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Mizanur Rahman Washim
Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha, Khulna, Bangladesh

Syed Lutfor Rahman
Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha, Khulna, Bangladesh

Shamsun Nahar
Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha, Khulna, Bangladesh

Correspondence
Mizanur Rahman Washim
Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha, Khulna, Bangladesh

Culture potential of SPF (specific pathogen-free) shrimp (*Penaeus monodon*) with special context of its growth and production performance in South-west coastal region of Bangladesh

Mizanur Rahman Washim, Syed Lutfor Rahman and Shamsun Nahar

Abstract
Culture potential of SPF (specific pathogen-free) shrimp (*Penaeus monodon*) was assessed for the period of 63 days in nine on station ponds of Brackish water Station from June to August 2015. Three different stocking densities were examined for each treatment *viz.*3 PL/m^2 (T_1), 5PL/m^2 (T_2) and 7 PL/m^2 (T_3). Water quality parameters were not significantly ($P>0.05$) different among the treatments except dissolved oxygen (DO). The growth and survival of SPF (specific pathogen-free) shrimp were higher in T_1 than those obtained from T_2 and T_3 respectively and did not differ significantly ($P>0.05$). The final weight gain was significantly ($P<0.05$) higher in T_1 than the other two treatments. Significantly ($P<0.05$) the lowest food conversion rate (FCR) was in T_3 then followed by T_1 and T_2. The production was highest in T_3 than those of T_2 and T_1 but showed significant ($P<0.05$) difference among the treatment. However, the highest net benefit was derived from T_3 where the stocking density was 7 PL/m^2. Meanwhile, the benefit cost ratio (BCR) of T_2 found significantly ($P<0.05$) higher than T_1 and T_3 which implies that stocking density of 5 PL/m^2 is more feasible than others. The present study reveals that, lower stocking density of SPF shrimp with short culture period would be appropriate to achieve higher growth, production and net benefit.

Keywords: SPF (specific pathogen-free) shrimp, PL (Post larvae), MKA hatchery, growth, production

1. Introduction
Of all the major hurdles of world shrimp farming industry, disease is the most critical one, which causes an innumerable loss every year particularly to the marginal shrimp farmers of Bangladesh. The majority of shrimp production in Bangladesh is produced by small farmers. Although Bangladesh produces 2, 23,582 MT farmed shrimp/prawn in 2014-15 [1], disease have repeatedly impacted our shrimp production negatively. Since the inception of viral disease outbreak in Bangladesh (1994) particularly in the semi-intensive shrimp culture system in Cox's Bazar [2], several endeavors had been undertaken by Government, NGO's and International organizations to control this epidemics. However, the innovation of SPF (specific pathogen-free) shrimp (*Penaeus monodon*) may abate the drawbacks of our shrimp aquaculture industry. The farmers depend on shrimp hatcheries for stocking of PL (Post larvae) in their Ghers. Meanwhile, hatcheries have depended on wild broodstock that frequently carry diseases to produce these larvae which often passed on to farmer's pond from the hatcheries and limit production on shrimp farms.
To address these barriers to shrimp farmers production, Americans Hawaii-based Moana Technologies with support from Feed the Future Partnering for Innovation, started shipping Specific Pathogen Free (SPF) breeding stock in Bangladesh in 2014 [3]. Moana is a marine biotechnology company that, after more than a decade of research, developed its own breed of SPF black tiger shrimp and this can be produced more than twice as fast as wild shrimp, and command a significantly higher price than other varieties in the export market [4]. SPF shrimp are those which are produced from biosecurity facilities, have been repeatedly examined and found free of specified pathogens using intensive surveillance protocols, and originate from broodstock developed with strict founder population development protocols and these founder populations are generated by extensive quarantine procedures that result in SPF F1 generations derived from wild parents [5]. Scientists have already been succeeded on the discovery of other

SPF penaeid species many years before rather SPF-*Penaeus monodon* shrimp PL. The first commercial shipment of SPF *Penaeus vannamei* broodstock from the Americas to Asia was from Hawaii to Taiwan Province of China in 1996 [6]. SPF *Penaeus stylirostris* had also been experimentally introduced to many Asian countries (including Brunei, Taiwan Province of China, Myanmar, Indonesia and Singapore) from secure breeding facilities in Mexico and the United States of America and these introductions began in the year 2000 [7].

However, the introduction of SPF shrimp (*P. monodon*) PL have been unveiled a new dimension to our shrimp aquaculture challenging against disease. The successful development and domestication of SPF broodstock is performed by MKA Hatchery in Cox's Bazar, Bangladesh and they were able to produce 1.9 million shrimp PL in 2014 and now commercially producing and meeting the supply of SPF post larvae in the country [3]. Meanwhile with the help of USAID (the United States Agency for International Development), in 2014 World Fish introduced pathogen-free post larvae or shrimp fries called specific pathogen free (SPF) in Khulna, Satkhira and Bagerhat which gives farmers extra security against loss from diseases, in particular the most common, the White Spot Syndrome Virus (WSSV), which poses a serious threat to the industry [8]. Till now several experiment/study has been performed about culture potential of different shrimp species both in home and abroad based on stocking density [9-11] and some authors have reported an inverse relationship between growth and stocking density [12, 13] but culture potential of SPF shrimp (*P. monodon*) has not yet been studied. Considering the above, the present study aimed to investigate the growth and production performance of specific pathogen-free (SPF) shrimp (*P. monodon*) with different stocking densities under a short culture period.

2. Materials and Methods

2.1 Site of the experiment

The present experiment was conducted in Brackishwater Station of Bangladesh Fisheries Research Institute (BFRI) situated in Paikgacha sub-district (upazila) is a part of Khulna city and located between longitudes 22^{0}28´ and 22^{0}43´ North and latitudes 89^{0}09´ and 89^{0}23´ East of Bangladesh.

2.2 Experimental design

The experiment was conducted in nine on-station earthen ponds of 0.1 ha each following the design furnished in the Table below.

Treatments	Stocking densities (No/m^2)	Replications
T1	3	3
T2	5	3
T3	7	3

2.3 Preparation of shrimp pond

The ponds were prepared by complete drying, liming $CaCO_3$ @ 250 kg/ha of soil and then filled with the tidal water by complete screening with fine meshed net up to a depth of 1.0 m. The entire farm was encircled up to 1.0 m height with fine meshed nylon net that ensures a biosecurity. Water was treated with chlorine @ 20 ppm to disinfect water and kill all animalcules those are the carrier of pathogen and disease. Molasses @ 80 kg/ha were applied to develop colour of water to prevent penetration of sunlight and then fertilized with urea and TSP @ 25 and 30 kg/ha, respectively for quick development of colour of water and production of plankton.

2.4 In-pond nursery preparation and stocking of post-larvae (PL)

An in-pond nursery was constructed at one corner of each pond by encircling nylon net fastened in bamboo frame. After seven days of fertilization required quantity of shrimp PL were stocked with acclimatization with pond water and reared for 14 days before releasing into the whole pond. In the nursery the stocked PL were fed with CP (Charoen Pokphand) nursery feed (NASA) twice daily.

2.5 Post stocking management

After two weeks of nursery rearing they were allowed to spread over the whole pond by opening the nursery enclosure. In the grow-out ponds, the shrimp were fed with CP feed depending on the biomass of shrimp. The feeding behavior and well-being of shrimp were checked every 1-2 days intervals through cast netting. Growth of fishes was monitored at weekly interval and feeds were adjusted accordingly. The water of the ponds was treated with $CaMg(CO_3)_2$ (dolomite) @ 15 ppm on monthly basis and fertilized with inorganic fertilizer whenever necessary. In the grow-out ponds Zeolite @ 4 ppm was applied whenever needed. Aeration has been provided to the ponds whenever necessary through agitating water by paddle wheel/airjet aerator.

2.6 Monitoring of water quality

The different hydrographical parameters *viz*., surface water temperature (ºC), depth (cm), transparency (cm), salinity (ppt), pH, DO (mgl^{-1}) and Total alkalinity (mgl^{-1}) were monitored at seven days interval using a Celsius thermometer, a graduated pole, a secchi-disk, a refractometer, a portable pH meter (HI 8424, Hanna Instruments, Portugal) and a portable dissolved oxygen meter (HI 9142, Hanna Instruments, Portugal) and Total alkalinity was determined following the titrimetric method according to the standard procedure and methods [14]. Dissolved oxygen (DO) was monitored almost daily after 50 days of culture.

2.7 Harvesting of shrimp

After 63 days (9 weeks) of grow out period, shrimps of all ponds were harvested, firstly with repeated cast netting and finally by complete dewatering with pump. Total weight and number of shrimp in each pond were recorded for data analysis. The survival rate as well as production, specific growth rate (SGR % day), feed conservation ratio (FCR), average daily gain (ADG g/pcs/day) and final weight gain (%) were also estimated. Specific growth rate (SGR % day) and feed utilization efficiency were calculated respectively as follows:

a. ADG (g) = Final weight (g)/Duration of culture (days)

b. Final weight gain % = (Final wt.-Initial wt. / Final wt.) x100

c. SGR (%/day) = 100 x [(LnWt-LnW_1)/t]

Where, Wt. = Final weight

W_1 = Initial weight

t = Days of culture

d. Survival rate % = 100 x (No. of shrimp harvested / No. of shrimp stocked)

e. Feed Conversion Ratio (FCR)

= Dry weight (g) of feed supplied / Live weight (g) of fish gained

2.8 Data analysis

The final data were expressed as mean value ± Standard deviation (SD) and analyzed by one-way analysis of variance (ANOVA) to find the level of significance of difference among different treatments and the significance were assigned at the 5% level ($P>0.05$). All statistical analysis was done by using the Microsoft Excel and SPSS (Statistical Package for Social Science) version-20.

3 Results

3.1. Physico-chemical parameters

The physico-chemical parameters of water *viz.* temperature, depth, transparency, dissolved oxygen; pH and alkalinity etc. recorded during the study period were found within optimum range. During the study period, the mean water temperature were 30.0±0.76, 30.10±0.60 and 29.66±0.76 ºC in T_1, T_2 and T_3, respectively but did not differ significantly ($P>0.05$) among the treatments. The mean depths were ranged from 124.60 to 125.89 cm (Table 1). The recorded transparency was (31.0±1.00 cm), (29.81±2.33 cm) and (30.33±0.58 cm) in T_1, T_2 and T_3 respectively. However, no significant ($P>0.05$) difference were recognized in water depth and transparency. The observed Dissolved Oxygen (DO) content ranged from 3.0 to 6.5 mgl^{-1} with mean values was 4.93±0.59 (T_1), 4.79 ±0.28 (T_2) and, 3.35±0.87 mgl-1 (T_3) and showed significant difference ($P<0.05$) among the treatments.

Table 1: Hydrographical properties of weekly sampling over the 63 days (9 weeks) study period

Variables	T_1 (3Nos/m^2)	T_2 (5Nos/m^2)	T_3 (7Nos/m^2)
Water Tempera-ture (ºC)	29.83±0.76 ^{a} (26.8-32.00)	30.10±0.60 ^{a} (27.5-30.6)	29.66 ± 0.76 ^{a} (27.3-30.2)
Depth (cm)	124.92±11.00 ^{a} (110-120)	124.60±2.81 ^{a} (115-130)	125.89± 8.56 ^{a} (125-140)
Transpa-rency (cm)	31.0± 1.00 ^{a} (27-36)	29.81± 2.33 ^{a} (28-40)	30.33± 0.58 ^{a} (25-35)
Salinity (ppt)	9.64± 0.13 ^{a} (5-18)	9.18±0.49 ^{a} (5-18)	9.23± 0.25 ^{a} (5-18)
Dissolved oxygen (mgl^{-1})	4.93± 0.59^b (4.5-6.5)	4.79±0.28^b (3.4-6.4)	3.35± 0.87^a (3.2-4.2)
pH	8.52±0.09^b (7.5-9.1)	8.30± 0.10^a (8.0-8.9)	8.43± 0.11^{ab} (7.7-8.5)
Total Alkalinity (mgl^{-1})	127.45±1.27^a (115-140)	126.40±0.65^a (118-136)	137.26±1.20^b (128-155)

Mean ±SD (Standard Deviation) and range in parentheses; Values in the same row having the same superscripts are not significantly different ($P>0.05$).

The observed mean salinity range between 5 ppt to 18 ppt (Table 1) and did not differ significantly ($P>0.05$) among the treatments. The mean values of pH were 8.52±0.09, 8.30±0.10 and 8.43±0.11 recorded in T_1, T_2 and T_3, respectively but did not differ significantly ($P>0.05$). The highest mean value of total alkalinity was recorded in T_3 (137.26±1.20 mgl^{-1}) and the lowest was in T_2 (135.00±12.65 mgl^{-1}) but the variations among the treatments were not statistically significant ($P>0.05$).

3.2 Growth and production of SPF shrimp

The growth of SPF shrimp *P. monodon* under different stocking densities at the end of the experiment in weekly sampling has been depicted in Fig 1 which indicates that the weight were always higher in T_1 than T_2 and T_3.

The highest mean final weight was obtained in T_1 (18.11±0.62 g) and lowest in T_3 (15.21±1.58 g). However, the mean final weight were showed significant difference ($P<0.05$) in T_1 followed by T_2 and T_3, when ANOVA was performed. The average daily gain (ADG) was significantly ($P<0.05$) lower in T_3 followed by T_2 and T_1. The Specific growth rate (SGR % day) were higher in T_1 (6.01±0.18) than those obtained in T_2 (5.87±0.36) and T_3 (5.71±0.02) and showed no significant difference. The highest survival rate (74.00±3.69%) was also observed in T_1, where the stocking density was 3Nos/m^2 whereas the lowest was (70.93±4.89%) in T_3, where the stocking density was 3Nos/m^2 and there were no significant differences among the treatments. The Food Conversion Ratio (FCR) of T_3 (1.13±0.057) was found to be lower than T_2 (1.17±0.015) and T_3 (1.16±0.015) which showed significant ($P<0.05$) difference among the treatments. However, the highest production was recorded in T_3 (755.05± 91.6 Kg/ha) and the lowest was produced in T_1 (401.14±33.76 Kg/ha) but the variation was significant ($P<0.05$) among the treatments.

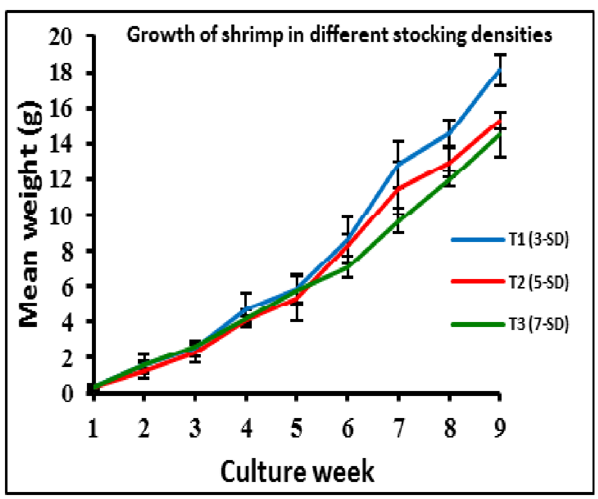

Fig 1: Growth of shrimp in weekly sampling at different stocking densities over the culture period of 63 days.

Table 2: Growth performance, survival, feed utilization and production performance of SPF shrimp (*P. monodon*) after 63 days (9 weeks) of culture period

Parameters	Treatments		
	T_1(3 Nos/m^2)	T_2(5 Nos/m^2)	T_3(7 Nos/m^2)
Initial weight (g)	0.38±0.080 ^{a} (.31-.47)	0.33±0.047 ^{a} (.30-.39)	0.39± 0.02 ^{a} (.37-.41)
Final weight (g)	18.11±0.62 ^{b} (17.48-18.72)	15.27±0.32^a (14.95-15.60)	15.21±1.58 ^{a} (13.54-16.70)
% wt gain	4387.6±505 ^{a}	4410±684 ^{a}	3598.0±37 ^{a}
Specific growth rate (SGR) (% day)	6.01± 0.18 ^{a} (5.84-6.20)	5.87±0.36 ^{a} (5.56-6.27)	5.71±0.02 ^{a} (5.69-5.74)
ADG (g/pcs/day)	0.28±0.007 ^{a}	0.25±00 ^{b}	0.23±.014 ^{c}
Feed conversion ratio (FCR)	1.16± 0.015 ^{b}	1.17±0.015 ^{b}	1.13±0.057 ^{a}
Survival (%)	74.00± 3.69 ^{a} (70-77)	73.40±3.46 ^{a} (69-76)	70.93±4.89 ^{a} (65-74)
Production (Kg/ha)	401.14±33.76 ^{a} (368-435)	560.45±25.80 ^{b} (531-580)	755.05± 91.64^c (700-860)

Values are means of data obtained ± Std. Deviation (mean ± SD) of weekly sampling. Values in the same row having the same superscripts are not significantly different (*P*>0.05).

3.3 Cost-benefit analysis

The total cost of production was the lowest in T1 (BDT 1, 60,420/ha) than those in T_2 (BDT 1, 82, 930/ha) and T_3 (BDT 2, 55, 940/ha). The net benefits generated from 63 days (9 weeks) culture period was calculated as BDT 60, 130, 86,086 and 1,06,484/ha for T_1, T_2 and T_3, respectively and showed significant (P<0.05) difference among them. However, the net benefits of BDT 1, 06, 484/ha in T_3 was significantly (P<0.05) higher than obtained from T_2 and T_1 where SPF shrimp (*P. monodon*) post-larvae stocked in 7No/m^2 stocking density (Table 3).

Table 3: Cost of production and economic returns of SPF shrimp (*P. monodon*) from 1ha ponds for a culture period of 63 days (9 weeks).

Items	Treatments		
A. Cost	T_1 (BDT)	T_2 (BDT)	T_3 (BDT)
Pond lease (BDT8,000/ha/yr)	1350 (65 days)	1350 (65 days)	1350 (65 days)
Bleaching (BDT 40/Kg)	22,000	22,000	22,000
Lime (BDT 14/Kg)	3,100	3,100	3,100
Molasses (BDT 35/Kg)	2,800	2,800	2,800
Urea+ TSP (BDT 16+25 /Kg)	1050	1050	1050
PL (BDT .90/Pcs)	27,000	45,000	63,000
CP Feed: a. Nursery (BDT 130/Kg)	2,730	4,330	6,015
b. NASA-2002 (BDT 130/Kg)	65,890	68,796	1,22,125
Labour (2 labour @BDT 250/day)	31,500	31,500	31,500
Miscellaneous	3,000	3,000	3,000
Total costs	1,60,420	1,82,930	2,55,940
B. Gross benefit			
Selling price of shrimp	2,20,550	2,69,016	3,62,424
Net benefits (B-A)	60,130± 1616 ^{a}	86,086± 4234 ^{b}	1,06,484± 2928 ^{c}
BCR	1.37±0.02 ^{a}	1.47±0.02 ^{c}	1.41±0.01 ^{b}

Mean ±SD (Standard Deviation) and range in parentheses; Values in the same row having the same superscripts are not significantly different (*P*>0.05)

4. Discussion

Studies suggested that the growth and survival of shrimps are affected by temperature, salinity and dissolved oxygen concentration [15-17]. Temperature is an essential parameter that influences the photosynthesis in water, physiological responses of culture organisms, decomposition of organic matter, subsequent bio-chemical reactions and also one of the most important factors controlling growth of marine shrimp [18]. The mean range of temperature was (26.8 to 32 ºC) in the grow out ponds which is closely similar to the observation of Debnath [19] and Chiu [20]. Salinity level (5-18 ppt) at present study agrees with the reports Shivappa [21] and Collins [22], which stated that *P. monodon* adapted quite well in freshwater conditions and wide range of salinity tolerance 5 ppt to 35 ppt Gunarto [23]. Although Rmanathan [24] have opined that the optimum range of pH 6.8 to 8.7 should be maintained for maximum growth and production. However, variation in pH in the present trial was within the range of (7.5-9.1) which is closely consistent with the observation of Pushparajan [25] and Boyd [26] best range of water pH for shrimp culture is 7-9. The secchi disc reading should be 30-40 cm MPEDA [27] whereas the transparency of present study was between the ranges of 25-40 cm. Over the study period, lower dissolved oxygen (DO) observed in T_3 appeared to be related to stocking density where stocked with a high density. This might be due to the higher consumption rate of oxygen by the higher density of shrimp and other aquatic organisms that agree with Boyd [28]. However, the DO level (3.2 to 6.5 mg/l) was within the acceptable ranges in all the experimental ponds although they differ significantly (P<0.05).

Final weight, average daily gain and survival rate of SPF tiger shrimp (*P. monodon*) in T_1 were significantly (P<0.05) higher, where stocking density was lower (3Nos/m^2) compared to T_2 (5Nos/m^2) and T_3 (7Nos/m^2) although the same feed was adjusted accordingly with the weekly monitoring of growth rate. Krantz and Norris [25] have stated that, survival rates of 60 to 80% are to be expected for *P. monodon* culture under suitable rearing conditions. However, the survival rate (65-77%) of present experiment was higher than Islam [10], Saha [32] and Islam [29]. On the other hand specific growth rate (SGR) also observed higher in T_1 (6.01±0.18) but showed no significant difference among them and the results are closely consistent with Islam [29] and Apud [30] reported a lower growth rate of shrimps as stocking density increased. The study showed that production of SPF shrimp was significantly (P<0.05) higher in T_3 (755.05±91.64) kg/ha than that of obtained from T_2 (560.45±25.80) kg/ha and T_1 (401.14±33.76) kg/ha and these production are closely agrees with the findings of Latif [10] (achieved a production of

759.14±19.002 kg/ha at 5/m^2 stocking density in 120 days culture period) and Mazid [33] (reported a yield of shrimp *(P. monodon)* of 350.0-500.0 kg/ha at 1.0-2.5 PL/m^2 density). The FCR value was significantly ($P<0.05$) lower in T_3 (1.13±0.057) than the other two treatments but it closely agrees with the observation of Debnath [19], Gunarto [23] and Pushparajan [25] although their culture period was longer. The cost and economic benefit analysis showed that the higher net return was achieved in treatment T_3 (where SPF shrimp-*P. monodon* stocking density was 5Nos/m^2) than T_2 and T_1 but the BCR value in T_2 (1.47±0.020) is significantly ($P<0.05$) higher than other two treatment which implies that percentage of net economic return is higher in 5Nos/m^2 stocking density compared to others which is similar to the findings of Latif [10]. So, a stocking density *viz.* 5Nos/m^2 with a short culture period of SPF shrimp would be suitable for this region.

5. Conclusion
The general conclusion obtained from the present study is that, the growth, survival and production performance of specific pathogen- free (SPF) shrimp (*P. monodon*) with short culture period is apparently satisfactory where no outbreak of disease occurred. However, further research should be performed about optimum stocking density, culture period and performance in different culture types likewise traditional, other modified and intensified culture system before planning for massive extension to farmer level.

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