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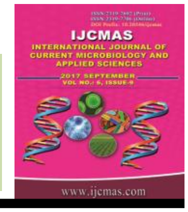

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Effect of Stocking Density on Growth and Survival of *Fenneropenaeus merguiensis* (de Man, 1888) Post Larvae

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A B S T R A C T

The present study evaluated the effect of stocking density on growth and survival of post larvae (PL) of *Fenneropenaeus merguiensis* (de Man, 1888), commonly known as banana shrimp. PL was stocked at four different densities i.e., 10, 20, 30 and 40/m^2 (T10, T20, T30 and T40, respectively) and reared for a period of 60 days. Water quality and growth performance parameters of different treatments were compared during the experiment. A significant ($p<0.05$) decrease in dissolved oxygen (DO) concentration relative with the higher stocking density was observed. Though some of the parameters showed significant ($p<0.05$) difference among the treatments, the water quality remained within safe level, throughout the experiment. Increase in stocking density led to significant reduction ($p<0.05$) in BWG and SGR of PL. The highest value of FCR was in T40 and lowest in T10. Stocking density also showed an inverse relationship with survival percentage of shrimp. Results of the present study revealed that high stocking density (≥30/m^2) can seriously impair the growth of *F. merguiensis* PL.

Introduction

Shrimp, which earns huge revenue through export, is an important aquaculture commodity in India. Several studies revealed that *Fenneropenaeus merguiensis*, popularly known as banana shrimp, which are found throughout the Asian and Australian tropical and subtropical waters, is a promising candidate species for aquaculture (Zacharia and Kakati, 2002). *F. merguiensis* has attracted researchers and farmers because of certain advantages like captive breeding in pond, tolerance to low water quality, euryhaline and eurythermal nature, it's readily availability in the wild, good performance during high stocking densities, low protein requirement, minimal size variation during grow out phase, and cheap availability of wild brooders. Above that, rearing of larvae and post larvae of *F. merguiensis* is comparatively easy (Hoang, 2002).

According to FAO (2010), *F. merguiensis* is a high value commodity for shrimp culture with the ability to attain fast growth rate and forms 5.5 % of the total shrimp (Truong *et al.*, 1995) globally.

Growth and survival of aquatic organisms, which together determine the ultimate yield, are influenced by a number of ecological parameters and managerial practices. At present, a transitional nursery phase between the hatchery and grow-out ponds is a common practice in shrimp farming industry in many countries (Correia *et al.*, 2014). This three phasing farming system has lots of benefits. Nevertheless, high stocking density in the nursery phase may affect the growth and survival of shrimp due to an increase in cannibalism and degradation of water quality (Wasielesky *et al.*, 2013). Determining the optimum stocking density of the animals being reared is thus very important for the farmers, to maximize production and profitability. However, so far, no studies have been undertaken to investigate the influence of stocking density on the growth and survival of *F. merguiensis* PL under captive condition. Hence, the present work was aimed at studying the performance of *F. merguiensis* post larvae reared in nursery tanks at four different stocking densities.

Materials and Methods

Experimental animal

Post Larvae (PL15-20) of banana shrimp, *Fenneropenaeus merguiensis* (de Man, 1888), (30 days old), proured from ICAR-CIBA, Chennai, were procured from "Soil and Water Management Research Unit", Navsari Agriculture University (NAV), Gujarat. The average length and weight of PL were 1.02 cm and 0.01g, respectively.

Site of the experiments

The experiments were conducted in the wet lab of Veraval Regional Centre of ICAR-Central Marine Fisheries Research institute (CMFRI) at Veraval, Gujarat. Subsequently, analysis work was carried out in the water and soil chemistry laboratory of the same institute. Feed formulation and related analysis were undertaken in the feed mill and biochemistry laboratory in the fish processing section of Veraval Research Centre of ICAR-Central Institute of Fisheries Technology (CIFT) at Veraval, Gujarat.

Acclimatization of *F. merguiensis* post larvae

Post larvae of *F. merguiensis* were acclimatized in the rectangular tanks (100×50×50 cm) for 15 days. During this period, optimum water quality parameters (Dissolved oxygen, pH, temperature, salinity, TAN) were maintained. Feeding, siphoning, aeration and water exchange were done meticulously. Feeding frequency was maintained at four times (06.00hrs, 12.00hrs, 18.00hrs and 24.00 hrs.) with 100 % body weight.

Experiment

An experimental trial of 60 days was conducted to understand the effect of stocking density on the growth and survival of hatchery raised post larvae of *F. merguiensis*, in laboratory conditions. After acclimatization for a period of fifteen days, *F. merguiensis* PL were stocked in a FRP tank of 70 L capacity in the wet laboratory. Healthy PL were segregated and stocked in pre-cleaned tanks at different stocking densities i.e., 10 (T10), 20 (T20), 30 (T30) and 40 (T40) numbers/m^2. Aggregate initial weight of PL was taken using an electronic balance. The average weight of PL was found in the range of 0.35±0.05 g. All the experimental units received 10 % water exchange daily and 100 % exchange of water on weekends. The commercial feed "Godrej Agrovet" was used to feed the shrimp PL. Aeration was paused at the time of feeding and siphoning of the tank while continuous aeration was maintained for rest of the time. Siphoning was done eight times per day, after an hour of feeding for collecting the left over feed and before the next feeding schedule for collecting the excreta. Physico-chemical quality parameters of water in the experimental tanks and growth performance parameters of PL of *F. merguiensis* were analyzed at regular intervals throughout the experiment.

Analysis of physico-chemical quality parameters of water

Temperature of water in the experimental tanks was measured in the morning and evening using mercury thermometer and expressed as ^oC. pH (hydrogen ion concentration) of the water in all the experimental tanks was tested using a digital pH meter and salinity of the water was measured by salinity refractometer. The DO level in water was estimated according to Winkler's method (APHA, 1995). The level of total ammonia (NH3)-N (TAN) was estimated using spectrophotometer at a wavelength of 640 nm by phenate method (APHA, 1998) and nitrite-N (No_2-N) and nitrate-N (No_3-N) concentration was measured (APHA, 1998) at 543 nm using UV-VIS spectrophotometer and compared with standard graph. Phosphate (PO4-P) concentration was measured (APHA, 1998) at 693 nm using UV-VIS spectrophotometer and concentration was expressed as mgL^{-1}.

Evaluation of growth performance parameters

Growth performance parameters of PL of *F. merguiensis* like % body weight gain (BWG), specific growth rate (SGR), feed conversion ratio (FCR), feed conversion efficiency (FCE), protein efficiency ratio (PER) and survival percentage were analyzed. The percentage BWG and SGR of *F. merguiensis* were evaluated by taking its body weight at 15 days interval. Digital weighing balance was used to measure the weight of the shrimps. The following formulas were used to evaluate the growth of *F. merguiensis*.

BWG (%) = (Final weight – Initial weight) × 100/ Initial weight

SGR (%) = [(Ln final weight - initial weight)/number of days] ×100

$$\text{PER} = \frac{\text{Wet weight gain (g)}}{\text{Dry protein intake (g)}}$$

$$\text{FCR} = \frac{\text{Dry feed intake (g)}}{\text{Wet weight gain (g)}}$$

FCE (%) = Wet weight gain (g)/Feed consumed (g) x 100

$$\text{Survival (\%)} = \frac{\text{Total number of shrimps harvested}}{\text{Total number of shrimps stocked}} \times 100$$

Statistical analysis

The data were analyzed by statistical package for social science (SPSS) software version 16.0. Significance difference between means was determined using Duncan's multiple-range test (DMRT). The level of significance was set up at $p \leq 0.05$.

Results and Discussion

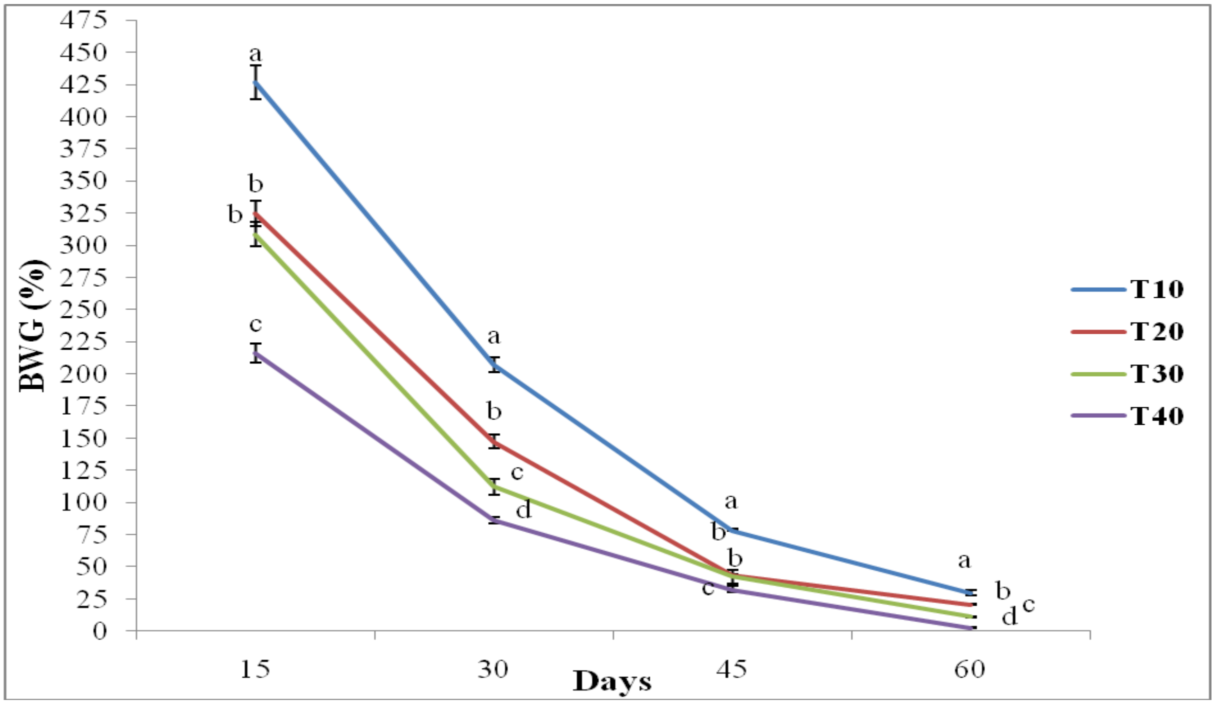
Stocking density plays an important role in growth and survival of any cultured organism. Globally, super-intensive production of shrimp is gaining popularity as a possible way to improve aquaculture production (Liu *et al.*, 2017). However, there are few studies on growth performance of *F. merguiensis* PL stocked at different densities. In the present study, different stocking densities (10 (T10), 20 (T20), 30 (T30) and 40 (T40) numbers/m^2) were used for optimization and to analyze its effect on growth and survival of *F. merguiensis*.

Physico-chemical quality parameters of water

Stocking density can affect the shrimp, if water quality declines with increasing density. The deteriorated water quality can cause stress in shrimp leading to reduction in growth (Liu *et al.,* 2017, Aguilar *et al.*, 2012). Hence, the quality parameters of water need to be checked regularly during shrimp rearing. The physico-chemical quality parameters of the water in the present experimental tanks are shown in table 1. Temperature is a very important environmental factor, which strongly influences the growth of shrimps (Bray and Lawrence, 1992). Temperature ranging from 24 to 32^{o}C is optimum for penaeid larval culture. Throughout the experimental period, the water temperature of the experimental tanks was found between 27.32 ºC to 27.69 ºC. There was no significant difference ($p>0.05$) in the temperature of the tanks with increase in stocking density. Water used for experiment was pre-collected from sea shore and after filtering, stored for further use. Evaporation loss is maintained by adding fresh water, to keep the salinity constant. Although salinity of water in the tanks decreased, when stocking density increased, the decrease was not significant ($p>0.05$). Salinity of water in the tanks during experimental period ranged from 31.73± 0.29 to 32.01± 0.23 ppt as given in table 1. Hudinaga (1942) and Cook and Murphy (1969) reported that salinity from 27 to 34 ppt was suitable for development of penaeid larvae. Saldanha and Achuthankutty (2000) suggested that the optimum pH for the growth of *F. merguiensis* is 7.8 to 8.7. The values of pH in the present experiment, match with the findings of Wang (2004) in *L. vannamie* culture. The pH values of water ranged from 7.9±0.05 to 8.1 ± 0.20, for experimental tanks with different stocking densities. There was no significant ($p<0.05$) difference observed in pH values with the higher stocking density. Several previous researchers have suggested that optimum dissolved oxygen level for penaeid culture is 5.23 to 7.10 mg/L. During the experimental period, DO of the water in the tanks was found in the range of 5.18 ± 0.04 to 6.16 ± 0.90 mg/L, which was well within the acceptable range. In general, dissolved oxygen level varies with water temperature, metabolic rate, biomass density etc. In the current study, highest DO level was recorded in T10 and lowest in T40 corresponding to lowest and highest stocking density. Similar observation was done by Rahman and Rahman (2003). The DO level of water in various tanks showed significant difference ($p<0.05$) with increase in the stocking density as observed by Balakrishnan *et al.,* (2011) in pond condition for *L. Vannamei* and Shyne Anand *et al.,* (2014) for *F. merguiensis.*

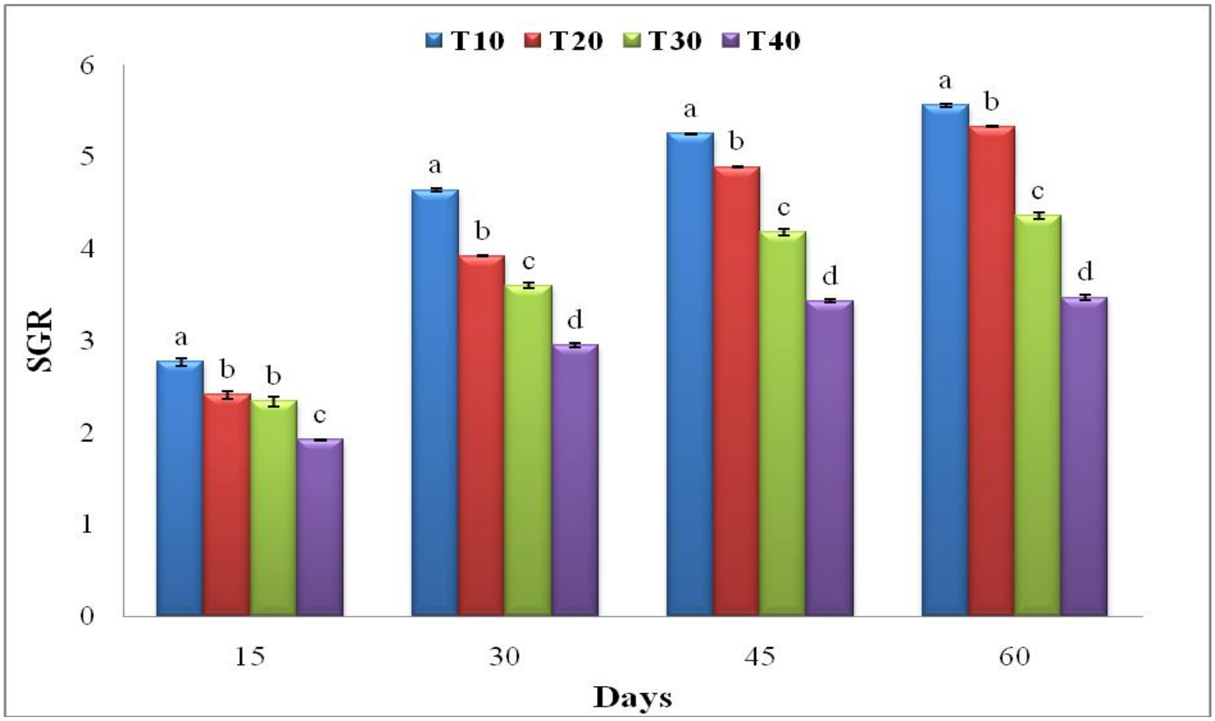
Total ammonia (NH3)-N (TAN), Nitrite (NO_2-N) and Nitrate (NO_3-N) are important water quality parameters, which indirectly hamper the growth and survival of shrimp. TAN is very crucial for culture of aquatic organisms. In the present study, though there was a significant increase ($p<0.05$) in the level of TAN, mean values of TAN in all the treatments remained below toxic levels throughout the experimental period. The observed values of TAN were from 0.20 ± 0.03 to 0.34 ± 0.01 mg/L and the values for NO_2-N, during the experiment, ranged from 0.12 ± 0.02 to 0.17 ± 0.03 mg/L as shown in table 2. The level of NO_2-N increased from T10 to T40 and did not differ significantly. Mean values of NO_3-N fell in the range from 0.22 ± 0.02 to 0.41 ± 0.04 mg/L. There was significant ($p<0.05$) increase in the NO_3-N level with increase in stocking density.

Fig.1 BWG (%) of PL of *F. merguiensis* during rearing of 60 days in FRP tanks at different stocking density



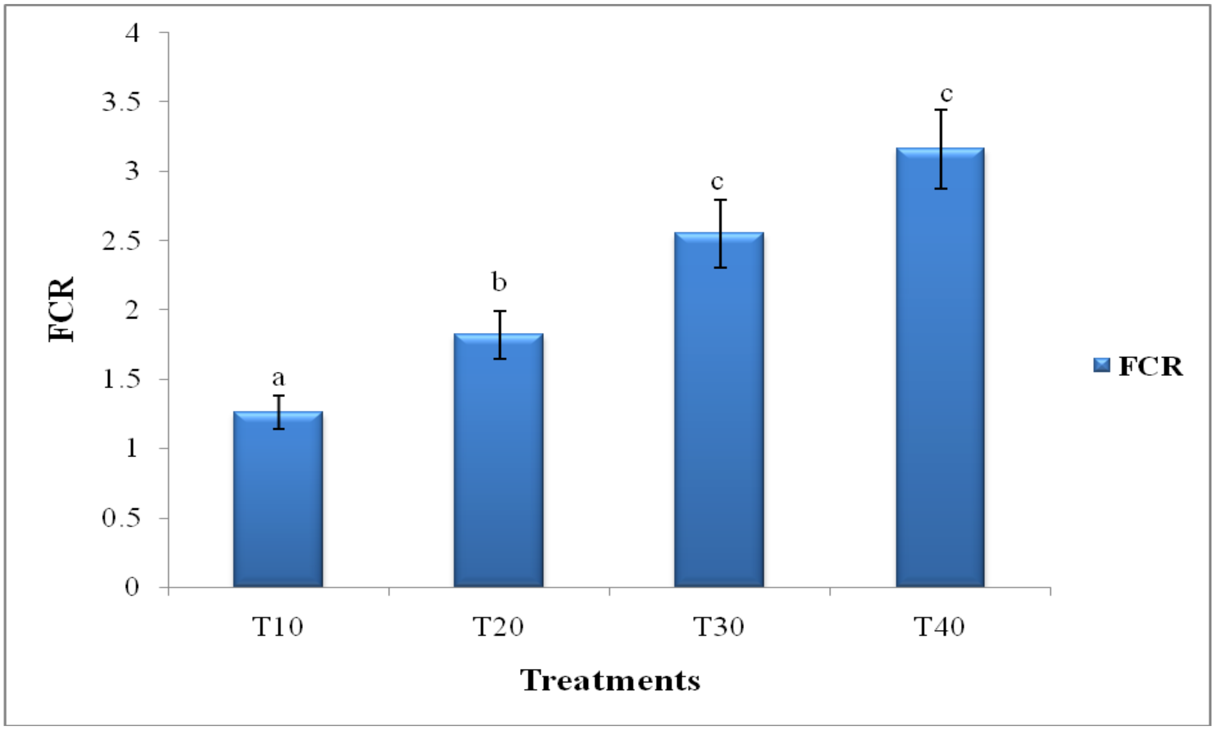
*Means ± SD in the same column with different superscripts are significantly different (p<0.05)

Fig.2 SGR of PL of *F. merguiensis* during 60 days rearing in FRP tanks at different stocking density



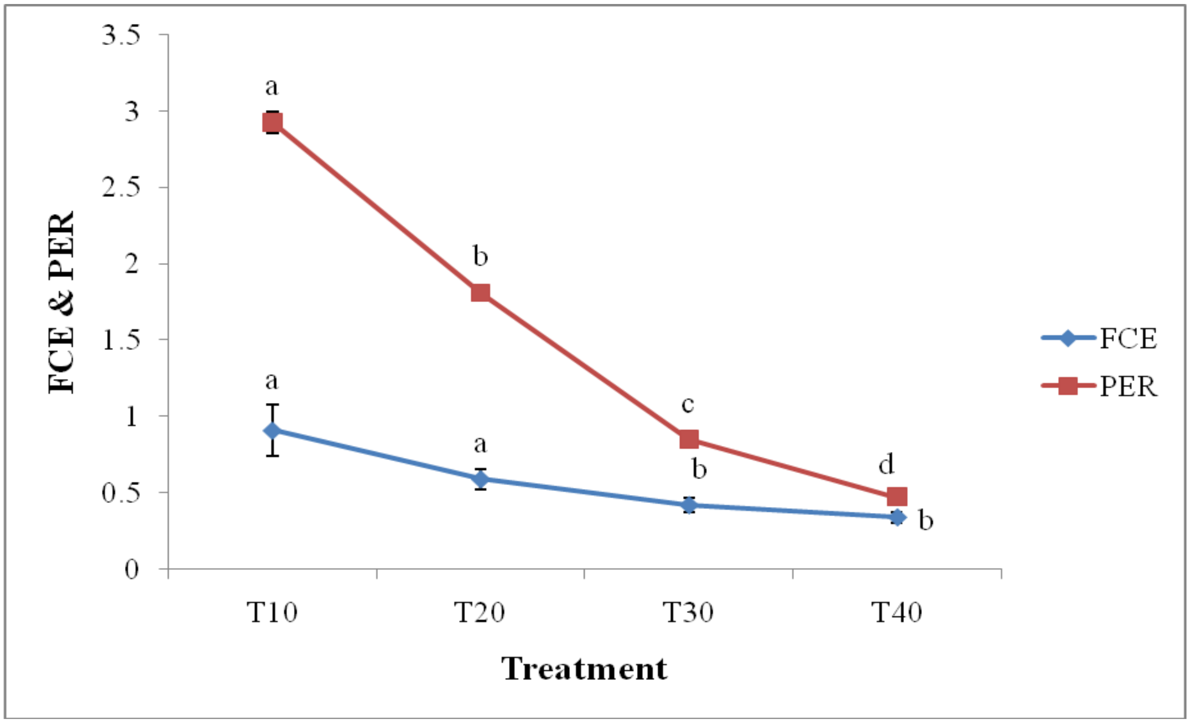
*Means ± SD in the same column with different superscripts are significantly different (p<0.05)

Fig.3 FCR of PL of *F. merguiensis* reared for 60 days in FRP tanks at different stocking density



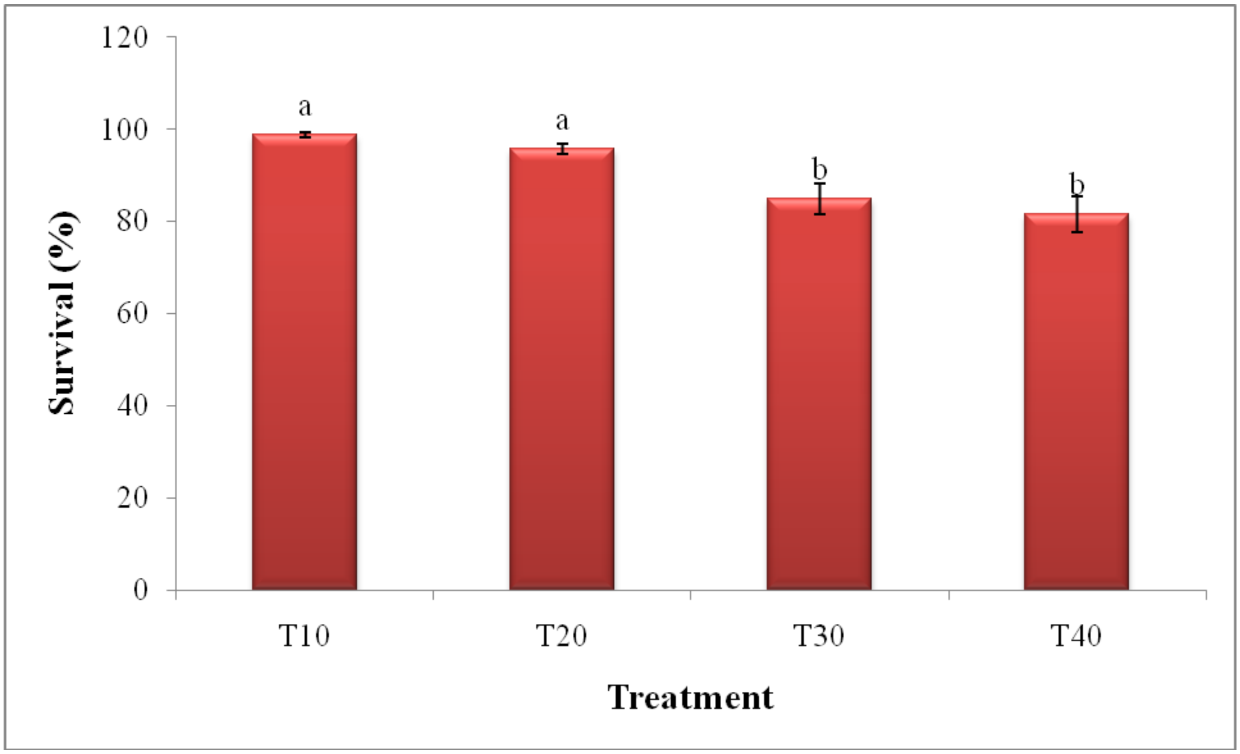
*Means ± SD in the same column with different superscripts are significantly different ($p<0.05$)

Fig.4 FCE and PER of PL of *F. merguiensis* reared for 60 days in FRP tanks at different stocking density



*Means ± SD in the same column with different superscripts are significantly different ($p<0.05$)

Fig.5 Survival (%) of PL of *F. merguiensis* reared for 60 days in FRP tanks at different stocking density



*Means ± SD in the same column with different superscripts are significantly different (p<0.05)

Table.1 Temperature, salinity, pH and DO of water in the experimental tanks with Different stocking densities

Treatment	DO (mg/L)	Temperature (°C)	Salinity (mg/L)	pH
T10	6.16±0.09[a]	27.32±1.11[a]	32.01±0.33[a]	8.1±0.20[a]
T20	5.84±0.12[b]	27.47±1.23[a]	32.07±0.34[a]	8.0±0.15[a]
T30	5.53±0.06[c]	27.51±1.21[a]	31.84±0.34[a]	7.9±0.05[a]
T40	5.18±0.09[d]	27.69±1.33[a]	31.73±0.32[a]	7.9±0.05[a]

* Mean ± standard deviation in the same column with different superscripts are significantly different (p<0.05)

Table.2 TAN, NO_2-N, NO_3-N and PO_4-P content of water in the experimental tanks with Different stocking densities

Treatment	TAN (mg/L)	NO_2-N (mg/L)	NO_3-N (mg/L)	PO_4-P (mg/L)
T10	0.20±0.03[a]	0.122±0.02[a]	0.216±0.02[a]	0.18±0.04[a]
T20	0.29±0.03[a]	0.135±0.03[a]	0.290±0.02[ab]	0.33±0.03[ab]
T30	0.31±0.02[b]	0.148±0.02[a]	0.372±0.03[bc]	0.55±0.09[b]
T40	0.34±0.01[b]	0.171±0.03[a]	0.405±0.04[c]	0.92±0.19[c]

* Mean ± standard deviation in the same column with different superscripts are significantly different (p<0.05)

Lowest value of NO_3-N was observed in T10 and highest value was noted for T40. The values of NO_2-N and NO_3-N were also well below safe levels. Similar result was obtained by Arnold *et al.,* (2006) for tiger shrimp (*Penaeus monodon*) intensively grown from PL_{15} for 56 d in tank systems at different stocking densities, with and without the addition of artificial substrates. The detected phosphate level in the experimental tanks was from 0.18 ± 0.04 to 0.92 ± 0.19 mg/L. The level of PO_4-P was found significantly different ($p<0.05$) among the treatments. Water quality declined at high stocking densities (T30 and T40) and nitrogenous compounds and phosphate were higher. But, it was observed that in general, mean water quality parameters in all the treatments were within the level recommended for culturing penaeids.

Growth performance parameters of PL of *F. merguiensis*

It is well known that stocking density influences growth and survival of shrimp and an inverse relationship between these factors are generally reported (Wasielesky *et al.,* 2013). In the present experiment, the average final individual weight of PL of *F. merguiensis* decreased significantly ($p<0.05$) with increasing stocking density. At the end of the experiment, the final biomass found significantly ($p<0.05$) higher in T10 and T20 compared to T30 and T40. The mean final weights found in T10, T20, T30 and T40 were 5.95, 4.83, 3.83 and 3.27 g respectively, at the end of 60 days of the experiment. Similar result was declared by Sookying *et al.,* (2011) for pacific white shrimp. They revealed a significantly higher mean final weight in the low density groups compared with higher densities. Body weight gain (%), among the treatments, for PL of *F. merguiensis* ranged from 216.19 ± 7.45 to 426.67 ± 12.95 as shown in figure 1. Increase in stocking density led to significant reduction ($p<0.05$) in BWG and SGR of PL. Similar observation was found by Balakrishnan *et al.,* (2011). They revealed average body weight increased when stocking density was low. BWG (%) on 15th day of current experiment showed significant difference ($p<0.05$) among the treatments. The highest BWG observed in T10 having stocking density of 10 shrimp PL/tank and the lowest BWG was seen in treatment T40 with stocking density of 40 shrimp PL/tank. On 30th day, BWG was in the range of 86.14 ± 2.36 % to 207.23 ± 5.5 % and it varied from 32.74 ± 2.39 % to 78.8 ± 0.99 % on 45th day. At the end of the experiment, BWG was highest in T10 (29.95 ± 1.85 %) and lowest in T40 (2.79 ± 0.62 %). The negative relationship between stocking density and average body weight was also reported by Krishna *et al.,* (2015). Values of SGR obtained for PL of *F. merguiensis* reared under captive condition at different stocking density is shown in figure 2. Significant ($p<0.05$) differences in SGR of *F. merguiensis* were found between the tanks with different stocking density. Highest SGR were observed in the T10 and lowest in T40. Similar observation was done by Araneda *et al.,* (2008), Williams *et al.,* (1996) and Sookying *et al.,* (2011), who reported that that increasing density and shrimp biomass had negative effect on growth.

FCR is always a prime concern for shrimp farmers. At the end of the study, the FCR was found in the range of 1.25±0.12 to 3.16±0.28, as depicted in figure 3. FCR increased with increased shrimp density and significant ($p<0.05$) differences in FCR were found among the treatments. Highest value of FCR was obtained for T40, whereas lowest one was recorded in T10. Similar result was obtained by Shakir *et al.,* (2014) for *Penaeus monodon.* FCR in T40 and T30 was significantly higher than T10. The values of FCE obtained for banana shrimp PL in the experiment is shown in figure 4. The FCE ranged from 0.34 ± 0.03 to 0.91 ± 1.71. Highest FCE were recorded in T10 and the lowest in T40. Significant differences ($p<0.05$) were observed for FCE of T10 and T40. T10 showed no significant ($p<0.05$) difference in FCE value from T20. Protein efficiency ratio (PER) indicates the better utilization of dietary protein. In the present study, values of PER varied from 2.90±0.07 to 0.47±0.01 for PL of *F. merguiensis* reared for 60 days. Highest PER value was recorded in T10 and lowest in T40 as depicted in figure 4. It may be due to overcrowding and competition for food and space, which ultimately leads to stressful conditions for the cultured shrimps. Observed results match with the findings of Chakraborty *et al.,* (1997) for tiger shrimp (*Penaeus monodon* fab.) fed on commercial formulated diets. Survival percentage of PL of *F. merguiensis* ranged from 98.81±0.61 to 81.46 ± 3.85 (Figure 5). Significant differences ($p<0.05$) were found in survival percentage among the treatments. Survival rate was significantly ($p<0.05$) lower in the treatments with higher stocking density (T30 and T40) and highest rate of survival was observed in T10. Similar result was reported by Apud (1981) for *P. monodon*, where high stocking density resulted significant ($p<0.05$) reductions in survival. Li *et al.,* (2006) also observed that high stocking density negatively influenced the growth and survival rate of Chinese shrimp, *Fenneropenaeus chinensis*. Survival percentage observed in the present study suggested that stocking density $\geq 30/m^2$ had affected shrimp survival.

Stocking density is a very important factor in shrimp culture. It significantly affects the growth, survival and yield of shrimp growth. Many of the previous researchers have also reported that growth of shrimp generally decreased, when stocking density increased. The reduced water quality, along with the physical stress caused by crowding at higher stocking densities, could be the reasons for lower growth performance. Similarly, results of the present study revealed that high stocking density ($\geq 30/m^2$) can seriously impair the growth of *F. merguiensis* PL.

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