Vol. 20 No. 6

FISHING CHIMES

Quality Assessment of Hatchery reared Post-Larvae of Tiger Shrimp *Penaeus monodon* (Fabricius)

Imelda Joseph*, L.H.Rao, S.S.Mishra *and* P.S.Sudheesh

Central Institute of Brackishwater Aquaculture, 101/B, Mahalingapuram, Chennai - 600 034

The authors followed a simple and practical methodology for determining the post-larval (PL) quality of *Penaeus monodon* produced in eight commercial shrimp hatcheries in and around Chennai. Studies were carried out by taking into consideration various parameters like visual observations, microscopic studies and stress tests by salinity drop to less than 50% of the ambient and exposure to formalin at 100 ppm and 200 ppm concentrations for 2 hours. PL from three of the hatcheries were found to be not of good quality as per the studied norms. MBV occlusions were present in 15% to 30% PL from another two hatcheries. Poor quality of post-larvae encountered in the present study can be attributed to lack of hygiene, improper feeding regime, and high stocking density which is stress inducing.

The induced maturation and breeding of *Penaeus monodon* in captivity was first developed in India in seventies (Alikunhi *et al* 1977; Alikunhi and Hameed Ali 1977; Muthu and Laxminarayana 1977). With the later standardisation of hatchery systems with designs, feeding schedules etc., amenable for convenient operation, shrimp hatcheries started proliferating not only along the Indian coastline but also along the coastline of the countries of the sub-continent.

It is reported that shrimp larval mortality is a global phenomenon and this is attributed to increased stocking density, inappropriate and insufficient feeding and occurrence of major disease problems (Liao 1985).

One of the major diseases of *P. monodon* was identified as monodon baculovirus (MBV) (Lightner and Redman 1981; Lightner 1983). It was found that a major factor for mortalities in shrimp ponds that occurred mainly in Andhra Pradesh and Tamil Nadu during nineties could be due to the outbreak of MBV. In fact, a similar situation of MBV occurrence was reported to be the cause of mass mortality of shrimps in Taiwanese shrimp ponds in 1988 (Liao 1989). In this background, the need for quality control measures for PL was emphasised and there were followed by several hatchery owners as well as farmers. PL harvest in hatcheries, transportation of PL to farms, their acclimation to farm conditions etc. are important aspects of shrimp farming (Olin and Fast 1989). There is a dearth information on PL quality studies and the present one was directed at evaluating the quality of PL of *P. monodon* produced in the eight commercial shrimp hatcheries.

Materials and Methods

A practical methodology was followed for quality evaluation of *P. monodon* post larvae. Various criteria followed for this include length and weight measurements, visual observations like movement, behaviour, feeding and pigmentation. Muscle to gut ratio (MGR) was estimated by microscopic observations using a calibrated oculomotor, where, the ratio of diameter of the sixth abdominal muscle to width of gut directly above it is determined (Bauman and Scura 1990). Further, moulting conditions, muscle deformities, hind gut appearance, appendages, setae, rostral spine count were also observed (Olin and Fast 1992). Routine microbiological observations for disease diagnosis (Bray and Lawrence 1989) like bacterial necrosis, filamentous forms, fungus, protozoan attack etc., were done, and MBV infections were diagnosed by their characteristic occlusion bodies in wet mounts of hepatopancreas using 0.05% malachite green stain. Stress tests by exposing PL to 100 ppm and 200 ppm formalin and salinity drop to 10 ppt from ambient for 2h (Tackaert *et al* 1989); Bauman and Jamandre 1990) were also done and found to be practicable and convenient for application to assess PL quality. Hatcheries were selected on random basis and samples were collected during February to May. PL was obtained in two cycles from each of the hatcheries.

Results and Discussion

Many workers have reported that PL length and weight are dependent on the diet supplied to them (Wilkenfield *et al.*, 1984; Fuze *et al.*, 1985 and Samocha *et al.*, 1989). The present study also has shown that size of the PL is dependent on the diet given but not much related to quality. PL of 2 to 8 mg and 9 to 13mm lengths were encountered in the present observations. Table 1 shows the mean length and weight of PL along with standard deviation, from different hatcheries. PL is generally planktonic for 5 to 7 days following its metamorphosis from mysis stage (upto PL_5 to PL_7). During this time they actively swim using their pleopods with occasional abrupt movements by vigorous tail flicks. The age of PL samples in the present study varied from PL_5 to PL_{20}. Erratic behaviour like irregular, zig zag or spiral swimming is

*Present address : NRC of CIBA, Narakkal, Cochin-682 505, Kerala

Table 1 : Mean length, weight, MGR and rostral spine count along with standard of Penaeus monodon Post larvae from different hatcheries

Hatchery Code	Length(mm)	Weight(mg)	Muscle: Gut Ratio (MGR)	Rostral Spine
I	8.7±0.46	2.1±0.51	5.6±0.87:1	3/1
II	10.5±0.84	2.8±0.36	3.8±0.68:1	3/2
III	12.0±0.98	6.7±1.13	3.9±1.90:1	6/2
IV	11.3±1.10	6.1±1.02	8.7±1.80:1	4/2
V	10.9±0.94	3.6±1.57	6.0±0.91:1	6/3
VI	9.5±0.50	2.6±0.50	6.3±0.74:1	4/2
VII	9.4±1.0	2.3±0.68	4.2±0.61:1	5/2
VIII	10.7±1.0	3.2±0.49	7.7±1.79:1	5/2

Table 2 : Morphological, physiological and pathological observations of *Penaeus monodon* Post larvae from different hatcheries

Parameters studied for quality assessment	Hatchery Code							
	I	II	III	IV	V	VI	VII	VIII
Fulness of Stomach.(%)	90	70	60	80	100	100	45	85
Muscle Deformity (%)	Nil	5	10	Nil	Nil	Nil	Nil	Nil
Incomplete Moulting (%)	10	10	15	5	Nil	Nil	Nil	Nil
Swollen Hind Gut (%)	Nil	Nil	10	10	Nil	Nil	Nil	Nil
Broken Appendages (%)	Nil	10	15	10	Nil	Nil	20	Nil
Bacterial Necrosia (%)	Nil	5	15	Nil	Nil	Nil	15	Nil
Filamentous Forms (%)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Fungus (%)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
MBV Occlusion bodies (%)	Nil	Nil	15	Nil	Nil	Nil	15	Nil
Protozoans (%)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Others (%)	Nil	30	Nil	Nil	Nil	Nil	Nil	Nil

Table 3 Showing survival rate of Penaeus monodon Post larvae from different hatcheries on exposure to stress tests

Hatchery Code	% survival at 10ppt salinity	% survival at 100ppm formalin	% survival at 200ppm formalin
I	80	87	80
II	46	70	68
III	87.3	10	7
IV	88	93.3	90
V	80	90	90
VI	100	100	100
VII	75	65	60
VIII	100	100	100

indicative of either physical deformities or other problems of PL. They should neither be lethargic nor should bounce off from the bottom of rearing tanks (Olin and Fast 1992). It is observed in the present study that Pls (I,IV,V,VI & VIII) from five hatcheries were satisfactory in their visual qualities. These PLs were actively swimming, some benthic, well fed with normal pigmentation and well spread tail fan. Seeds from other hatcheries (II, III and VII) were slender, less active and were poorly fed with some abnormal pigmentation (II and III). Reddish pigmentation results from stressed hatchery conditions (Bauman and Jamandre 1990).

Table 2 shows the results of morphological, physiological and pathological observations of PL from different hatcheries. Feeding, as evidenced by full gut, is not clearly conclusive of PL quality. All PL from hatcheries coded as V and VI had full guts. Others had 45 to 95% PL with full gut. It was noticed PLs at hatcheries II, III and VII, which had 30% and more PL with empty gut were not of good quality. This is in conformity with the results of Bauman and Jamandre (1990) who reported that PL stock with 30% or more of it with empty gut was not of good quality as per stress test result obtained. Muscle deformity was observed in 5 to 10% of PL from hatcheries II and III. Broken appendages were present in the range of 10 to 15% PL from three hatcheries (II, III and IV). As per reports, for a healthy PL, the hepatopancreas and anterior gut should be full and well developed, without any swelling in the hind gut. The appendages should be in tact without any deformities (Olein and Fast 1992). The criteria were satisfactory in the case of PL from two hatcheries only (V and VIII).

The data on pathological observation (Table 2) shows 5 to 15% of PL from three hatcheries (II, III and VIII) were infected with bacterial necrosis. All PL from all the eight hatcheries were free from filamentous forms, fungi and protozoans. Hatchery II had 30% of its seed infested with *Zoothamnium* sp. MBV occlusion bodies were encountered in 15% of PL from two hatcheries (III and VII). The intra nuclear occlusion bodies in hepatopancreas have been reported from PL_2 stage onwards (Chen 1989a). In Thailand, PL are detected with MBV occlusions as early as PL_5 and PL_{10} (Nash 1990).

Table 4 : Recommendations and suggestions drawn out of *Penaeus monodon* quality assessment studies

Hatchery Code	Recommendations and Suggestions
I	PL are healthy, and of good quality, are well suited for stocking in ponds.
II	Quality not upto the mark and not suitable for stocking. Better hygienic practices are to be followed and feeding regime should be modified.
III	Quality not good enough for stocking in ponds. In this hatchery, stocking has to be reduced, needs more water exchange and health monitoring.
IV	Healthy and well fed PL : recommended for stocking in the ponds.
V	Healthy and well fed post larvae: recommended for stocking in the ponds.
VI	Healthy and good quality PL : recommended for stocking in ponds.
VII	Inferior quality PL : not recommended for stocking, MBV occlusion very high. Seeds are to be discarded.
VIII	Very healthy PL: Recommended for grow out stocking.

For quality assessment by pathological observations, it is important to note that a healthy PL does not accommodate any type of pathogenic bacteria, fungi or protozoa in its body. The setae and gill chambers will be clean of debris and bacteria (Olin and Fast 1992). In the present study also, healthy PL from three hatcheries were free of all types of bacteria and other forms.

The stress tests were intended to evaluate PL hardiness by subjecting them to sudden change in salinity and exposure to high concentrations of formalin. Formalin is used for the above test because, it is being widely used as a disinfectant or for fast moulting of PL in hatcheries. Also formalin is easily available in all the hatcheries to perform such tests. It was kept in view that higher concentration (100 and 200ppm) of formalin will inflict some extent of stress on the PL causing a sort of irritation. Exposure to low salinity to cause stress on PL is very much in practice. This bioassay would allow the identification of healthy post larvae, which is not evident otherwise (Gomez *et al* 1991). *P. monodon* could tolerate an abrupt reduction in salinity from 30 ppt with less than 20% mortality, if the range of reduction was within 20ppt. In the present study, it is observed that, out of the PL from one of the hatcheries (hatchery II) only 46% survived at 10ppt salinity. PL from all the other hatcheries showed 80 to 100% survival after 2 h exposure in 10ppt salinity (Table 3). Charmantier *et al* (1988) found that PL should be more tolerant to salinity variations upto PL_{20} stage. They also found out that age influences the acclimation capacity of penaeid post larvae. Ontogeny of osmoregulatory capabilities with larvae and post larvae of *P. japonicus* is reported by the above workers. Comparing the results obtained by the authors with those published for different species, the authors found that age of the PL and feeding regime followed during the larval rearing period assured good quality PL. Also, as stated by Gomez *et al* (1991), it can be concluded that strong salinity resistance may be a security factor for grow-out performance of PL. A study by Tackaert *et al* (1989) showed that resistance of PL to salinity stress could be enhanced with n-3 HUFA enrichment of Artemia when it is supplied as food source.

Data of survival percentage of PL in the two formalin concentrations (100 and 200 ppm) are shown in Table 3. Effects of two formalin concentrations on the fry do not differ significantly. PL from three hatcheries (II,III and IV) showed less than 70% survival. The rest were considered hardy and healthy PL. On exposure to 100 and 200 ppm formalin (35% aqueous solution) for 2 h, less than 30% mortality is expected for quality ones (Olin and Fast 1992).

The evaluation results showed that Muscle-Gut Ratio (MGR), diagnosis of MBV occlusion bodies and stress tests are suitable criteria, which are useful to differentiate healthy PL from others. It is recommended to discard PL with MBV occlusion bodies and MGR less than 4:1. Visual and other microscopic observations were not found to be truly conclusive of quality studies, though they were useful. Also it was found necessary to have details about larval rearing period for knowing the feeding regimes, water quality standards, stocking density and hygiene practices followed in various hatcheries. Age also determines the grow-out performance of PL and it is concluded that PL of *P. monodon* will be the best for stocking at PL_{18} to PL_{20} (10-12mm length) stages. All the tests followed for quality assessment of PL were found to be ideal for laboratory as well as field conditions and they do not require high scientific skill or sophisticated equipments.

Further rearing of PL from all hatcheries in post larval tanks for 10 to 20 days from the date of procurement was also carried out during this study. They were fed with chopped clam meat and particulate feed. Post-larvae from five hatcheries (I,IV,V,VI and VIII) had shown higher survival rate (above 80%).

Recommendations of the study along with remarks are given in Table 4. PL from hatcheries V, VI and VIII were of very good quality and those from II and IV were of quality above average. Post larvae from hatcheries II, III and VII were found unsuitable for stocking

in grow-out ponds. Also it should be borne in mind that good quality PL will get easily acclimatized to the stress during transport, harvest and to varying conditions in grow-out systems. It is possible to produce good quality PL only by following strict and optimal larval rearing practices.

Further, PCR techniques followed in quality assessment of broodstock and of post-larvae collected from wild as well as from hatcheries, would need more of sophistication.

Acknowledgement

The authors are thankful to the Director, Central Institute of Brackishwater Aquaculture (CIBA), Chennai for the facilities provided. Their thanks are also due to the various shrimp hatchery owners who supplied us the post larvae of *P. monodon*.

References

ALIKUNHI, K.H., A. POERNOMO, S.ADISUKRENSO, M.BUDINO AND S.BUSMAN, 1975. Preliminary observations on induction of maturation and spawning in *P. monodon* Fabricius and *P. merguiensis* de Man by eyestalk extirpation. *Bull Shr Cult Res Cent*, 1:1-11.

ALIKUNHI, K.H. and HAMEED ALI, 1978. Induction of maturation and spawning on pond grown stocks of penaeid shrimps for large-scale seed production (Abstract). Proceedings of first National Symposium on Shrimp Farming, 16-18, Bombay.

Bauman, R.H. and D.R. JAMANDRE, 1990. A practical method for determining quality of *Penaeus monodon* (Fabricius) fry for stocking in grow out ponds. In Technical and economic aspects of shrimp farming. Proceedings of the Aquatech '90 conference, ed. M.B New, H. de Saram and T. Singh, *Infofish*, Kuala Lampur, Malaysia, pp124-131.

BAUMAN, R.H. and E.D. SCURA, 1990. Determining the quality of *Penaeus monodon* post larvae (Abstract) World Aquaculture Society 21st annual conference, 101p.

CHARMANTIER, G., M. CHARMANTIER CAURES, N. BOURAVICHA, P.THUET, D.E. AIKEN and J.P. THRILLES, 1988. Ontogeny of osmoregulation and salinity tolerance in two decapod crustaceans: *Homarus americanus* and *Panaeus japonicus* Boll Bull., 175: 102-110.

COUCH, J. A., 1974. An enzootic nuclear polyhidrosis virus of pink shrimp: ultrastructure, prevalence and enhancement. *J Invert Pathol.*, 24: 311-331.

COUCH, J. A., 1981. Viral diseases of invertebrates other than insects. In: ed. E.W. Davidson, Pathogenesis of invertebrate diseases. Allanheld, Osmun Publ., Totowa, N.J. pp127-160.

FUZE, D.M., J.S. WILKENFIELD and A.L. LAWRENCE, 1985. Studies on the use of boiled chicken egg yolk as feed for rearing penaeid shrimp larvae, Texas *J Sci.* XXXVII: 371-388.

GOMEZ, R. D., J.M. RODRIGUEZ and J.MORALES, 1991. Stress tests: a practical tool to control post larval shrimp quality. In: ed. P.Lavens, P. Sorgeloos, E. Jaspers and F. Ollevier, Larvi'91-Fish and Crustacean Larviculture Symposium, *European Aquaculture Society, Special Publication*, No. 15, Gent, Gelgium.

HIRONO, Y. and M. LESLIE, 1992. Shrimp culture industry in Ecuador. In: eds. A.W. Fast and J. Lester, Marine Shrimp culture : Principles and practices. Elsevier Science Publishers pp783-835.

LIAO, I.C., 1985. A brief review of the larval rearing techniques of penaeid prawns. In: eds. Y.Taki, J.H. Primavera and J.A. Liobrena, Proceedings of the first international conference on the culture of penaeid shrimps, Iloilo City, Philippines, pp65-78.

LIAO, I.C., 1989. *Penaeus monodon* culture in Taiwan: Through two decades of growth. *Int J Fish Technol.*, 1:16-24.

LIGHTNER, D.V., 1983. Diseases of cultured penaeid shrimp. In: ed. J.P. McVey, CRC Handbook of Mariculture, Voll, Crustacean aquaculture, CRC Press. Inc., Boca Raton, Florida, pp289-320.

LIGHTNER, D.V., 1988. Diseases of cultured penaeid shrimps and prawns. In eds. C.J. Cinderman and D.V. Lightner, Disease diagnosis and control in North American marine aquaculture, Elsevier, Amsterdam, pp8-133.

LIGHTNER, D.V. and REDMAN R.M., 1981. A baculovirus caused disease of the penaeid shrimp, *Penaeus monodon*, *J Invert Pathol.*, 38: 299-302.

LIGHTNER, D.V., R.M. REDMAN and T.A. BELL, 1983c. Observations on the geographic distribution, pathogenesis and morphology of the baculovirus from *Penaeus monodon* Fabricius. *Aquaculture*, 32:209-233.

MUTHU, M.S. and A.LAXMINARAYANA, 1977. Induced maturation and spawning of Indian penaeid prawns. *Indian J Fish.*, 24: 172-180.

NASH, G.L., 1990. *Penaeus monodon* grow out diseases. In: eds., M.B.New, H de Saram and T.S.Singh, Technical and economic aspects of shrimp farming. Proceedings of the Aquatech '90 Conference, Infofish, Kuala Lumpur, Malaysia, pp124-131.

OLIN, P.G. and A.W. FAST, 1992. Penaeid PL harvest, transport, acclimation and stocking, In: eds, A.W. Fast and L.J. Lester, Marine shrimp culture: Principles and practices, Elsevier Science Publishers, B.V. pp301-329.

TACKAERT, W.P.ABELIN, P.DHERT and P.SORGELOOS, 1989. Stress resistance in post larval penaeid shrimp reared under different feeding procedures (Abstract), *J World Aquacult Soc.*, 20:74p.

WILKENFIELD, J.S., A.L. LAWRENCE and F.D.KUBAN, 1984. Survival, metamorphosis and growth of penaeid shrimp larvae reared in a variety of algal and animal foods, *J World Aquacult Soc.*, 15:31-49.