

stages in 9 to 11 days at 28°C C-33 ppt, 7 to 9 days at 33°C-33 ppt, and 13 to 15 days at 23°C-33 ppt.

Statistical analysis showed that salinity had highly significant effect on rates of hatching of eggs and survival from nauplius to first zoeal stage but not temperature although the latter had an apparent effect. However, both factors affected time of hatching of eggs and time of molting from nauplius to zoea. Interaction effect was significant only on rate and time of hatching. Different sources (spawners) of eggs and nauplii did not have significant effect on time of hatching and molting from nauplius to zoea, but significantly affected the hatching rate of eggs and survival rate of nauplii to zoea stage.

### The Influence of Temperature and Salinity on Oxygen Consumption of *Penaeus monodon* Postlarvae

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The effect of salinity and temperature on oxygen consumption at different developmental ages of *Penaeus monodon* postlarvae (P<sub>5</sub> to P<sub>60</sub>) was studied. The design was a 2 × 5 factorial, using two levels of temperature (15 and 30°C) and 4 levels of salinity (10, 15, 20 and 30 ppt). One-day old postlarvae (P<sub>1</sub>) were acclimated to various salinities prior to the start of the experiments. Oxygen consumption was determined after three hours using a YSI dissolved oxygen meter *vis-a-vis* Winkler titration method.

Respiratory activity as affected by temperature and salinity varies, dependent on the postlarval stage tested. Statistical analyses showed that temperature did not significantly influence oxygen uptake at early stages (P<sub>5</sub>-P<sub>8</sub>) until P<sub>25</sub>-P<sub>28</sub>. Its effect started to become apparent when the postlarvae were P<sub>35</sub>-P<sub>38</sub> and was most pronounced at P<sub>49</sub>-P<sub>52</sub>. In general, the postlarvae consumed more oxygen at higher temperature and the variation in the oxygen consumption of the postlarvae under the two temperatures become less obvious as the postlarvae were older. Salinity seemed to affect the oxygen consumption of the young postlarvae, P<sub>5</sub>-P<sub>8</sub> and P<sub>25</sub>-P<sub>28</sub>, more than temperature. Differences in rate of oxygen consumption at various salinities were greater in younger postlarvae (P<sub>5</sub>-P<sub>38</sub>) than in older postlarvae (P<sub>42</sub>-P<sub>60</sub>). The relationship between rate of oxygen consumption and body weight is nearly linear in the various salinity-temperature treatments. In all cases, the regression was significant at 1% level. *P. monodon* postlarvae behaved as respiratory conformers in all the salinities tested at ambient temperatures.

The least oxygen consumption rate was noted at salinities of 20 and 30 ppt at low temperature (15°C) and 20 ppt at high temperature (30°C). The importance of these findings is discussed and related to improvement of postlarvae transport methodology.

### Effect of Carrageenan Micro-Binded Diet on the Larval Stages of *Penaeus indicus*

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At present, most hatcheries depend on live food like diatoms, *Chlorella*, rotifer and brine shrimp to rear the larval stages of various penaeid species. Mass production of live feed requires much space (tanks) and labor, and is often affected by environmental conditions. The possibility of substituting live food with artificial diet for *Penaeus indicus* larvae was evaluated. Carrageenan micro-binded diet (C-MBD) was selected as test diet and its composition was modified from C-MBD designed for *P. japonicus* (about 45% protein).

Larvae stocked at 100/l and fed five times/day at 0.8 mg/larva/day had an average survival rate of 45% from Z<sub>1</sub> to M<sub>1</sub>. Water temperature was 26.5-30.5°C and salinity 32-33 ppt. An average survival rate of 70.2% from M<sub>1</sub> to PL<sub>1</sub> was attained when the stocking density was 30/l and feeding was three times/day at 0.3 mg/larva/day (water temperature 25.5-28.5°C, salinity 27-32 ppt). From PL<sub>1</sub> to PL<sub>5</sub> at stocking density of 20/l with feeding rate of 0.3 mg/larva/day (fed 3 times a day), the average survival rate was 64.9% (water temperature 25.5-28.5°C, salinity 28-32 ppt).

The results show that the present composition of C-MBD is highly effective for mysids up to the early postlarval stages of *P. indicus*.

### Effects of Diet on Reproductive Performance of Ablated *Penaeus monodon* Broodstock

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Four practical diets were compared for their effects upon ovarian maturation and spawning of ablated *Penaeus monodon* broodstock. Diets were formulated based upon the fatty acid profile of wild *P. monodon*. Diets 1 and 3 were cod liver oil-based while Diets 2 and 4 were soybean oil-based. Experimental treatments consisted of each of the formulated diets given in combination with natural food (squid, mussel, and annelids). An all-natural diet served as control. The fatty

acid composition and total lipid content of the diets and of *P. monodon* fed with these diets were assessed.

Reproductive performance was evaluated in terms of number of spawnings, fecundity, egg and nauplii production and hatching rate of eggs. Broodstock response was best in Diet 1 and comparable with the control, followed by Diets 3 and 4, and was poorest in Diet 2.

Broodstock performance appeared to be related to the fatty acid pattern of the diet. All pelleted diets contained similar levels of total lipids. However, there were differences in amounts of important polyunsaturated fatty acids (PUFA): 20:4 $\omega$ 6 (arachidonic), 20:5 $\omega$ 3 (eicosapentaenoic) and 22:6 $\omega$ 3 (docosahexaenoic) acids. The fatty acid profiles of Diets 1 and 3 more closely resemble the profile of maturing ovaries of wild *P. monodon*; the PUFA content of these diets and  $\omega$ 3/ $\omega$ 6 ratios were higher compared to Diets 2 and 4. Diet 2, showing the poorest profile among the diets, was low in  $\omega$ 3/ $\omega$ 6 ratio and contained minimal levels of PUFA.

### Study on the Larval Rearing of *Penaeus merguensis*

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Nursing postlarvae of *Penaeus merguensis* in the same tank as rearing always results in low survival rates, around 30%. One reason is that stocking density for P<sub>1</sub> is too high for postlarvae grown to P<sub>20</sub> size. Another reason may be that it is impossible to sufficiently clean a tank containing culture stock. In order to overcome the first constraint and to test whether the second is valid, rearing of nauplii to early postlarval stage was done in one tank, then early postlarvae were moved to another tank for nursing to P<sub>20</sub>.

Rearing was done in rectangular, concrete tanks (5 m  $\times$  5 m  $\times$  2m) of 50 ton capacity, with an initial stocking density of 20-40 nauplii/l. *Chaetoceros* sp. at a density of 3-4  $\times$  10<sup>4</sup> cell/ml, or *Tetraselmis* sp. at 1-3  $\times$  10<sup>4</sup> cell/ml were fed to zoea stage, then rotifer was given when the larvae metamorphosed to mysis stage. Within 8-10 days, when all of the larvae metamorphosed to postlarval stage, they were transferred to the nursing tank. Postlarval nursing was done in rectangular, concrete tanks with a capacity of 12 or 30 tons. The stocking rate was 12 postlarvae/l in the 12-ton tanks and 8 postlarvae/l in the 30-ton tanks. The early postlarvae were fed constantly with brine shrimp, and the older postlarvae were fed 4-5 times daily with squid meat. Fifty to seventy percent of seawater was exchanged, and siphoning of food remnants was done daily. The postlarvae grew to an intermediate size (1.0-2.5 cm total length) for stocking in grow-out ponds within 12 to 20 days.

The results of rearing in 50-ton tanks with an initial stocking density of 20-25 postlarvae/l, 25-30 postlarvae/l and 30-40 postlarvae/l produced survival rates of 74.3%, 63.6% and 47.6%, respectively. The survival rate for nursing in 12-ton

tanks, with stocking density of 12 postlarvae/l was 85.0% and for 30-ton tanks with stocking density of 8 postlarvae/l was 61.7%. These results seem to indicate that the rearing and nursing of shrimp would be more efficient if carried out in separate tanks.

### Characterization of Ovarian Maturation Stages in Wild Unablated *Penaeus monodon*

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At least five wild-caught *Penaeus monodon* from various maturation stages (initially classified *in vivo* as 0, I, II, III, IV, V) were measured, weighed and dissected for histological and histochemical studies. The anterior and posterior parts of the thoracic and abdominal regions of the ovary were sampled and stained with Mallory trichrome, alcian blue-periodic acid-Schiff (AB-PAS) and Sudan black.

Results showed that the ovary is composed of the ovarian wall and its extensions, zone of proliferation, follicle cell layer and oocytes. The proliferating cells are less than 10  $\mu$ m, have thin rims of cytoplasm, and increase in size as maturation proceeds. Based on histology, the stages were finally classified into groups (1) previtellogenic (stage 0), (2) vitellogenic (stages I and II), (3) cortical rod (stages III and IV), and (4) spent (stage V). The previtellogenic group consists only of perinucleolar oocytes (46-72  $\mu$ m) which are stained negatively with AB-PAS and Sudan black. Oocytes bigger than 55  $\mu$ m are enveloped by a single layer of follicle cells. The vitellogenic group is composed mostly of yolky oocytes (121-211  $\mu$ m) with the following cytoplasmic inclusions: small granules of glycoproteins, medium-size globules of lipoglycoproteins, and few large lipid droplets. The cortical rod group consists mostly of yolky oocytes (288-408  $\mu$ m) with additional rod-like bodies which contain acid and basic mucopolysaccharides but no lipid. The presence of cortical rods is a characteristic feature of mature penaeid ovaries. The spent group is similar to the previtellogenic group but contains some yolky oocytes, thicker follicle cell layers, or irregularly shaped perinucleolar oocytes. The GSI ranges of the four groups are 0.899-1.937, 3.099-7.598, 5.631-12.000 and 1.848-2.919, respectively.

### The Use of Haptophyceae in Rearing Experiments on Larval *Penaeus orientalis*

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The food value of five clones of Haptophyceae, *Coccolithus pelagicus*, *Dicrateria zhanjiangensis*, *Isochrysis galbana*,