Khulna University Studies Volume 19(1): 43-54 : 2022

ORIGINAL ARTICLE LIFE SCIENCE

GROWTH COMPARISON OF MONO AND MIXED-SEX GIANT FRESHWATER PRAWN (*MACROBRACHIUM ROSENBERGII*, DE MAN) AND STOCKING OPTIMIZATION OF MALE MONOSEX FARMING

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KUS: 21/14: 210921

Manuscript submitted: September 21, 2021 Accepted: January 17, 2022

Abstract

Most often male freshwater Prawn (*Macrobrachiumrosenbergii*) exhibits higher growth compared to the female counterpart. The current study has been conducted to evaluate the production performance and the survival rate of mono-sex male freshwater prawn culture compared to mono-sex female and mixed-sex culture. Conjointly the study also appraises the production competence of mono-sex male *M. rosenbergii*at different stocking densities. The experiment was conducted in two different phases. The first experiment comprised of three treatments, *viz.* mono sex male (T_1), mono sex Female (T_2) and both male & female (T_3) with the same stocking density (3 ind/m^2) and maintaining the sex ratio 100% male and female for T_1 and T_2, and 50:50 ratio for T_3. The second experiment was conducted with all-male prawn (100% male) with three different stockings densities (i.e. 1, 2 and 3 ind/m^2 successively as D_1, D_2 and D_3). The results showed that in the first experiment, mono-sex males performed better growth, survival and production than mono-sex females and mixed-sex populations. In the second experiment, the highest individual growth was found to be 57.81±0.12 g in D_1, 52.22±0.14 g in D_2 and 40.97±0.1g in D_3. Similarly, D_1 showed the highest survival compared to D_2 and D3. In contrast, the highest production (888.05±9.05kg/ha) was found in D_2, followed by D_3 (826±42.07kg/ha) and D_1 (496.18±34.86kg/ha). Hence, this research suggests practicing mono-sex male *M. rosenbergii* culture at stocking of 2/m^2 for accomplishing maximum profits.

Keywords: Mono-sex, stocking density, growth, survival, production performance, aquaculture

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DOI: https://doi.org/10.53808/KUS.2022.19.01.2114-ls

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Introduction

Prawn culture is now one of the leading global aquaculture sectors contributing source of food and nutrition (Islam et al*.,* 2008; Kutty, 2005; Paul et al*.,* 2016). This sector is contributing to the economy of Bangladesh due to its extensive culture. Bangladesh is blessed with a suitable agro-climatic condition, and fresh, marine and brackish water for prawn culture (Ahmed et al., 2008; Azad et al., 2009; Wahab et al., 2012). Among 24 freshwater prawn species of Bangladesh, Giant Freshwater Prawn *Macrobrachiumrosenbergii* is the most economically important species (Ahmed et al., 2008; Akand & Hasan, 1992). Due to the wide geographical distribution, this species is widely cultured in India, Bangladesh, China, Thailand and Taiwan (Islam et al*.,* 2008; Kutty, 2005; Michael Bernard New, 2005; Paul et al*.,* 2016).

Recently, the culture area of *M. rosenbergii* occupied 1,48,093 ha and this area is increasing rapidly over time (Ghosh et al., 2016). Although, the prawn culture occupies a large area, the target production is still poor (e.g. 713 kg/ha) compared to other neighboring countries (Ghosh et al., 2016), such as Thailand 2338 kg/ha (Vicki, 2007), Vietnam 1000–1500 kg/ha (Ridmontri, 2002), China 1500 kg/ha (Weimin & Xianping, 2002) and India 600–1000 kg/ha (Raizada et al., 2005). In Bangladesh still, prawn is cultured through an extensive and traditional culture system at low stocking density, and mostly in gher and rice fields with other finfish species (Ahmed et al., 2010; Hasanuzzaman et al., 2011). The culture of mono-sex fish and shellfish populations is advantageous in growth and production. On the other hand, stocking density in the culture is another major factor affecting the production, growth, and survival of prawn (Azad et al., 2009; Hutchings & Villarreal, 1996).

Today, the climatic change and other anthropogenic activities are changing the distribution and availability of some natural broodstock, thereby prawn aquaculture decreasing over the decades (Ali, 1999; Woodward et al., 2010). Hence, significant changes in the structure and composition of the prawn industry of the country are observed. In the integrated culture of prawn and finfish, intra-species competition and cannibalism of prawn lead to higher mortality, therefore, tumbling the prawn production (Ahmed et al., 2010; Hasanuzzaman et al., 2011). So, people are now changing their plan to single-species culture with higher stocking densities for higher production.

In Bangladesh, several studies have been found on the optimization of stocking density of several aquaculture species for achieving maximum yield (Chakraborty et al., 2006; Paul et al., 2016; Rahman et al., 2005). We have huge prawn farming area mostly practice traditional culture with other carp species, therefore, the overall production is lower compared to other Asian countries. The lower production might be due to lack of suitable production technology, poor stocking practices, socioeconomic and environmental issues (Ahmedet al., 2008; Azad et al., 2009; Hossain & Chakraborty, 2017; Kunda et al., 2008). Due to the traditional method of cultivation, many species are cultivated simultaneously by overlooking the stocking density which does not give the desired results of prawn production. Moreover, studies have shown that male species of prawn grows faster and are become larger in size than females. So, it is necessary to focus monoculture of prawn as well as to intensify the culture system with different stocking densities to get higher production. This study aimed to investigate the growth comparison of mono and mixed-sex giant freshwater prawn and the production performance of mono-sex male at different stocking densities.

Materials and Methods

Experimental setup

This experiment was conducted in pond complex II at Fisheries and Marine Resource Technology Discipline of Khulna University, Bangladesh. The culture period was divided into two distinct phases and the culture duration of each phase was 210 days (including 60 days nursing and 150 days culture period) from June to December in two consecutive years.Due to the late rain, pump related problems in the culture facility and lack of seed, we could not avoid the winter season. Both the experiments were carried out in nine experimental ponds (40 m^2 of each). The first phase was intended with three treatments, *viz.* mono sex male (T_1), mono sex female (T_2) and both male and female (T_3). The stocking density was kept 3 juvenile/m^2, as Hasanuzzaman et al. (2011) and references therein reported that the traditional stocking density of prawn culture in Bangladesh varies between 1-4 PL/m^2.In the second experiment, the mono-sex male prawn was cultured at three different stocking densities, such as 1/m^2 (D_1), 2/m^2 (D_2) and 3 juvenile/m^2 (D_3). Each of the experiment was conducted in triplicate.

Pond Preparation and management

The experimental ponds were dewatered with a pump and dried under sunlight for two weeks to allow exhume on the bottom mud. The pond bottom and dike were renovated by digging, repairing and removing unwanted aquatic weeds and fauna. The pond bottom was disinfected by liming at 250 kg/ha (Quick lime $CaCO_3$). After pouring the water, the ponds were fertilized with urea and Triple Super Phosphate (TSP) at the rate of 25 and 12.5 kg/ha respectively. The water level of the ponds was raised to 1.5 m before stocking. The total area of the ponds was fenced with a fine-meshed nylon net to protect the experimental area from unwanted dwellers.

PL nursing and stocking

The post larva (PL) of *M. rosenbergii* was collected from a commercial hatchery of Rupsha, Khulna. All the PLs were reared in the nursery pond for 60 days with a stocking density of 25 PL/m^2. After 60 days of nursing, the juveniles (1.74±45 g) were sorted by sex through hand segregation and stocked in the grow-out ponds at the stocking density of 3 ind/$m^2$and maintaining the sex ratio 100% male and female for T_1 and T_2, and 50:50 ratio for T_3. In the second experiment, mono-sex male juveniles (1.77±34 g) were stocked in the grow-out ponds at the rate of 1/m^2, 2/m^2 and 3 juvenile/m^2 which was within the range (1-4/m^2) of traditional culture practice in Bangladesh (Hasanuzzaman et al., 2011). After nursing period, sex was identified by manual observations and 100% mono-sex male juvenile prawn was stocked in the grow out ponds. We were careful to set the stocking density of mono-sex male prawn as they show cannibalistic behavior and compete for shelter.

Food and Feeding management

In the nursery ponds, the larvae were fed with a commercial Saudi-Bangla Nursery Feed having 35 % protein level with a feeding frequency of 3 times/day. Feed was given according to body weight and the age of the prawn (Table 1). The prawns in the grow-out ponds were fed with SABINCO supplementary quality pellet feed containing 30% protein, and the feed was given at 10-3% of the average prawn's body weight.

Table 1. Feeding rate administered at prawn nursery ponds.

Week	Rate of feeding (for 100 PL) g per each administration	Week	Rate of feeding (for 100 PL) g per each administration
1^{st}	63	6^{th}	232
2^{nd}	88	7^{th}	262
3^{rd}	128	8^{th}	296
4^{th}	162	9^{th}	330
5^{th}	188	10^{th}	350

Water quality Parameter measurement

Basic water quality parameters were measured fortnightly in the water chemistry laboratory of Fisheries and Marine Resource Technology Discipline at Khulna University. Salinity was measured by Refractometer (ATAGO CO. LTD, Japan, Master- T 2312, Salinity range 0–100 ppt), Digital Thermometer (DIGITAL

THERMOMETER, made in China, model no WT-2, Temperature range−20 –80°C) was used to measure temperature. pH was measured by using pH meter (HACH, Sension 3, USA). Transparency was measured by Scheci dice and dissolved oxygen was measured by DO meter (PDO-519, made in Taiwan, Lutron). Water parameters were taken and measured at every fifteen days interval throughout the culture period.

Data collection and growth monitoring

Sampling was done fortnightly and thirty individuals were taken from each pond for the measurement of length and weight. Hand cast net were used to catch the fishes during sampling and immediately after catching, fishes were places in sampling box and measured length weight very carefully. Bodyweight was recorded by electric balance to the nearest 0.001 g accuracy and length was recorded as cm by a cm scale. At the end of the experiment, all the ponds were dewatered by a water pump and all the prawn was harvested completely to determine the production and survival rate.

$$Survival\ (\%) = \frac{Nt}{N0} \times 100\%$$

Where N_t is the number of prawns at the end of the experiment and N0 the initial number of prawns.

$$Average\ weight\ gain(WG) = Wt - W0$$

In which W_t is the final body weight and W0 the initial body weight of prawn.

$$Daily\ weight\ gain\ (DWG)\ (g/day)\ = (Wt - W0)/t$$

In which W_t is the final body weight and W0 the initial body weight of prawn, and t is the duration of the growth interval.

$$Specific\ growth\ rate\ (SGR) = \frac{ln(Wt) - ln(W0)}{t} \times 100$$

In which W_t is the final body weight and W0 the initial bodyweight of the prawn, and t is the duration of the experiment in days.

$$Feed\ conversion\ ratio\ (FCR) = \ Total\ amount\ of\ feed\ given\ (g)/Weight\ gain\ (g)$$

Statistical analysis

One-way analysis of variance (ANOVA) was used after being confirmed the homoscedasticity of variances (Levene's test) and normality of the data distribution (Shapiro-Wilk test) to determine the significant difference of growth and survival between the treatments. When there were significant differences ($P < 0.05$), the Tukey-HSD test was done to show the comparison between the treatments. All the statistical analyses were performed using SPSS 25.0.

Results

Growth performance in experiment 1

In the first experiment, mono-sex male, mono-sex female and mixed-sex prawn were cultured at the same stocking density (3 ind/m^2). The results showed that mono-sex male has a higher mean body weight at the end of the culture period than mixed-sex and mono-sex female (Figure 1).

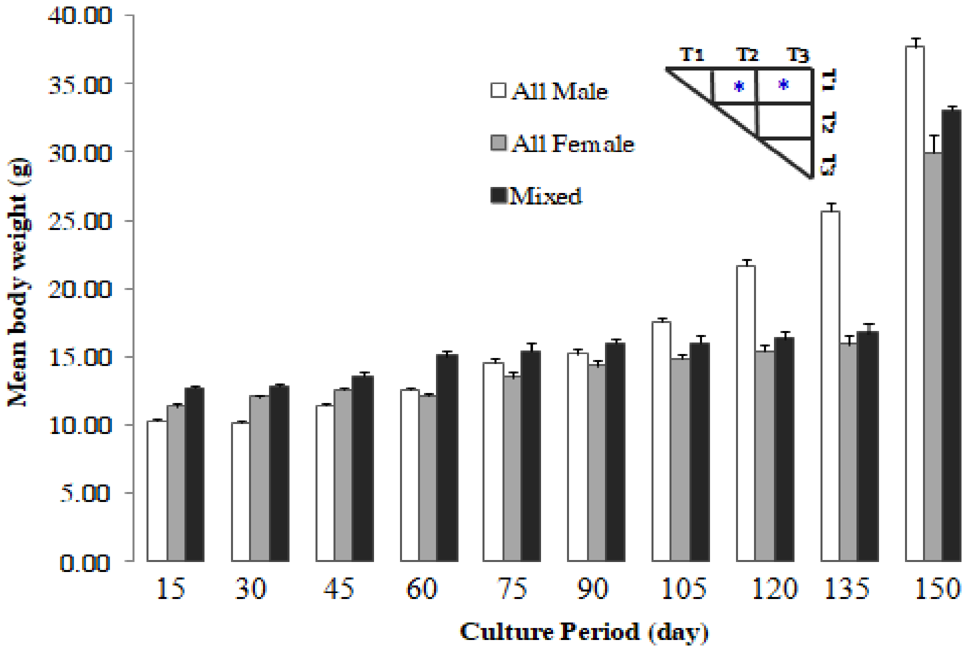

Figure 1. The bodyweight of mono-sex male, mono-sex female and the mixed-sex prawn revealed by fortnightly sampling. Error bars represent the standard deviation of thirty individuals. Mean values significantly different from each other are noted by asterix (*) (One-way ANOVA, $P < 0.05$).

The mono-sex male treatment group was found to have higher individual final weight in T_1 (38.83±3.6 g), followed by T_3 (33.07±5.8 g) and T_2 (28.33±3.9 g). Similarly, the highest weight gain and gross production were found in T_1, followed by T_3 and T_2 ($P< 0.05$) (Table 2).

Table 2. Production performance and feed utilization (mean ± standard deviation) of mono-sex male, mono-sex female and mix-sex prawn after 150 days of culture. Different superscript letters in the same row indicate significant differences between the treatments (One-way ANOVA, $P< 0.05$).

Growth parameters	T_1 (Mono-sex male)	T_2 (Mono-sex Female)	T_3 (Mixed Sex)
Initial Weight (g)	1.74 ± 45^{a}	1.74 ± 45^{a}	1.74 ± 45^{a}
Final Weight (g)	38.83 ± 3.6^{a}	28.33 ± 3.9^{b}	33.07 ± 5.8^{c}
Weight Gain (g)	37.09 ± 9.04^{a}	26.59 ± 11.37^{b}	31.33 ± 14.21^{c}
DWG (g/day)	0.25 ± 0.05^{a}	0.18 ± 0.06^{a}	0.21 ± 0.05^{a}
(%) SGR	2.07 ± 0.03^{a}	1.86 ± 0.02^{a}	1.96 ± 0.02^{a}
FCR	1.35 ± 0.04^{a}	1.57 ± 0.02^{a}	1.28 ± 0.02^{a}
Survival rate (%)	67.78 ± 5.9^{a}	64.72 ± 0.96^{a}	63.05 ± 2.17^{a}
Gross Production (kg/ha)	789.61 ± 85.6^{a}	550.14 ± 44.2^{b}	625.51 ± 22.8^{b}

SGR=Specific growth Rate, DWG=Daily Weight Gain, FCR= Feed Conversion ratio

Growth performance in experiment 2

In the second experiment, final weight, weight gain, daily growth rate and gross production were found to be higher in D_1 and D_2 compared to D_3. In contrast, the results showed better survival in D_1 and D_2, compared to D_3(P<0.05).

Table 3. Production performance (mean ± standard deviation) of mono-sex male prawn at three different stocking densities (D_1=1/m^2, D_2=2/m^2, D_3=3/m^2) after 150 days of culture. Different superscript letters indicate the significant difference between the treatments (One-way ANOVA, P < 0.05).

Growth parameters	D_1	D_2	D_3
Initial weight (g)	6.44±0.32^a	5.29±0.58^b	4.56±0.54^b
Final weight (g)	57.81±0.93^a	55.22±1.13^a	40.97±0.77^c
Weight gain (g)	51.37±0.86^a	46.93±0.56^b	36.4±1.13^c
DWG (g/day)	0.34±0.01^a	0.31±0.00^a	0.24±0.01^b
(%) SGR	1.46±0.03^a	1.52±0.05^a	1.47±0.08^a
Survival rate (%)	85.83±3.05^b	80.41±3.15^b	67.22±3.05^a
Gross production (kg/ha)	496.18±34.86 ^{a}	888.05±9.05 ^{a}	826±42.07 ^{b}

Water quality parameter

In the present study, important water quality parameters, like pH, temperature, dissolved oxygen (DO), transparency and salinity were monitored (Table 4). All the water quality parameters were within the acceptable range for freshwater prawn culture.

Table 4. Water quality parameters (mean ± standard deviation) in the culture ponds during culture period of the experiment

Month	Days	pH	DO (ppm)	Salinity (ppt)	Temperature (°C)	Transparency (cm)
July	1st 15 days	7.72±0.12	5.82±0.12	0.33±0.24	31.10±1.10	21.50±0.24
	2nd 15 days	7.75±0.12	5.60±0.19	0.50±0.00	30.94±1.00	21.33±0.47
August	1st 15 days	7.72±0.07	5.40±0.00	0.50±0.00	30.41±0.55	26.33±0.00
	2nd 15 days	7.87±0.05	5.42±0.07	0.42±0.12	30.41±0.49	26.00±0.47
September	1st 15 days	7.67±0.05	5.10±0.19	0.42±0.12	31.14±0.64	27.00±1.89
	2nd 15 days	7.65±0.12	5.03±0.24	0.33±0.24	31.11±0.59	27.83±0.24
October	1st 15 days	7.65±0.07	4.57±0.38	0.92±0.59	28.06±4.33	27.17±0.24
	2nd 15 days	7.75±0.02	4.75±0.31	0.83±0.47	28.44±4.07	25.83±0.24
November	1st 15 days	7.63±0.09	3.96±0.20	2.33±0.60	20.07±1.33	27.67±1.73
	2nd 15 days	7.60±0.09	3.90±0.15	2.44±0.42	19.91±1.09	27.78±1.50

Discussion

Like other crustaceans, the growth and physiology of *M. rosenbergii*are influenced by many factors, including environmental factors, sex and sexual maturity, stocking density, cellular metabolism, size and feeding habits (Aflalo et al., 2012; Hirose et al., 2013; Whiteley, 2011). In experiment 1, ponds stocked with all-male prawns produced numerically superior results compared to all females and mixed-sex. So, the culture of the all-male population produces a significantly higher marketable yield than an all-female or mixed-sex culture (Mohanta, 2000; New et al., 2000; Rungsin et al., 2006). Today, mono-sex male or female population of fish species is grown from the concept of diverting breeding energy to increase body mass (Nair et al., 2006), whereas sexual dimorphism causes reduced growth of the female mono-sex population. Apparently, upon attaining sexual maturity, females start to divert much of their energy intake to ovarian maturation compared to growth, whereas males continue to grow at the same rate (Ling, 1969).

In Prawn culture, Stocking density plays an significant role in survival, growth, population structure and production (Azad et al., 2021). There is significant relationship between the growth rate and the stocking density of Prawn, because suitable stocking density facilitates best food utilizations, thus accelerate highest growth maintaining healthy pond environment (Azad et al., 2021; Ghosh et al., 2016). Sometime high stocking density negatively impact on the production because of the competitions of food and shelter arising, risk of disease outbreak consequence higher mortality and poor growth of prawn Probably, due to the same effects in combination with shelter competition, cannibalism and male morphotype caused a lower survival of prawn at higher stocking densities (Azad et al., 2019, 2021; Hossain & Chakraborty, 2017).Compering to our neighboring country, in India the stocking density of *M. rosenbergii* varies from 2 to 3/m^2 in mixed culture and 1–2/m^2 in all male monoculture (Nair & Salin, 2012). Whereas in Bangladesh, it varies from 2 to 3/m^2, and 2/m^2 has been recommended as the most profitable density (Asaduzzaman et al., 2009; Azad et al., 2021; Ghosh et al., 2016).In the first experiment, several growth parameters, such as final weight, weight gain, and gross production were found higher in mono-sex male prawn culture compared to mono-sex female and mixed-sex populations (Table 2). The results also showed that mixed-sex culture showed better performance than mono-sex female might be due to the higher growth rate of male counterparts or due to the sexual homogeneity in the community. Similarly, faster growth and higher weight of male prawn was found by Aflalo et al. (2006). They reported that higher growth and production characteristics of male prawn might give more economic opportunity in prawn culture. Hence, we conducted the second experiment for finding a suitable stocking density to achieve higher production from a mono-sex male prawn culture. The suitable marketable size of prawn varies 45 to 60g and its gain this commercial size within 6 to 8 months culture period (Azad et al., 2019; Ghosh et al., 2016; Hasanuzzaman et al., 2011.

In the second experiment, higher weight gain, final weight, daily weight gain, and survival rate of the mono-sex male population at the stocking density 1 and 2 individuals per m^2 were might be high due to the less competition among the individuals on space and food. Baysa & Whangchai, (2007) obtained significantly (P<0.05) higher growth performance, survival and production of prawns stocked at 25 ind/m^2 density than at 50/m^2 density in case of intensive culture. In their study, lower production was obtained in the case of 50/m^2 stocking density due to extremely high stocking density that might lead the individuals to have serious competition among them. Siddiqui et al. (1997)evaluated the effects of stocking density and mono-sex culture on growth, survival, yield and feed conversion ratio of freshwater prawn (*Macrobrachiumrosenbergii)* at densities of 5, 10, 15 and 20 prawns/m^2 in triplicate concrete tanks in Saudi Arabia. They reported that the mean body weight decreased with the increasing density, being highest at 5 prawns/m^2 (29.6 g) and lowest at 20 prawns/m^2 (17.4 g). An on-farm trial with 4 different treatments including 3, 6, 8 and 10 PL/m^2 in Mekong Delta, Vietnam for one year provided that the growth performance for the treatments 3, 6 and 8 PL/m^2 were significantly higher than that of treatment 10 PL/m^2(Phuong et al., 2008).

Higher survival of prawn in D_1 might be due to the proper utilization of supplemented feed, and better pond quality. A feeding try was used to measure the feed consumption. On the other hand, a higher mortality rate is observed at higher stocking density due to the higher male competition. Probably, increased density might create pressure on the dominant males which revealed a higher survival through balanced social interaction (Banu et al., 2016).

The effect of stocking densities on growth and production also experimented on a wide range of fish and crustacean species. Almost similar patterns of results were obtained from the experiments conducted so far including polyculture of prawn and carps (Ranjeet & Madhusoodana Kurup, 2002) white shrimp, *Penaeusvannamei* (Araneda et al., 2008) black sea turbot, *Psetta maxima*(Aksungur et al., 2007); mullet, *Mugil platanus* (Sampaio et al., 2001). In most cases, the growth performance and survival were higher at lower stocking densities, but the production and profitability depend on stocking density where the most production may come from the optimized higher stocking density.

The water quality parameters were recorded within the suitable range as mentioned for the nursery and grow-out cultivation of freshwater prawns. The water quality parameters did not significantly vary among the treatments which might be due to change stocking density of prawn as reported in other studies (Azad et al., 2019, 2021; Ghosh et al., 2016). In the present experiment, the water temperature range was 16-27 ºC (Table 4) which was tolerable for prawn (not optimum for biological activities) as the optimum range of water temperature for aquatic animals is 25-30 º C (Passy, 2007). The lower water temperature was attributed due to the winter season and this might lead to the comparatively lower growth performance of the prawns. Standard water transparency in a prawn culture pond should be between 25-35 cm (Nandlal & Pickering, 2006). In the present experiment, the values ranged between 19-34 cm (Table 4) that were compatible with the standard value with little variation. In the present experiment, the pH range was found to be 7.4-8 (Table 4) throughout the experimental period. The suitable pH range for fishes, crustaceans and other aquatic organisms is 6.5-8 (Ramesh & Anbu, 1996).

One of the most important factors to indicate the water quality is DO which is essential for the respiration of every organism. The optimum range of DO for fish, crustaceans and other aquatic animals is 5-8 ppm (Ragothaman & Trivedy, 2002). In both of the experiments, the DO levels were within the suitable ranges varied between 5.8 and 6.6 mg/L (Table 4).

Still, there is a dearth of information on the suitable stocking density of giant freshwater prawn (*Macrobrachiumrosenbergii*) culture for ensuring better production and profitability. The findings of this research work would be useful by getting better stocking density for sustainable prawn aquaculture. We suggest further studies should be conducted on intensifying mono-sex male prawn production through ecological approaches for maximum production and economic efficiency.

Conclusion

This study supports that mono-sex male has a higher mean body weight as compared to mixed-sex and mono-sex female. Besides, our study shows the maintenance of stocking density 2 prawns/m^2 in mono-sex male culture for better growth performance and production. As *M. rosenbergii*s a prime and promising aquaculture species in the tropical and sub-tropical areas, the culture of mono-sex male prawn will be interested in the farmers. However, special attention must be given to producing mono-sex male PL of prawn in large quantities to meet the increasing demand of prawn farmers. Otherwise, a negative impact will interfere with the natural population balance in male-female availability.

Acknowledgement

The authors are grateful to the Bangladesh Bureau of Education Information & Statistics (BANBEIS), Ministry of Education of Government of the People's Republic of Bangladesh USDA for financial support to conduct the research.

Conflict of interest

The authors declared no conflict of interest in producing this manuscript.

Author contributions

Shikder Saiful Islam: Field research, data acquisition, data analysis, project administration, writing: original draft, , writing: review and editing; Md Rony Golder: Writing: original draft, data analysis; Joyanta Bir: Data

analysis, writing: original draft, writing: review and editing; Saroj Kumar Mistry: Field research and data collection; Md. Nure Alam Siddique: Field research and data collection; Md. Rayhan Ali: Resources, writing: review and editing; Wasim Sabbir: Supervision, writing: review and editing; Md. Abul Kalam Azad: Field research and data collection; Khandaker Anisul Huq: Fund acquisition, supervision, project administration, writing: reviewing and editing.

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