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Comparative quality assessment of wild and hatchery-produced black tiger shrimp (*Penaeus monodon*) post-larvae in pre-stocking nursing condition

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Abstract. Farmers of Bangladesh like to stock wild post-larvae (PL) of Black tiger shrimp (*Penaeus monodon*). However, wild PL collection is detrimental to aquatic biodiversity, and the supply of hatchery PL is sufficient. The study was conducted in Cox's Bazar, Bangladesh, to compare the quality aspects of wild and hatchery PL in terms of size variation, survival, tolerance, and morphology. The study had one month duration and consisted of treatment 1 (T1, wild PL) and treatment 2 (T2, hatchery PL) with three replications. Two stocks named C1 (wild PL) and C2 (hatchery PL) were maintained to measure the size variation and stress tolerance. Stocking density was 5 PL/liter; feeding rate was 90 *Artemia* nauplii/shrimp PL/day. Temperature, salinity, and pH were maintained at 27.6±1.3 ^{0}C, 25.2±0.9 ppt, and 7.9±0.2. The findings showeda greater size range (PL 1 to PL ≥ 15) in C1 than C2 (PL9 to PL15); more tolerance of wild PL than hatchery PL inthe salinity stress test, and morphologically excellent quality PL of 88.8% (T1) and 88.7% (T2). Other findings included mean growth of 10.16±1.3 mm (T1) and 9.67±0.4 mm (T2), the specific growth rate for length/day of 2.25% (T1) and 1.95% (T2), and final survival of 78% for both T1 and T2. These findings can contribute to identifying the quality issues in shrimp PL production and reduce the negative impacts of wild PL collection on coastal and marine fisheries.
Keywords: *Penaeus monodon*, Shrimp post larvae, Shrimp nursing

Introduction

Giant tiger shrimp (*Penaeus monodon*) is a marine crustacean of the Penaeid family. It is one of the most commercially-cultured species in the world (FAO 2016). It is considered the white gold of Bangladesh for its economic role from export earnings. In 2019-20, shrimp production was 2,41,281 metric tons (MT) and export value was 1,988.56 core BDT (DoF 2020). The supply of quality post-larvae (PL) is the prerequisite of shrimp production (Arnold *et al.* 2009). There are two sources of shrimp PL supply for aquaculture; harvesting wild PL and hatchery production (Hossain and Hasan 2017).There are 43 registered private shrimp hatcheries in Bangladesh with 792.952 crores shrimp PL production in 2019-20 (DoF 2020). Wild PLcollection rate is significant although the hatchery-produced shrimp PLs are available (Ahamed *et al.* 2012). About 2 billion wild PLs are collected annually from the coastal areas of Bangladesh (Banks 2003). There are many challenges of PL production and supply from hatcheries. The most significant challenge is maintaining the quality. Farmers usually prefer to stock wild PL to hatchery PL because they perceive that wild PLs have better survival and good local availability on-demand (Ahmed *et al.* 2005).

Wild PL collection negatively impacts the stock, ecology, and biodiversity of coastal and marine fishes and other aquatic species. During PL collection, several other finfish, shellfish, and aquatic species entangle to the net in larval or juvenile stages (Ahamed *et al.* 2012). For collecting single shrimp PL, collectors discard about 99 finfish and other species of shrimps (Rashid, 2000). Indiscriminate wild PL collection hampers the existence of endangered and threatened species. It

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also interrupts the conservation and biodiversity maintenance efforts (Hoq 2007). To combat wild PL collection, the Government of Bangladesh formed a regulation in 2000 (MoFL 2000). The effectiveness of this regulation is not apparent because of the scarcity of alternative livelihood options for PL collectors. Most importantly, there is necessary to spread scientific information among shrimp farmerson the actual quality of wild PL and hatchery PL.

There are several criteria to assess the quality of shrimp PL. These include visual observation, microscopic examination, stress tests with salinity drop and formalin exposure at 100 ppm and 200 ppm concentrations for 2 hours (Imelda *et al.* 2000). Visual and microscopic observations include muscle to the gut ratio (MGR), eye appearance, body colour, shell appearance, tail muscle condition, uropod condition and melanization or necrosis in body parts. Quality assessment criteria also include swimming activity and behaviour against external stimuli and survival rate (Saurabh *et al.* 2006). The study aimed to compare wild and hatchery-produced shrimp (*Penaeus monodon*) PL in terms of qualitative and quantitative aspects. The qualitative comparison involved differences in life stage according to rostrum spine count, morphological aspects and salinity stress tolerance (SST). The quantitative comparison involved measuring growth by length (mean growth and SGRL/day - specific growth rate for length per day) and survival in pre-stocking nursing conditions. The finding this study may help reduce farmers' preference for wild PL to hatchery PL in terms of quality. It may also contribute to reducing the negative impacts of wild PL collection on coastal and marine fisheries.

Materials and Methods

Study site: The study was conducted at Marine Hatchery of "Coastal Biodiversity, Marine Fisheries, and Wildlife Research Centre, CVASU", Cox's Bazar. The wild PLs were collected from Rejukhal, and the hatchery PLs were collected from a local shrimp hatchery named "Niribili Plus Shrimp Hatchery'. The study duration was one month, from October 1, 2021, to October 30, 2021.

Experimental design: The study consisted of two treatments with three replications for each. Treatment-1 (T1) was used for wild PL (T1R1, T1R2, and T1R3), and treatment–2 (T2) for hatchery PL (T2R1, T2R2, and T2R3). The treatment units were of 5 litre plastic tanks. Two stock tanks (C1: wild PL; C2: hatchery PL) of 100 liters were maintained for measuring size variation and performing salinity stress test (SST).

Water preparation, PL collection and stocking: Seawater (32 ppt) was collected, filtered, anddiluted to 24 ppt. Each tank of T1 and T2 was filled with 5 liters of diluted seawater, and aeration was provided. Two hundred (200) of each wild PLs and hatchery-produced PLs were collected and transported to the study area. The collected PLs were acclimatized in a 100 L tank for 1 hour to the salinity and temperature condition of the experiment. Then the PLs were stocked at a density of 5 PL/liter (20 PL in each T1R1, T1R2, and T1R3; T2R1, T2R2, and T2R3). The remaining PLs were kept in stocking tanks (C1 and C2) with proper aeration.

Artemia nauplii were provided as feed at a rate of 90 *Artemia* nauplii/shrimp PL/day and at a feeding frequency of 3 times per day (30 *Artemia* nauplii/shrimp PL/time). The quantity of required *Artemia* nauplii and decapsulation method was followed from Stappen (1996). The temperature, salinity, and pH values were measured with a digital salinity meter (Hanna – HI98319) and a digital pH cum thermometer (Hanna – HI98107). The water was diluted at five-

day intervals to compensate for the salinity increase by evaporation. The experimental tanks were siphoned regularly to remove faecal matter and uneaten feed.

Comparison of wild and hatchery PL

Qualitative comparison: Qualitative comparison between wild PL and hatchery PL involved differences in life stageaccording to rostrum spine count (Table 1), morphological aspects and salinity stress tolerance (SST). Life stage observation and SST was performed on 30 samples from C1 and C2. Morphological aspects wereexamined from the PLs of T1 and T2. The information of Limsuwan and Ching (2013) was followed to interpret the life stages of PL (Table I). SST procedure of Tackaert *et al.* (1989) was followed with some modifications in this study. The test involved 3 hours retention of PL at a reduction of 50% salinity (from initial 24 ppt to 12 ppt) and then to 0 ppt. Finally, the number of survivors was counted in each salinity condition. In the case of morphological comparison, some of the crucial morphological quality aspects mentioned by Saurabh *et al.* (2006) and Imelda *et al.* (2000) were observed.

Table I. Life stage interpretation of shrimp PL against rostrum spine number*

Rostrum spine no.	Life-stage of PL
Below 3 spines	PL 5-10
Three (3) completely developed spines and a bud of the 4th spine	PL 10-15
Above 4 spines	PL 15-20

***Limsuwan and Ching (2013)

Quantitative comparison: The quantitative comparison involved measuringgrowth from length (mean growth and SGRL/day - specific growth rate for length per day) and survival evaluation. The initial TL (total length) of 30 wild and 30 hatchery PL samples was measured from C1 and C2. After 30 days of rearing, the final TL from T1 and T2 was measured. The obtained data were used to calculate the mean growth and SGRL/dayusing equation1 and equation 2.The number of live PLs in T1 and T2 was counted regularly for survival observation. Normal movement and swimming indicated live PL during counting. The dead PLs were removed regularly by siphoning. At the end of the experiment, the average survival (%) of T1 and T2 was calculated (equation 3) and compared.

Equation 1: Mean growth = mean final length – mean initial length

Equation 2:

$$\text{SGRL/day (\%)} = \frac{(\log \text{final length}) - (\log \text{initial length})}{\text{Days of rearing}} \times 100$$

Equation 3:

$$\text{Survival (\%)} = \frac{\text{The final number of shrimp PL}}{\text{The initial number of shrimp PL}} \times 100$$

Water quality parameters: Mean temperature, salinity and pH was maintained at 27.6±1.3 ^{0}C, 25.2 ±0.9 ppt, 7.9±0.2 in T1 and T2. Temperature, salinity and pH ranged from 25.3 ^{0}C to 29.7 ^{0}C; 24 ppt to 28.1 ppt and 7.3 to 8.2 during the rearing period.

Statistical analysis: The collected data were organized, categorized, analyzed and visualized using Microsoft Excel software (version 2019).

Results

Life-stage variation according to rostrum spine count: The life stage variation was greater in C1 (PL1 to PL ≥ 15) than C2 (PL 9 to PL 15). The samples of C1 were within PL 12-15 (47%), PL 9-12 (20%), PL 6-9 (17%), PL 3-6 (3%) and PL 1-3 (10%). On the other hand, the samples of C2 were within PL 12-15 (90%) and PL 9-12 (10%), respectively (Figs. 1 and 2).

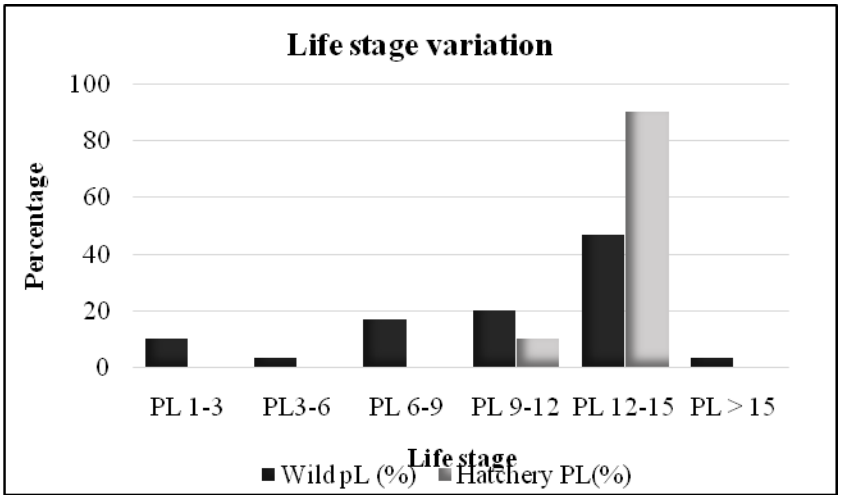

Fig. 1. Comparative life stage variation of wild and hatchery PL according to rostrum spine count.

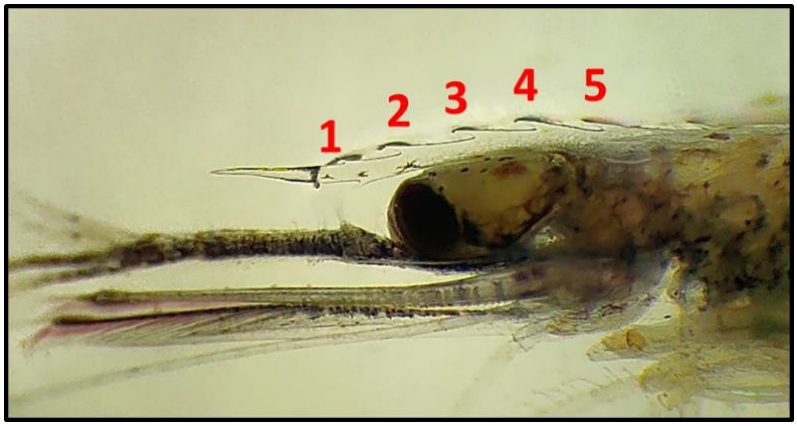

Fig. 2. Rostrum spine count of shrimp PL under microscope.

Salinity stress test (SST): The salinity stress tolerance of C1 was found better than that of C2 after 3 hours of retention in SST. At 50% reduction of salinity (24 ppt to 12 ppt), PL survivors ofC1 was 100% but reduced to 45% in C2. At 0 ppt, survivors of C1 was 40% while reduced to 0% in C2 (Fig. 3).

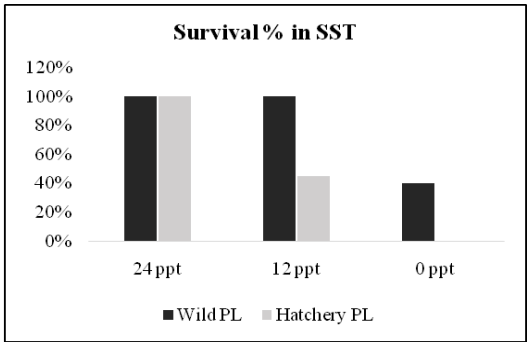

Fig. 3. Comparative survival of wild and hatchery PL in salinity stress test.

Morphological quality: From observing morphological quality aspects (Fig. 4), excellent quality PLs of 88.8% and poor quality PLs of 10.5% were found in T1. On the other hand, the percentage of excellent quality PLs and poor quality PLs was found 88.7% and 11.3% in T2. The findings, along with the morphological quality criteria, were summarized in Table II.

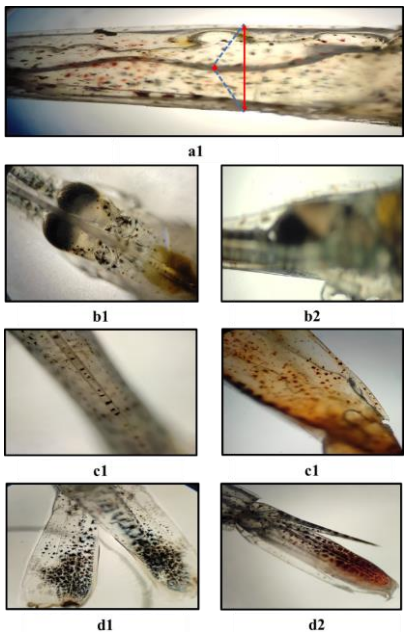

Fig. 4. Morphological quality aspects of shrimp PL (a1: Muscle to gut ratio; b1: clean and distinct eye-stocks, b2: opaque, indistinct eye-stocks; c1: transparent tail muscle with a few pigmentation spots, c2: opaque and white tail muscle; d1: noticeably open uropod, presence of pigment cells, d2: closed uropod, not pigmented)

Table II. Quality status of PL according to morphological quality aspects

Criteria	Observation	Comment	Wild PL % (T1)	Hatchery PL % (T2)
Muscle to gut ratio (MGR)	3:1	Excellent	96	100
	1-3:1	Fair	4	0
	< 1:1	Poor	0	0
Eye	Clean and distinct eye-stocks	Excellent	86	93
	Opaque, eye-stocks are not distinct	Poor	14	7
Body color	Transparent bodies with star-like brown or dark-brown pigmentation	Excellent	82	85
	Pink or red coloration, irregular pigmentation	Poor	18	15
Shell	Clean shell, luster, and shine on the shell	Excellent	85	83
	Dirt, organic matter and necrosis (black spots or brown lesions) on the shell, no luster and shine on the shell	Poor	15	17
Tail muscle	Transparent with a few pigmentation spots	Excellent	88	79
	Opaque and white	Poor	12	21
Uropod	Noticeably open, presence of pigment cells	Excellent	96	92
	Closed, not pigmented	Poor	4	8
Total (%)		Excellent	88.8	88.7
		Poor	10.5	11.3

Growth and survival: Mean growth by TL in T1 and T2 was found 10.16±1.3 mm and 9.67 ± 0.4 mm after 30 days of rearing. SGRL/day was greater in T1 (2.25±0.9 %) than T2 (1.95±0.2 %). The average survival was found similar (78%) for both T1 and T2 at the end of the rearing period. However, the weekly survival trend differed between T1 and T2. At the end of week 1, week 2 and week 3, there was 85%, 80% and 78% survival in T1; 90%, 83% and 82% survival in T2 (Fig. 5).

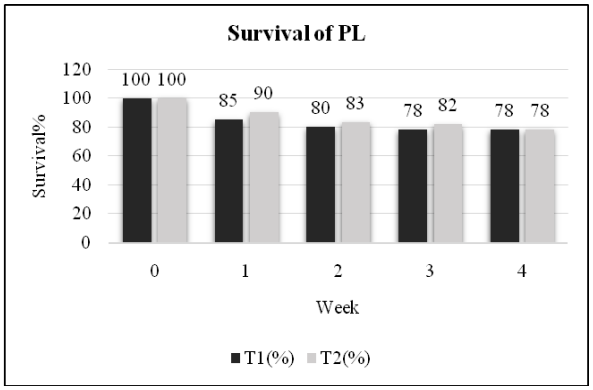

Fig. 5. Weekly survival of wild PL and hatchery PL during the rearing period.

Water quality: The mean value of temperature, salinity and pH was maintained 27.6±1.3 ^{0}C, 25.2±0.9 ppt, 7.9±0.2 in T1 and T2. Temperature, salinity and pH ranged from 25.3 ^{0}C to 29.7 ^{0}C; 24 ppt to 28.1 ppt and 7.3 to 8.2.

Discussion

Life-stage variation according to rostrum spine count: Life stage variation was considered a quality criterion of shrimp PL in this study. Primavera *et al.* (1998) mentioned the life stage as a good criterion to assess quality before culturing *Penaeus monodon*. Life-stage variation in T1 was greater with a range of PL 1 to PL ≥ 15 than T2 with a range of PL 9 to PL 15 in this study (Fig. 1). Castille *et al.* (1993) found uniform and higher shrimp growth rates with small size variations. Hence, the present research infers that hatchery PLs may grow more uniformly than wild PLs because of small size variations. The life stage range of T2 represented 90% of individuals above PL10 in this study. This finding resembled the recommended size of PL stocking by FAO (2007). They recommended stocking after PL10 stage because gill development completes at this stage. A large size range of PL in a single batch is undesirable for stocking to grow-out systems. Thus, the greater life stage variation in wild PL in the present study indicates lesser suitability for stocking than hatchery PL.

Salinity Stress Test (SST): SST was used to evaluate the comparative tolerance between wild PL and hatchery PL. There was greater tolerance in the samplesof C1 than C2 after 3 hours of retention in 12 ppt (50% reduction) and 0 ppt (Fig. 3). Tackaert *et al.* (1989) suggested a stress test to assess crustacean larva quality. Shrimp PL with great survival in stress tests was considered as high quality by Gallardo *et al.* (1995). Therefore, wild PL was considered more tolerant than hatchery PL in this study. Exposure to frequent salinity changes affects the survival and growth of Penaeid shrimp during the nursing stage (Kumlu *et al.* 2000). The tolerance range of shrimp PL differs with salinity changes (Criales *et al*. 2011). These findings logically support the survival variation between C1 and C2 in 3 different salinity conditions in the present study.

Morphological quality: This study observed several morphological criteria (Table 2) to compare the quality of wild PL and hatchery PL and found very little differencebetween T1 (88.8%) than T2 (88.7%). Racotta *et al.* (2003) mentioned some standard indicators to evaluate the quality of larva. Size, growth rate, the status of nutrition, general appearance, biochemical constituents, stress tolerance, disease symptoms, and molecular methods are some indicators for larval quality assessment in recent times (Kim *et al.* 2020). The present study considered some morphological features mentioned by Kim *et al.* (2020) and Saurabh *et al.* (2006). The quality of PL is one of the most important elements affecting the production of shrimps, but there are few scientific studies on it (Mirzaei *et al.* 2021). Therefore, no comparative study between wild PL and hatchery PL quality was found to validate and justify the present study's findings. The study was limited to some morphological quality aspects, but a more intensive quality assessment of shrimp PL is needed.

Growth and survival: Carapace length (CL) and total length (TL) are the morphometric criteria used to measure the growth of Penaeid shrimp (Gautam *et al.* 2014), but only TL was used in this study. The present study found 10.16 ±1.3 mm and 9.67±0.4 mm mean growth by TL in T1 and T2, respectively. The higher mean growth of T1 resembled the mean growth found by Nahavandi *et al.* (2010). SGRL/day for T1 was greater (2.25±0.9 %) than T2 (1.95±0.2 %) in this study. The SGRL/day for T1 and T2 was higher than the findings of Jain *et al.* (2006) and lower than the

findings of Foes *et al.* (2016) for *Litopenaeus vannamei*. Survival analysis was mentioned as a way for the post-larval quality control of shrimp by Imelda *et al.* (2000). Castille *et al.* (1993) mentioned the importance of survival as a good quality index of post-larva. Therefore, the survival of shrimp PL was compared between wild and hatchery PL in this study. At the end ofthe rearing period, no difference was found in the average survival (78%) between T1 and T2 in the present study (Fig. 5). This finding resembled the findings of Tao *et al.* (2021), who found no difference in survival between the two treatments. Maintenance of proper nursing conditions yielded more survival in this study. Thus, proper nursing condition maintenance before stonkingcould be used to improve the survival of shrimp PL.

This study investigated the quality aspects of shrimp PL of both wild and hatchery sources in terms of size variation, survival, tolerance, and morphological criteria. The result revealed that wild PL sare better in terms of tolerance, but hatchery PLs are better in appearance and uniformity. Moreover, the final survival of wild and hatchery PL was found similar in controlled conditions of nursing. However, the percent reduction in survival with time was gradual in wild PL. The findings of this study suggest ensuring proper pre-stocking nursing to improve the survival and viability of hatchery PL. Quality PL supply is the prerequisite to ensuring desired survival, growth, and production of shrimp. Moreover, intensive culture technologies require strong and disease-resistant shrimp PL. Therefore, more intensive research is necessary on the quality assessment of hatchery-produced shrimp PL in Bangladesh to facilitate intensive shrimp culture and export of shrimp.

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