

RESEARCH ARTICLE

A comparative study on the quality of postlarvae of Black Tiger Shrimp, *Penaeus monodon*, produced in hatcheries in Sri Lanka

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Abstract: Stocking of high quality postlarvae in grow-out systems is regarded as one of the key factors that influences the quality of cultured shrimp at harvest. Therefore it is important to investigate whether there are differences in the quality of postlarvae produced in hatcheries with different management systems. A survey was carried out and shrimp hatcheries situated in the North Western Province were categorized into 3 major types as hatcheries with a high level of management, medium level of management and low level of management, considering the facilities available and production procedures adopted in them. A less expensive scoring system was developed to assess the quality of postlarvae using external appearance to the naked eye, behavioural patterns, length variation, appearance under the microscope, gut to muscle ratio, gut fullness, survival under stress tests and occurrence of certain disease symptoms. Nine shrimp hatcheries, three from each level of management were then selected, five random samples each containing more than three hundred postlarvae ($PI_{15} - PI_{18}$) were obtained from each hatchery and the quality of postlarvae was assessed using the scoring system developed.

When mean total scores received from the scoring system were compared, it was found that the quality of postlarvae produced in the hatcheries with high and medium levels of management was significantly higher than that of postlarvae produced in the hatcheries with low level of management ($p < 0.05$) while there was no significant difference between the quality of postlarvae produced in the hatcheries with high and medium levels of management ($p > 0.05$).

Key words: Black tiger shrimp, cultured shrimp, *Penaeus monodon*, postlarvae, Sri Lanka.

INTRODUCTION

Most Asian countries producing cultured shrimp have faced difficulties since early 1990's in relation to disease conditions and quality of shrimp at harvest.¹ Major importers such as the European Union have set new detectable limits for banded antibiotics in imported shrimp.² Bangladesh receives 10% less for its value of shrimp than its competitors because of perception worldwide that the shrimp of Bangladesh are not

clean.³ Thailand also suffered a setback due to stunting syndrome. The cause was multifactorial and one of the factors was poor quality postlarvae.⁴ The quality of postlarvae is one of the key factors which influences successful production of high quality marketable cultured shrimp.⁵

Different countries employ different strategies to determine the quality of postlarvae, before stocking in grow-out systems. These include Polymerase Chain Reaction (PCR) technology to detect the presence or absence of certain disease causing agents.^{4,6,7} In Sri Lanka, there are few private and government laboratories with PCR technology to check whether the postlarvae are carriers of White Spot Syndrome Virus (WSSV). This is an expensive test and a majority of Sri Lankan shrimp hatchery managers is unable to use PCR as a routine test to assess the quality of stocks of postlarvae. They would certainly prefer a less expensive quality assessing method that could be performed at hatchery level for routine assessments, the results of which could be confirmed by PCR testing just before the postlarvae are sold to grow-out farmers.

The quality of postlarvae is influenced by the production procedure adopted in hatcheries which depends on the level of management.⁸ The present study contributes to develop a method to assess the quality of postlarvae produced under different management levels.

METHODS AND MATERIALS

A survey was carried out using a questionnaire and visits to twenty seven shrimp hatcheries situated in the North Western Province and these hatcheries were categorized

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into 3 major types as hatcheries with a high level of management, medium level of management and low level of management considering the facilities available and the production procedure adopted in them (Table 1). Nine shrimp hatcheries, three from each level of management were selected to study the quality of postlarvae produced; the study was carried out from February to August 2003. Shrimp hatcheries that employed similar feeding regimes in larval rearing were selected for this study in order to minimize the effect of food and feeding on the quality of postlarvae.

Five random samples, each containing more than three hundred, 15 d - 18 d old postlarvae (Pl_{15} to Pl_{18}), were obtained from each hatchery, transported to the laboratory and were released into separate glass aquaria

(60 cm x 30 cm x 30 cm) giving minimum stress. The aquaria were first filled with water obtained from respective shrimp hatcheries from where the samples of postlarvae were obtained. Continuous aeration was arranged and the postlarvae were allowed to acclimatize for 24 h and fed with the same feed and same ration (similar to how they were fed at the respective hatcheries) over the last 18 h of acclimatization. At the end of acclimatization, postlarvae were subjected to quality assessment tests (Table 2) and the quality of each sample of postlarvae obtained from each hatchery was assessed using the scoring system developed (Table 3). Mean total scores assigned to the quality of postlarvae produced in hatcheries with different management levels were then compared using one way analysis of variance (ANOVA) and Tukey's pairwise comparison tests.

Table 1 : Facilities available and production procedures adopted in hatcheries with three different management levels.

Hatchery type: Level of management	Features / Facilities available
1. High	* A water treatment system (with sand filters, mechanical filters and UV or ozone sterilization) is available. * An indoor algae culture room is available with pure species of unicellular algae for scaling up as required to feed protozoa larvae. * An in-house laboratory is present with at least one trained personnel to, i. monitor growth of shrimp larvae at each developmental stage ii. monitor health status of shrimp larvae including inherited abnormalities, weaknesses etc. iii. monitor water quality iv. monitor quality of algae * In-house broodstock maturation tanks (with a quarantine tank, a treatment tank, etc.) are available. * Properly built Larval Rearing Tanks (LRT) and nursery tanks are available; size (length, width and depth), shape etc. of these tanks are most appropriate for the developmental stage reared in.
2. Medium	* A water treatment system (with filters and facilities for sterilization) is available. * Algal stocks are obtained from other hatcheries (where indoor algae culture is done) as required. * No in-house laboratory; but a skilled personnel with scientific knowledge is available to monitor health of developmental stages of shrimp larvae by external observations / behavioural changes. * In-house broodstock maturation tanks are available; however sometimes nauplii are obtained from other hatcheries and further rearing is done (upto Pl_{18} - Pl_{20}). * Properly built larval rearing tanks and nursery tanks are available.
3. Low	* No water treatment system. * Algae are produced in outdoor tanks by fertilization of raw sea water. * No in-house laboratory; no skilled personnel; managed by people with a low level of knowledge acquired trial and error methods / experience. * No broodstock maturation tanks; copulated gravid females are collected from the sea and are used to obtain nauplii. * Larval rearing tanks are available; these tanks may or may not be built properly to suit the different developmental stages.

Table 2 : The scoring system developed to assess the quality of shrimp postlarvae.

Parameter	Maximum score	Description	Marks allocated
1. External appearance to the naked eye			
a) Colour and appearance of the shell	3	Percentage of postlarvae having oily shells of light to dark brown colour 75 - 100 50 - 75 < 50	3 2 1
b) Colour of hepatopancreas ^a	4	Percentage of postlarvae with orangish yellow coloured hepatopancreas 75 - 100 50 - 75 < 50	4 2 1
c) Appearance of appendages	3	Percentage of postlarvae with complete appendages 75 - 100 50 - 75 < 50	3 2 1
2. Behavioural pattern of postlarvae			
a) Swimming direction ^b	4	Percentage of postlarvae that swam against the water current 75 - 100 50 - 75 < 50	4 2 1
b) Position of first antennae when swimming	3	Percentage of postlarvae with 'V' shaped first antennae 75 - 100 50 - 75 < 50	3 2 1
c) Condition of uropod when swimming	3	Percentage of postlarvae with spread uropod 75 - 100 50 - 75 < 50	3 2 1
d) Reaction to disturbance ^c	5	Percentage of postlarvae jumped off 75 - 100 50 - 75 < 50	5 3 1
3. Length variation (coefficient of variation) ^d	10	< 5 5 - 10 10 - 12 12 - 15 > 15	10 8 6 4 0
4. External appearance under microscope			
a) Condition of the shell ^e	5	Percentage of postlarvae with transparent shells 75 - 100 50 - 75 < 50	5 3 1
b) Shape of chromatophores ^f	5	Percentage of postlarvae with star shaped chromatophores 75 - 100 50 - 75 < 50	5 3 1

a = a prominent indicator of the health condition

b = a good indicator in identifying postlarval quality

c = one of the best indicators and also easy to observe

d = a sensitive indicator

e = transparency is a good indicator in quality assessment

f = very important to identify the health condition

Table 2 continued

Parameter	Maximum score	Description	Marks allocated
c) Condition of the hepatopancreas	3	Percentage of postlarvae having hepatopancreas without black spots 75-100 50-75 < 50	3 2 1
d) Number of rostral spines	3	Percentage of postlarvae with more than 5 rostral spines 75-100 50-75 < 50	3 2 1
5. Gut : muscle ratio ^g	10	Percentage of postlarvae with 1:4 gut : muscle ratio 85-100 70-85 50-70 < 50	10 7 5 3
6. Gut fullness ^h	10	Percentage of postlarvae with full gut 85-100 70-85 50-70 < 50	10 7 5 3
7. Stress tests		Percentage survival rate	
a) Salinity stress test	5	> 80 < 80	5 0
b) Formalin stress test	5	> 80 < 80	5 0
c) Temperature stress test	4	> 80 < 80	4 0
8. Disease symptoms of postlarvae			
a) External fouling organisms; debris	5	Present (P); Present (P) Present (P); Absent (Ab) Absent (Ab); Present (P) Absent (Ab); Absent (Ab)	0 3 3 5
b) Necrosis	5	Percentage of postlarvae with necrosis on the shell 0-20 20-50 < 50	5 3 0
c) MBV occlusion bodies in the hepatopancreas	5	Present Absent	0 5

g = regarded as one of the best parameters to assess the quality

h = an important parameter in quality assessment

Table 3 : Allocation of marks to the samples of postlarvae obtained from different hatcheries using the scoring system developed.

Feature	Hatchery type: Level of management								
	High			Medium			Low		
	1	2	3	1	2	3	1	2	3
1. External appearance to the naked eye (Total marks 10)									
* Mean % ($\pm$ SE) of Pl having healthy colour and appearance of shell (Marks 3)	93 ± 1.2	96 ± 2.1	95 ± 1.5	90 ± 2.7	85 ± 1.8	82 ± 1.2	70 ± 2.4	82 ± 1.6	80 ± 2.2
Marks allocated	3	3	3	3	3	3	2	3	3
* Mean % ($\pm$ SE) of Pl having healthy colour of hepatopancreas (Marks 4)	84 ± 2.2	80 ± 1.8	82 ± 1.0	74 ± 1.4	80 ± 1.4	70 ± 2.1	68 ± 2.0	74 ± 1.1	70 ± 2.4
Marks allocated	4	4	4	2	4	2	2	2	2
* Mean % ($\pm$ SE) of Pl with complete appendages (Marks 3)	99 ± 1.0	96 ± 1.1	99 ± 0.5	88 ± 1.8	80 ± 1.2	80 ± 2.1	76 ± 3.1	80 ± 4.1	76 ± 1.7
Marks allocated	3	3	3	3	3	3	3	3	3
2. Behavioural pattern (Total marks 10)									
* Mean % ($\pm$ SE) of Pl that swam against the water current (Marks 4)	90 ± 1.2	93 ± 1.1	86 ± 1.4	90 ± 2.4	88 ± 2.2	84 ± 3.0	74 ± 2.8	80 ± 1.9	80 ± 2.0
Marks allocated	4	4	4	4	4	4	2	4	4
* Mean % ($\pm$ SE) of Pl keeping first antennae in a 'V' shape when swimming (Marks 3)	84 ± 1.2	80 ± 1.1	88 ± 1.7	80 ± 2.4	84 ± 1.6	78 ± 1.8	68 ± 2.5	72 ± 1.4	78 ± 1.9
Marks allocated	3	3	3	3	3	3	2	2	3
* Mean % ($\pm$ SE) of Pl with a spread uropod when swimming (Marks 3)	78 ± 1.2	72 ± 0.8	76 ± 0.9	70 ± 2.4	66 ± 1.9	60 ± 3.5	60 ± 2.4	64 ± 3.1	66 ± 2.9
Marks allocated	3	2	3	3	2	2	2	2	2
* Mean % ($\pm$ SE) of Pl jumped off as a response to sudden disturbance (Marks 5)	78 ± 1.2	82 ± 1.0	84 ± 1.4	80 ± 1.6	80 ± 2.8	76 ± 1.5	46 ± 3.8	56 ± 5.1	84 ± 2.7
Marks allocated	5	5	5	5	5	5	1	3	5
3. Variation in body length (Total marks 10)									
* Mean percentage cumulative variance of length of Pl ($\pm$ SE)	6.53 ± 0.18	6.75 ± 0.80	7.23 ± 0.16	8.14 ± 0.90	9.95 ± 1.80	5.09 ± 2.60	11.82 ± 1.20	9.14 ± 1.40	5.94 ± 2.80
Marks allocated	8	8	8	8	8	8	6	8	8

Table 3 continued

Feature	Hatchery type: Level of management								
	High			Medium			Low		
	1	2	3	1	2	3	1	2	3
4. External appearance under the microscope (Total marks 16)									
* Mean % ($\pm$ SE) of PI with transparent shells (Marks 5)	84 ± 1.4	76 ± 0.4	80 ± 1.1	56 ± 2.8	48 ± 1.9	64 ± 3.3	30 ± 1.7	46 ± 2.6	40 ± 1.8
Marks allocated	5	5	5	3	1	3	1	1	1
* Mean % ($\pm$ SE) of PI with star shaped chromatophores (Marks 5)	97 ± 2.2	92 ± 1.3	94 ± 0.9	88 ± 1.7	80 ± 1.6	96 ± 2.0	50 ± 1.3	60 ± 1.6	72 ± 2.2
Marks allocated	5	5	5	5	5	5	3	3	3
* Mean % ($\pm$ SE) of PI without black spots on hepatopancreas (Marks 3)	96 ± 1.8	84 ± 2.2	88 ± 1.3	84 ± 2.0	76 ± 1.7	80 ± 1.8	68 ± 2.3	80 ± 1.9	70 ± 1.6
Marks allocated	3	3	3	3	3	3	2	3	2
* Mean % ($\pm$ SE) of PI with 5 to 6 rostral spines (Marks 3)	88 ± 1.5	85 ± 1.4	89 ± 2.0	72 ± 1.6	88 ± 1.2	76 ± 2.0	60 ± 1.9	64 ± 1.8	68 ± 3.0
Marks allocated	3	3	3	2	3	3	2	2	2
5. Gut to muscle ratio (Total marks 10)									
* Mean percentage ($\pm$ SE) of PI having gut to muscle ratio of 1:4	86 ± 1.1	60 ± 2.1	71 ± 2.2	55 ± 2.2	60 ± 2.7	56 ± 1.9	40 ± 3.0	40 ± 1.9	42 ± 2.1
Marks allocated	10	5	7	5	5	5	3	3	3
6. Gut fullness (Total marks 10)									
* Mean percentage ($\pm$ SE) of PI with the gut full of food	85 ± 2.2	90 ± 1.1	76 ± 2.1	76 ± 2.2	52 ± 1.5	86 ± 1.1	56 ± 2.4	62 ± 3.0	74 ± 3.1
Marks allocated	10	10	7	7	5	10	5	5	7
7. Symptoms of certain diseases (Total marks 15)									
* Mean percentage of survival of PI under salinity stress ($\pm$ SE) (Marks 5)	100 -	100 -	100 -	98 ± 0.2	98 ± 0.8	100 -	76 ± 2.1	78 ± 3.5	78 ± 2.8
Marks allocated	5	5	5	5	5	5	0	0	0
* Mean percentage of survival of PI under formalin stress ($\pm$ SE) (Marks 5)	100 -	96 -	100 -	90 ± 2.2	96 ± 2.5	94 ± 2.8	78 ± 3.2	84 ± 2.6	75 ± 3.8
Marks allocated	5	5	5	5	5	5	0	5	0
* Mean percentage of survival of PI under temperature stress ($\pm$ SE) (Marks 4)	100 -	96 ± 2.2	96 ± 1.8	88 ± 2.5	96 ± 2.4	90 ± 3.0	62 ± 1.9	74 ± 2.6	76 ± 2.7
Marks allocated	4	4	4	4	4	4	0	0	0

Table 3 continued

Feature	Hatchery type: Level of management								
	High			Medium			Low		
	1	2	3	1	2	3	1	2	3
8. Symptoms of certain diseases (Total marks 15)									
* Presence (P) or absence (Ab) of external fouling organisms / debris in PI (Marks 5)	Ab/ Ab	Ab/ Ab	Ab/ Ab	Ab/ Ab	Ab/ Ab	Ab/ Ab	P/P	Ab/ P	Ab/ P
Marks allocated	5	5	5	5	5	5	0	3	3
* Mean % ($\pm$ SE) of PI with necrosis on the exoskeleton (Marks 5)	10 ± 1.1	8 ± 0.9	4 ± 0.2	24 ± 2.0	34 ± 1.9	10 ± 0.9	64 ± 2.5	32 ± 1.9	48 ± 2.3
Marks allocated	5	5	5	3	3	5	0	3	3
* Presence (P) or absence (Ab) of MB V occlusion bodies in hepatopancreas of mid gut of PI (Marks 5)	Ab	Ab	Ab	Ab	Ab	Ab	P	Ab	Ab
Marks allocated	5	5	5	5	5	5	0	5	5
Total score received by the sample of PI from each hatchery	98	92	92	83	81	88	36	60	59
Mean total score received by each type of hatchery ($\pm$ SE)	94.0 $\pm$ 1.999			84.0 $\pm$ 2.082			51.7 $\pm$ 7.839		

RESULTS

Facilities available and production procedures adopted in the 3 major types of shrimp hatcheries situated in the North Western Province are given in Table 1, and Table 2 illustrates the scoring system developed. Marks allocated under the scoring system to the samples of postlarvae obtained from each hatchery is given (Table 3) with the mean total score received by the postlarvae from each type of hatchery. One way analysis of variance (ANOVA) showed that the mean values of total scores of samples of postlarvae from three different hatcheries were significantly different ($p < 0.05$). Tukey's pairwise comparisons showed that the mean total scores of the postlarvae produced in the hatcheries with high and medium levels of management were significantly higher than that of postlarvae produced in the hatcheries with a low level of management ($p < 0.05$; Table 3). This indicates

that the quality of postlarvae produced in the former two types of hatcheries were significantly higher than that of postlarvae produced in the latter type of hatcheries. Similarly, the comparison of the mean total scores of samples of postlarvae showed that there was no significant difference between the quality of postlarvae produced in the hatcheries with high and medium levels of management ($p > 0.05$).

DISCUSSION

Many developing, industrialized and Low Income Food Deficient (LIFD) countries are trying to produce quality shrimp products because 'quality' is very significant in the present global market.⁹⁻¹² Sri Lanka also has to pay greater attention to the quality of its shrimp products to maintain a position at the world trade of shrimp as disease outbreaks and the effect of environmental degradation could cause adverse effects on the demand and prices of Sri Lankan shrimp products.

The quality of cultured shrimp harvests is highly influenced by the quality of postlarvae stocked in grow-out systems. Therefore, the shrimp hatchery sector needs to ensure a stable supply of high quality postlarvae in response to the demand of grow-out farmers. This can be approached by quality assurance of postlarvae at the point of sale to farmers and by improved quality management in the production procedures.¹³ During larval stages, the shrimp will grow and develop various organs and appendages of the body that play different roles in activities like swimming, holding, biting and digesting food, producing secretions such as enzymes, hormones and secretions responsible for osmoregulation. The processes of this development are influenced by nutrition and the environmental conditions created by the rearing procedure. Thus the quality of postlarvae is influenced by the production procedure in hatcheries and in turn the quality of postlarvae influences the further viability of the growing shrimp.⁸

All shrimp hatcheries share the same basic infrastructure.¹⁴ However, according to the information gathered by the present study, major differences could be seen in shrimp hatcheries with regard to the size, available facilities and availability of services of trained personnel resulting in differences in the production procedure.¹⁵ Therefore, it is important to know whether there is a relationship between these differences in shrimp hatcheries and the differences in the quality of postlarvae produced in these hatcheries.

Fifteen to eighteen day old postlarvae (PL₁₅ to PL₁₈) were used for the quality assessment tests during the present study considering the preferred age of stocking of postlarvae in grow-out systems. It is also stated that⁴ fifteen day old postlarvae (PL₁₅) are suitable for quality assessment.

Though the criteria used for evaluating shrimp postlarvae is far from perfect, it has become an effective tool for hatchery practices during larval rearing and provides indices for good postlarval selection.¹⁶ There are many methods to assess the quality of postlarvae, but commonly used methods include the stress tests, gut fullness and size variation.⁶

The quality of postlarvae could be assessed by observations made with the naked eye as well as observations made under a light microscope.⁸ During the present study, almost all available methods were combined, except molecular biological methods, in assessing the quality of postlarvae produced in hatcheries

with different levels of management. Lack of standardization of postlarval quality assessing methods makes it difficult to draw conclusions or assess the worth of any particular test.⁶ The current interest of shrimp farming countries will lead to the development of a more standardized system for assessing the quality of postlarvae and even predicting pond performances (performances in grow-out systems) of these postlarvae.⁶ This system can be evaluated for accuracy and cost effectiveness.

Quality assessment procedure (scoring system) developed during the present study could be regarded as an effective method to compare and assess the quality of postlarvae produced in Sri Lankan hatcheries with different management levels.

According to a definition presented,¹⁷ high quality postlarvae should adapt well to the environmental conditions prevailing in grow-out ponds, grow faster accepting the given feed and reach the expected size within the culture cycle, with high survival under good management practices. During the present study it was found that the quality of shrimp postlarvae produced in hatcheries with high and medium levels of management were significantly higher (Table 3) than that of the postlarvae produced in hatcheries with a low level of management. It is essential to investigate how these postlarvae perform in grow-out ponds and the quality of marketable shrimp at harvest so that the quality of postlarvae assessed by the scoring system developed could be related to the quality of shrimp at harvest. Such an investigation would facilitate the prediction of pond performance of a stock of postlarvae.

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