearing water as well as in larval samples. This study confirmed the anti-*Vibrio* activity of *Tetraselmis suecica* and recommends the use of the algae in hatcheries to control *Vibrio* population in tanks. Such *Vibrio* reduction would to a considerable extent bring down the chances of diseases caused by pathogenic *Vibrio*, in hatcheries.

One of the major problems in the low water exchange hatchery systems (which are gaining popularity) is the accumulation of toxic nitrogenous substances namely ammonia and nitrite. The fluctuation in ammonia (NH_3), nitrite (NO_2) and pH in a low-water exchange larval rearing system was monitored for 14 days. The results indicated that NO_2-N had

comparatively more effect on the larval survival at postlarvae 5 stage than NH_3-N. The regression coefficient values for nitrite and ammonia on survival were 0.84 and 0.21 respectively. Results say that regardless of NH_3-N values, when NO_2-N value rises in the tank water to 34 µg/l and above, no more than 10% survival could be expected. The study strongly recommends that low water exchange systems regularly monitor and maintain nitrite and ammonia within optimum levels to ensure acceptable survival.

Processed microalgae (spray dried *Isochrysis* sp.) was tried as larval food, by using it at various levels replacing live algae. Results from experiments up to postlarva 1 stage demonstrated failure of spray-dried algae to replace live algae 100%. With *Artemia* inclusion in the feeding regime (for mysis and postlarva), spray-dried algae could substitute up to 66 % of live algae without much compromise on survival, percentage metamorphosis and development rate. The study stresses the need to improve the quality of algal product and also to reduce storage losses.

Efforts were made to study the role of two individual HUFAs or their combination on larval development, survival and growth by selecting and feeding two different microalgae one rich in EPA (*Chaetoceros muelleri.*) and the other in DHA (*Isochrysis* sp.). The larvae were fed on any one of the algae or a combination of two. Fatty acid analysis of different larval stages (from zoea up to mysis) indicated gradual reduction in EPA, DHA and ARA content of larvae, even with those fed with algae rich in particular nutrient. However, the quantity of particular HUFA in mysis 1 sample was directly related to the concentration of the same in algae on which it was fed. Mixed algae with its balanced HUFA content and more comprehensive range of fatty acids gave the best results with larval survival, growth

and development index. So, it is prudent to go for mixed algal feeding in hatcheries and the criteria for selecting a microalga for larviculture should also include the fatty acid profile of microalgae, especially for HUFA content.

Mass cultured rotifers enriched with microalgae or commercial enrichment emulsions were used to replace the costly and scarce *Artemia,* by feeding the enriched rotifers to zoea and mysis stage larvae. The effect of replacement was analysed on larval performance. With rotifers reared with Culture Selco and Yeast and enriched with another emulsion (DHA-Protein Selco), *Artemia* could be replaced totally for the mysis 1 stage larvae, without significant decrease in postlarva 1 survival, growth and percentage metamorphosis. Further knowledge on the fatty acid requirement of larvae and a need based enrichment of rotifers could make it possible to replace *Artemia* to further advanced larval stage.

Use of antibiotics to control proliferation of pathogens results in antibiotic-resistant bacterial strains. One recent method of disease control is by using probiotics. The daily addition of commercial probiotic (Aqualact) resulted in the significant increment in nauplii to postlarvae 3 survival, growth and disease resistance of postlarvae. Encouraging results were obtained with 0.4, 0.6 and 0.8 g/ton dosage addition to zoea, mysis and postlarval stage tanks respectively. Bacterial analysis of rearing water and larval samples confirmed the control of *Vibrio* population by the probiotic. No negative effect was noticed with probiotics usage as the water quality and total bacterial counts were not affected.

Other than the combination of bacteria, individual bacterial strain also acts as probiotic by inhibiting the growth of pathogens. Putative probiotic bacteria were isolated from rearing water of well performed larval tanks. The bacteria were identified and analysed for its colonisation potential and for control of deleterious *Vibrio* spp. associated with culture, through preemptive colonisation of the culture medium. The effect on larval performance was also analysed in the presence of putative probiotic bacteria. Characterisation confirmed the bacteria as *Vibrio alginolyticus*. The inoculation of mass cultured probiotic bacteria in a larval tank, resulted in the dominance of same and in simultaneous disappearance of the inoculated pathogen. The probiotic bacteria also improved the larval survival, growth and disease resistance.

A strategy for disease control, in addition to improving environmental conditions, must also include measures to increase larval resistance to infection. Enhancing the defence mechanisms of the host by immunostimulants is one way of achieving this. Three commercially available immunostimulants were attempted in the larval rearing tanks, at various dosages, regimes and routes of administration (immersion and oral). Results from the experiments favour the following method of immunostimulant administration, dosages and exposure periods for over all best survival, growth and disease resistance of larvae and postlarvae. For yeast glucan, it was the immersion of zoea 1, mysis 1 and postlarvae 1 and 3 for duration of 4.5 h in 0.075 mg/ml concentration. Vetrigard and levamisole gave the best results when incorporated in the feed (oral route) at a level of 20 mg/kg level and fed to first two stages of zoea, mysis and postlarva. Among the three immunostimulants, glucan was the most effective with the immersion method and vetrigard with oral route. Levamisole was found to be the least suitable for shrimp larviculture.

Restriction of *Artemia* feeding only during the initial stages of postlarva demands another source of exogenous enzymes and other nutrients supplied by live feed. In experiments conducted with 8 day old postlarvae (PL_8), the fresh biological feeds were evaluated as supplementary diet in the nursery tanks. Evaluation tools included growth analysis, survival and RNA/DNA ratio of PL_{20}. The supplementation of fresh feeds in a regime including commercial diet improved postlarval weight gain and survival. Nursery tanks which were fed with combination of commercial diet, fresh feeds and microalgae recorded the highest RNA/DNA ratio and growth. The results also demonstrated the assimilation of microalgae by postlarvae and advise its addition to nursery tanks. The RNA/DNA ratio exhibited strong relationship with shrimp growth rate (r^2=0.94) and very well demonstrated the greater sensitivity of the RNA/DNA index as an indicator of the physiological condition of shrimp postlarvae.